Toxicological profile for

Cardamom oil, seed oil

This ingredient has been assessed to determine potential human health effects for the consumer. It was considered not to increase the inherent toxicity of the product and thus is acceptable under conditions of intended use.

1. Name of substance and physico-chemical properties

1.1. IUPAC systematic name

Not applicable.

1.2. Synonyms

CAS 8000-66-6: Cardamom oil; Cardamom oleoresin / extract; Cardamom resinoid; Cardamom seed oil; Cardamom seed oil absolute (Elletaria cardamomum (L.) Maton); Cardamon; Elettaria cardamomum oil; FEMA No. 2241; Oil of cardamom; Oils, cardamom; Cardamom oil (PubChem)

CAS 85940-32-5: Cardamom, ext; Cardamom (Elletaria cardamomum (L.) Maton); EINECS 288-922-1; FEMA No. 2240; EC 288-922-1 (PubChem)

1.3. Molecular formula

“Unspecified”

1.4. Structural Formula

Not applicable.

1.5. Molecular weight (g/mol)

Not applicable.

1.6. CAS registration number

8000-66-6; 85940-32-5

1.7. Properties

1.7.1. Melting point

(°C): 1.5 (EPISuite, 2017) (CAS RN 8000-66-6).

1.7.2. Boiling point

(°C): 176.4 (EPISuite, 2017) (CAS RN 8000-66-6).

1.7.3. Solubility

3500 mg/L at 21^{o}C (EPISuite, 2017) (CAS RN 8000-66-6).

1.7.4. pKa

No data available to us at this time.

1.7.5. Flashpoint

(°C): No data available to us at this time.

1.7.6. Flammability limits (vol/vol%)

No data available to us at this time.

1.7.7. (Auto)ignition temperature

(°C): No data available to us at this time.

1.7.8. Decomposition temperature

(°C): No data available to us at this time.

1.7.9. Stability

“The typical shelf-life of pure flavouring compounds is stated to be at least 12 months, when stored in tightly closed containers under standard conditions” (EFSA, 2019)

1.7.10. Vapor pressure

1.9 mmHg at 25°C (EPISuite, 2017) (CAS RN 8000-66-6).

1.7.11. log Kow

2.74 (EPISuite, 2017) (CAS RN 8000-66-6).

2. General information

2.1. Exposure

Cosmetics: Yes (Cosmetics Bench Ref, 1996) Food: Yes (Ash, 1995) Environment: No evidence (Merck, 1996) Pharmaceuticals: Yes (Martindale, 1993)
FEMA estimated the PADI (possible average daily intake) of cardamom seed oil [presumably from its use as a flavouring] as 54.685 mg (Burdock GA, 2010).
“Cardamom is used extensively as a domestic spice in curries, breads, and cakes; also in coffee, especially in India, Britain, Germany, Scandinavia, the Middle East, and Latin America. Both cardamom seed and its oil arewidely used as flavor components in most categories of food products, including alcoholic and nonalcoholic beverages, frozen desserts, candy, baked goods, gelatins and puddings, meat and meat products, condiments and relishes, and gravies, among others. Highest average maximum use level reported for the seed is 0.5% in gravies and about 0.01% (117 ppm) for the oil in alcoholic beverages.”

As taken from Khan IA and Abourashed EA, 2010.

Reported uses (ppm): (FEMA, 2007)

Food Category	Usual	Max.
Alcoholic beverages	111.14	120.00
Baked goods	51.50	70.00
Chewing gum	50.00	4500.00
Condiments, relishes	61.84	70.00
Frozen dairy	5.62	10.00
Gelatins, puddings	12.91	15.00
Gravies	5.00	10.00
Hard candy	50.00	3500.00
Meat products	36.18	55.00
Nonalcoholic beverages	2.29	4.04
Soft candy	6.74	8.03

As taken from Burdock G.A (2010). Fenaroli's Handbook of Flavor and Ingredients. Sixth Edition. CRC Press. ISBN 978-1-4200-9077-2

Elettaria cardamomum seed oil (CAS RN 8000-66-6/85490-32-5) is used as a tonic, fragrance and perfuming agent, Elettaria cardamomum seed extract (CAS RN 85940-32-5) as a tonic and perfuming ingredient, Elettaria cardamomum seed powder (CAS RN 85940-32-5) as a skin conditioning ingredient in cosmetics in the EU.

As taken from CosIng, undated.

Cardamom seed absolute, cardamom seed oil, cardamom seed extract, cardamon seed, cardamom seed CO2 extract and cardamom seed distillate (all CAS RN 8000-66-6) are listed as fragrance ingredients by IFRA.

Cardamom seed oil (Elettaria cardamomum (L.) Maton) (CAS RN 8000-66-6) and cardamom (Elettaria cardamomum (L.) Maton) (CAS RN 85940-32-5) are listed as fragrance ingredients in the US EPA InertFinder Database.

Elettaria cardamomum seed oil (CAS RN 8000-66-6) is listed as an ingredient in inside the home (at <1% where specified) and personal care (at <1%) products, and cardamom extract (CAS RN 85940-32-5) as an ingredient in a personal care product, by the CPID.

"Although individual consumption figures for the EU are not available, the Fenaroli's handbook of flavour ingredients (Burdock, 2010) cites intake values of 0.026 mg/kg bw per day for cardamom seed and 0.0046 mg/kg bw per day for cardamom seed oil."

As taken from EFSA, 2019.

According to Health Canada's Natural Health Products (NHP) database, the following substances are used for the indicated purposes in non-medicinal NHPs:

Cardamom essential oil [no CAS RN listed] is used as a fragrance ingredient and as a flavour enhancer for oral use;

Elettaria cardamomum seed extract [no CAS RN listed] is used as a fragrance ingredient and skin-conditioning agent for topical use;

Oleoresin cardamom [no CAS RN listed] is used as a flavour enhancer.

As taken from Health Canada, 2021.

2.2. Combustion products

This ingredient was investigated in a pyrolysis study. Results are given in JTI Study Report (s).

Compound	Two stage heating		One stage heating	
	Abundance	Area%	Abundance	Area%
limonene	318710412	2.28	510899374	3.34
1,8-cineole	2456288380	17.56	2557739653	16.71
linalool	1471985629	10.52	1482149933	9.68
beta-terpineol + unknown	197145914	1.41	209473121	1.37
4-terpineol	243587856	1.74	256325734	1.67
alpha-terpineol	1667804008	11.92	1844482738	12.05
gamma-terpineol	481422992	3.44	512564417	3.35
linalyl acetate	582879560	4.17	640861243	4.19
beta-terpinyl acetate	450598517	3.22	513644916	3.36
geraniol	160946484	1.15	202233985	1.32
terpinyl acetate isomer	207968689	1.49	266536659	1.74
alpha-terpinyl acetate	3381842011	24.17	3905160903	25.51
eugenol	175252171	1.25	276275575	1.80
nerolidol	151599266	1.08	250237838	1.63
Total ion chromatogram	14036969074	100	15352014601	100

This ingredient was investigated in a pyrolysis study. Results are given in Baker and Bishop (2005) J. Anal. Appl. Pyrolysis 74:145–170.

Ingredient Name & CAS Number	Max. cig. appln. level (ppm)	Composition of pyrolysate (Compound, %)	Max. level in smoke (nü¡g)
Cardamom seed oil 8000-66-6	76	Alpha-Terpinolene 37.5 Cineole 26.5 Gamma-Terpinene + linalyl acetate 4.4 Linalool 3.4 Sabinene 2.6	14 10 2 1 1

2.3. Ingredient(s) from which it originates

Distilled from the seeds of Elletaria cardamomum (Burdock GA, 2010). “Source: Elettaria cardamomum (L.) Maton var. cardamomum (syn. E. cardamomum var. miniscula Burkill) (Family Zingiberaceae). Perennial reed-like plant with lance-shaped leaves borne on long sheathing stems, up to about 4m high; native to tropical Asia; now cultivated extensively in tropical regions, particularly India (Malabar coast), Sri Lanka (Ceylon), Laos, Guatemala, and El Salvador. Parts used are the dried, nearly ripe fruits with seeds from which an essential oil is obtained by steam distillation. The long wild native cardamon of Sri Lanka is obtained from E. cardamomum var. major Thwaites (syn. E. cardamomum var. miniscula Burkill), which has comparatively more elongated fruits (up to approximately 4 cm) than var. cardamomom, and dark brown pericarps with coarse striations, the oil of the which is used as a natural flavoring in liqueurs.”

As taken from Khan IA and Abourashed EA, 2010.

Elettaria cardamomum seed extract is an extract from the seeds;

Elettaria cardamomum seed powder is the powder obtained from the dried, ground seeds;

Elettaria cardamomum seed oil is the volatile oil obtained from the dried ripe seeds;

and Elettaria cardamomum fruit extract (no CAS RN given) is the extract of the fruit …

… of the Cardamom, Elettaria cardamomum, Zingiberaceae. The seed oil contains eucalyptol, sabinene, D-alpha-terpineol and its acetate, borneol, limonene, terpinene, L-4-terpinenol and its acetate and formate.

As taken from CosIng, undated

3. Status in legislation and other official guidance

This ingredient is a well characterized material that has been evaluated and approved as a food additive by expert bodies including US FDA, FEMA and the CoE.

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Cardamom seed, oil (Elletaria cardamomum (L.) maton; CAS RN 8000-66-6) is included on the US FDA's list of Substances Added to Food (formerly EAFUS) as a flavoring agent or adjuvant, and is generally recognised as safe (GRAS) under 21 CFR section 182.20 (Essential oils, oleoresins (solvent-free), and natural extractives (including distillates) (FDA, 2022, 2023).

ADI/TDI:
A 1976 publication notes that "cardamom" (not cardamom seed oil, specifically) is included in the list of "natural flavouring substances consisting of, or derived from, vegetables, herbs or spices to be used in small quantities as additives to food provided that they are not used in amounts exceeding those occurring naturally in food" (MAFF, 1976).
Codex Alim.: Not listed C of E no.: 180 FEMA no.: 2240 and 2241 TLV / OEL: Not listed Cosmetics (UK): Not listed in Schedule 1
Oils, cardamom (CAS RN 8000-66-6) are not -registered under REACH (ECHA).

There is a REACH dossier for cardamom, ext. (CAS RN 85940-32-5) (ECHA).

Cardamome HE (CAS RN 8000-66-6), cardamom, ext. (CAS RN 85940-32-5) are not classified for packaging and labelling under Regulation (EC) No. 1272/2008 (ECHA, 2024). Cardamom seed oil (Elettaria cardamomum (L.) Maton) (CAS RN 8000-66-6) and cardamom

(Elettaria cardamomum (L.) Maton) (CAS RN 85940-32-5) are listed in the US EPA InertFinder Database as approved for fragrance use pesticide products.

Oils, cardamom (CAS RN 8000-66-6) is listed in the US EPA Toxic Substances Control Act (TSCA) inventory and also on the US EPA 2024 CDR list (Chemical Data Reporting Rule).

US EPA Substance Registry Services (SRS) – TSCA and CDR lists.

Cardamom seed oil (FEMA no. 2241) (Waddell et al. 2007) and cardamom (Elettaria cardamomum (L.) Maton; FEMA no. 2240) (Hall and Oser, 1965) have been given GRAS (generally recognized as safe) status by FEMA, and the former has also been affirmed as GRAS (Fukushima S et al. 2020).

“In the absence of toxicological data of the feed additive itself, a component based approach was applied to assess the safety of the essential oil [from Elettaria cardamomum (L.) Maton] as a mixture. Based on structural and metabolic similarity, the components of cardamom oil were allocated to seven assessment groups. Assuming the absence of toxicologically relevant interactions among components, dose addition was applied within each assessment group by calculating the combined margin of exposure as a basis for risk characterisation. The FEEDAP Panel concluded that the additive under assessment is safe at the proposed use level of 5 mg/kg in feed for all animal species. A concentration of 5 mg/L water for drinking is considered safe for all animal species. The use of cardamom essential oil in animal feed is considered safe for the consumer.”

As taken from EFSA, 2019.

Cardamom essential oil and oleoresin cardamom [no CAS RNs listed] are classified as Natural Health Products (NHPs) for medicinal use under Schedule 1 item 2 (extract) of the NHP Regulations.

As taken from Health Canada, 2021.

4. Metabolism/Pharmacokinetics

4.1. Metabolism/metabolites

No data available to us at this time.

4.2. Absorption, distribution and excretion

“Cardamom is a strong antioxidant plant, so it is called the queen of spices. In the present study, we explored the potentials of cardamom on developmental, learning ability and biochemical parameters of mice offspring. Thirty pregnant mice were allocated to three groups of ten animals in each. Groups Π and -¿ received pilsbury's Diet containing 10 and 20% of cardamom (w/w) respectively, whereas Group I used as control. Cardomom was administered from the first day of pregnancy and was continued until post-natal day 15 (PD 15) and thereafter the mothers were switched to plain pilsbury's Diet. During the weaning period, three pups in each litter were color marked from the others, and were subjected to various tests (Physical assessment such body weight and eye opening and hair appearance; the neuromaturation of reflexes like righting, rotating, and cliff avoidance reflexes; learning ability and memory retention; estimation of monoamines neurotransmitters like dopamine and serotonin, non-enzymatic oxidative stress such as TBARS and GSH in forebrain at different ages of pups). The results indicated that the body weight gain was declining significantly. Hair appearance and eyes opening were delayed significantly. Righting, rotating, and cliff avoidance reflexes were delayed in treated animals. Exposure to cardamom led to enhance learning and memory retention as compared to control. Monoamines (DA, 5-HT) and GSH were elevated, whereas TBARS was inhibited significantly. In conclusion, perinatal cardamom exposure enhanced learning and memory as compared to control. Cardamom and its benefit compounds were transported via placenta or/and milk during lactation. Cardamom needs more

researches to investigate its benefits on other kinds of behavior." As taken from Abu-Taweel GM. 2018. Saudi J. Biol. Sci. 25(1), 186-193. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29379379

4.3. Interactions

"The plant of Elletteria cardamom was collected and phytochemical studies were made with different solvents using ethanol and water. The extracts showed different extractive values (2.0 and 9.0 respectively) and showed the presences of different bioactive compounds like Alkaloids, Saponins, flavonoids, Terpenes, Glycosides steroids. Based upon the literature this given us the positive signal which may induce cardio protective activity. Treatment with Ellettaria cardamom extract high dose 500mg/kg.b.w lowers the LDH levels, SGOT levels, SGPT levels, Total protein levels, Serum albumin levels, Alkaline phasphatase levels and Triglycerides in doxorubicin induced cardiac rats. Treatment with low dose 100mg/kg.b.w of Elletteria cardamom extract lowers the Triglycerides levels in doxorubicin cardiac rats. Treatment with high dose 500mg/kg.b.w of Elletteria cardamom extract lowers the total cholesterol levels in doxorubicin cardiac rats. Treatment with high dose 500mg/kg.b.w of Elletteria cardamom extract improves the HDL Cholesterol levels in doxorubicin cardiac rats. Treatment with high dose 500mg/kg.b.w of Elletteria cardamom extract lowers the LDL Cholesterol levels in doxorubicin cardiac rats. Treatment with high dose 500mg/kg.b.w of Elletteria cardamom extract lowers the VLDL Cholesterol levels in doxorubicin cardiac rats. Treatment with Elletteria cardamom extract high dose 500mg/kg.b.w lowers the total chloride levels in doxorubicin induced cardiac rats." As taken from Shahidullah M et al. 2017. Indian Journal of Research in Pharmacy and Biotechnology 5(6), 366-370. Available at https://www.ijrpb.com/issues/ijrpb%205(6)%202.%20Shahidullah%20366-370.pdf "Nowadays the use of new and safe antioxidant from plant, animal and microbial resources is increasing. The aim of this study was to investigate and compare the antioxidant properties and types of interaction (synergism and antagonism) of green tea and the Elettaria cardamomum extracts. Green tea and cardamom extract and BHT were prepared in 25, 50, 100, 150, 200 and 250 ?g/ml and combined extract in different combination to reach 50, 100, 150, 200 and 250 ?g/ml were prepared in different ratios (1: 1, 2: 1, 1: 2, 1: 3, 3: 1, 2: 2, 1: 4 and 4: 1). In this study, phenolic compound was evaluated by Folin-Ciocalteu's method. Their antioxidant activity was measured by four methods: DPPH free radical scavenging, FRAP assay, beta-carotene /linoleic and ability to prevent the oxidation of soybean oil. BHT was used as positive control for comparison. The results of the tests showed that, antioxidant properties of green tea extract was significantly more than from Elettaria cardamomum extract and BHT ($P<0.05$). In different ratio of combined extracts, free radical scavenging DPPH assay in all ratio, FRAP test in 8 ratio and beta-carotene-linoleic acid test in 5 ratios showed synergismtic effect. In the peroxide value assay, the chosen combination showed antagonism, although it was significantly ($p<0.05$) more effective than BHT in soybean oil stability." As taken from Arianfar A and Sardarodiyan M. 2018. Iranian Journal of Food Science and Technology 14(72), 25-36. Available at http://www.sid.ir/En/Journal/ViewPaper.aspx?ID=537458 "Background and Objectives: Lead exposure exerts extremely damaging effects over reproduction system. Elettaria cardamomum has several medicinal properties. This study was aimed to evaluate the protective effect of Elettaria cardamomum L. (dried fruit) hydroalcoholic extract on serum levels of gonadotropins and testosterone among lead acetate-induced adult male wistar rats. Methods: In this experimental study, 36 adult male wistar rats (220-250 gr) were randomly allocated equally into 6 groups. Animals in control group received 0.5 ml normal saline, while the other groups were; extract group 400 mg/kg, the group receiving lead acetate 500ppm in drinking water and experimental group receiving orally lead acetate 500ppm + extract group 200, 400 and 800 mg/kg. The duration of the test was 28 days. Treatment with the extract lasted for a week. In the end of examination, after anaesthetizing, blood samples were collected directly from heart and serum levels of testosterone, FSH and LH hormones were measured. Data were analyzed using SPSS software and one-way ANOVA P-values lower than 0.05 were considered to be significant. Results: The mean serum level of testosterone in experimental groups receiving lead acetate 500ppm +

Elettaria cardamomum L. hydroalcoholic extract was significantly increased in all three doses and the mean serum levels of LH and FSH was significantly decreased in comparison to the group receiving lead acetate. The effectiveness was markedly dose-dependent. Conclusion: The present study showed that Elettaria cardamomum L. hydroalcoholic extract have significant effects on the serum levels of testosterone and gonadotropins. However, more precise studies are needed to investigate the involved mechanisms." As taken from Elham R et al. 2017. Jorjani Biomedicine Journal 4(2), 21-33. Available at http://www.sid.ir/En/Journal/ViewPaper.aspx?ID=548374 "In this research work, the antioxidant and metabolomic profiling of seven selected medicinally important herbs including Rauvolfia serpentina, Terminalia arjuna, Coriandrum sativum, Elettaria cardamom, Piper nigrum, Allium sativum, and Crataegus oxyacantha was performed. The in vivo cardioprotective potential of these medicinal plants was evaluated against surgically induced oxidative stress through left anterior descending coronary artery ligation (LADCA) in dogs. The antioxidant profiling of these plants was done through DPPH and DNA protection assay. The C. oxyacantha and T. arjuna showed maximum antioxidant potential, while the E. cardamom showed poor antioxidative strength even at its high concentration. Different concentrations of extracts of the said plants exhibited the protection of plasmid DNA against H2O2 damage as compared to the plasmid DNA merely treated with H2O2. The metabolomic profiling through LC-MS analysis of these antioxidants revealed the presence of active secondary metabolites responsible for their antioxidant potential. During in vivo analysis, blood samples of all treatment groups were drawn at different time intervals to analyze the cardiac and hemodynamic parameters. The results depicted that the group pretreated with HC4 significantly sustained the level of CK-MB, SGOT, and LDH as well as hemodynamic parameters near to normal. The histopathological examination also confirmed the cardioprotective potential of HC4. Thus, the HC4 being safe and inexpensive cardioprotective herbal combination could be considered as an alternate of synthetic drugs." As taken from Afsheen N et al. 2018. Oxid. Med. Cell. Longev. 2018, 9819360. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29576858

"The goal of the present work was to study the protective role of the essential oil of Elettaria cardamomum (cardamom) against diethylnitrosamine (DENA)-induced oxidative stress in the kidney and brain of rats in comparison with geraniol, a pure compound as one of the main components of cardamom essential oil. Geraniol or cardamom essential oil was orally administered every day (100 and 200 mg/kg) for 1 week before DENA administration and continuously administered for 26 weeks. The levels of brain and kidney ornithine decarboxylase (ODC), brain acetylcholinesterase (AChE) and renal markers such as urea and creatinine were elevated after DENA administration. However, cardamom and geraniol administration decreased kidney and brain oxidative stress and lowered the activity of ODC in brain and kidney and the activity of AChE in brain. Cardamom or geraniol had significantly reduced the level of lipid peroxidation to almost half the value in DENA group and enhanced glutathione level by double its values in brain and kidney compared to glutathione levels in DENA treated-rats. In addition, geraniol or oil of cardamom improved the activities of the antioxidant markers; catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase, and glutathione-S transferase in the brain and kidneys of DENA-treated rats. The results support the chemopreventive, antioxidant, neuroprotective and anticancer effects of the essential oil of cardamom, which are mediated through its inhibition of ODC and AChE activities and activation of antioxidant markers." As taken from Elguindy NM et al. 2018. Beni-Suef University Journal of Basic and Applied Sciences 7(3), 299-305. Available at https://www.sciencedirect.com/science/article/pii/S2314853518300192

"This study was conducted to evaluate the potential cardioprotective effect of cardamom (CAR) against myocardial injuries induced by doxorubicin (DOX) in rats through investigation of histological alterations and the associated oxidative stress, apoptosis, inflammation, and angiogenesis. This study included 30 adult male albino rats that were randomized to 3 groups (n = 10/group): group I (control), group II (DOX) rats injected with DOX (2.5 mg/kg body weight [BW] i.p.) every other day for 2 weeks, and group III (CAR+DOX) received CAR extract (200 mg/kg BW) orally for 3 weeks, and 1 week later (starting from the 2nd week) they were injected with DOX (2.5

mg/kg BW i.p.) every other day for 2 weeks. Rats treated with DOX alone exhibited notable myocardial damage (discontinuity and disorganization of cardiac muscle fibers, mononuclear cell infiltration, and apparent increases in collagen fiber deposition) accompanied by loss of function (revealed by elevated serum levels of lactate dehydrogenase, creatine kinase, and cardiac troponin), induction of oxidative stress (indicated by higher levels of nitric oxide and malon-dialdehyde, and lower levels of superoxide dismutase, catalase, and glutathione peroxidase), apoptosis (evidenced by high caspase 3 activity and immunostaining), and inflammation (marked by high cardiac NFκB level). However, administration of CAR not only ameliorated all deleterious effects of DOX but also induced angiogenesis, as indicated by a significant increase in VEGF immunoreactivity. These data indicate that CAR could relieve DOX-induced cardiotoxicity, at least in part, via reductions in oxidative stress, apoptosis, and inflammation and increased tissue regeneration via induction of angiogenesis. Therefore, CAR could be a promising cytoprotective agent against DOX cardiotoxicity." As taken from Abu Gazia M and El-Magd MA. 2018. Cells Tissues Organs 206(1-2), 62–72. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/30716735/

"Single and combined antibacterial activities of cumin, cardamom, and dill weed essential oils against Campylobacter jejuni, Campylobacter coli, Escherichia coli, Staphylococcus aureus, and mixed cultures were determined by using the broth microdilution method for determining minimum inhibition concentrations. Among the bacteria tested, C. coli and C. jejuni were generally more susceptible to essential oils, with lower minimum inhibition concentrations. The minimum inhibition concentration values were obtained against Gram-negative and Gram-positive bacteria tested within the range of 0.012–15.00 and 3.75–15.00 μL/mL, respectively. Fractional inhibitory concentrations (FICs) were also calculated to evaluate the antimicrobial activities of essential oil combinations. Although the combined effects of essential oils changed depending on the strain and the type of essential oils used, generally the use of combinations increased the efficacy of the essential oils. Interestingly, according to the FIC index, the most synergetic effect was against C. coli and C. jejuni. This study has demonstrated the potential use of cardamom, cumin, and dill weed essential oils, and their combinations, against important pathogenic bacteria and their mixed cultures." As taken from Mutlu-Ingok A et al. 2019. Flavour and Fragrance Journal 34(4). Available at https://onlinelibrary.wiley.com/doi/abs/10.1002/ffj.3501

"Introduction: Liver cancer is ranked as the second most common cause of death globally as a result of its poor prognosis. It can be treated with sorafenib, but its use is limited due to its toxicity and adverse reactions. Lower doses of sorafenib with other complementary agents are recommended to minimize toxicity. Cardamom seeds are one of the most common ingredients of Indian and Chinese traditional medicine, and different studies have suggested that cardamom extract can display anti-cancer activities. Aim: this study aims to investigate the efficiency of Elettaria Cardamom Extract (ECE) on enhancement of Sorafenib-induced apoptosis in HepG2. Methods: Human liver cancer cell line (HepG2) were exposed to increasing concentrations of individual and combined treatments of Sorafenib and ECE for 24 h. The viability of cells was examined using MTT Assay. Clonogenicity and cell migration assays were carried out. Reactive oxygen species (ROS) generation and mitochondrial membrane potential (MMP) level were determined by DCFH-DA and JC-1 dye, respectively. Agarose gel electrophoresis and comet examinations were carried out to estimate the DNA damage. Results: Combined treatment of ECE with sorafenib suppressed the proliferation, colony formation and cell migration of HepG2 cells more than the sorafenib did alone. The half maximal inhibitory concentration (IC50), after 24h of incubation were 15 μM of sorafenib and 9 and 7.3 μM of sorafenib enhanced by 5 and 10 μg / 100 μl of ECE respectively. HepG2 treated cells displayed biochemical features of apoptotic cell death. The combined treatment increased the ROS production, reduced the level of MMP, increased Comet tail length and induced DNA fragmentation more than sorafenib did alone. Conclusions: These findings demonstrate that ECE enhanced the sorafenib effect in HepG2 cells and suggest that the ECE may be a promising agent for reducing sorafenib side effects in hepatocellular carcinoma (HCC)." As taken from Alghamadi HA et al. 2020. International Journal of

Pharmaceutical Research & Allied Sciences 9(2), 50-61. Available at https://ijpras.com/article/elettaria-cardamom-extract-synergistically-enhaning-sorafenib-induced-apoptosis-in-hepg2-cells

5. Toxicity

5.1. Single dose toxicity

For 8000-66-6: Acute oral LD50 rats: 5 g/kg bw (Ash, 1995). Acute dermal LD50 rabbits: >5 g/kg bw (Ash, 1995).
“Available data indicate cardamom oil to be nontoxic.“ As taken from Khan IA and Abourashed EA, 2010.

“Elettaria cardamomum is an aromatic spice (cardamom) native to the humid Asian areas, which contains some compounds with a potential anticonvulsant activity. Various pharmacological properties such as anti-inflammatory, analgesic, antioxidant, and antimicrobial effects have been related to this plant. This research was conducted to examine the probable protective impact of the essential oil and methanolic extract of E. cardamomum against chemically (pentylentetrazole)- and electrically (maximal electroshock)-induced seizures in mice. In addition, neurotoxicity, acute lethality, and phytochemistry of the essential oil and methanolic extract were estimated. The TLC method showed the presence of kaempferol, rutin, and quercetin in the extract, and the concentration of quercetin in the extract was 0.5 µg/mL. The major compounds in the essential oil were 1,8-cineole (45.6%), α-terpinyl acetate (33.7%), sabinene (3.8%), 4-terpinen-4-ol (2.4%), and myrcene (2.2%), respectively. The extract and essential oil showed significant neurotoxicity in the rotarod test at the doses of 1.5 g/kg and 0.75 mL/kg, respectively. No mortalities were observed up to the doses of 2 g/kg and 0.75 mL/kg for the extract and essential oil. The essential oil was effective in both the pentylentetrazole and maximal electroshock models; however, the extract was only effective in the pentylentetrazole model. The study suggested that E. cardamomum methanolic extract had no significant lethality in mice. Both the essential oil and methanolic extract showed movement toxicity. Anticonvulsant effects of E. cardamomum were negligible against the seizures induced by pentylentetrazole and maximal electroshock.” As taken from Masoumi-Ardakani Y et al. 2016. Planta Med. 82(17), 1482-1486. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27433883

5.2. Repeated dose toxicity

“BACKGROUND: Cardamom (Elettaria cardamomum) is an aromatic seed spice grown extensively in India and used as a flavoring in sweets. In this study, the anti-hypercholesterolemic effect of cardamom was evaluated in Wistar rats by inducing hypercholesterolemia with a high-cholesterol diet for 8 weeks. Dietary interventions were made with (a) cardamom powder (50 g kg-1), (b) cardamom oil (3 g kg-1 , equivalent to 50 g kg-1 cardamom) and (c) de-oiled cardamom powder (50 g kg-1). RESULTS: A significant reduction in blood total cholesterol (31%) and low-density lipoprotein cholesterol (44%) was observed by oral administration of cardamom oil in hypercholesterolemic rats, accompanied by a marked decrease in serum triglycerides by 42%. The

cholesterol content of cardiac muscle was beneficially lowered by 39% with administration of cardamom oil in hypercholesterolemic rats. Liver triglycerides were reduced by 33%. Incorporation of cardamom oil/powder in the diet did not alter feed consumption by rats. Compromised activities of hepatic antioxidant enzymes in the hypercholesterolemic situation were generally countered by dietary cardamom. Treatment with de-oiled cardamom as well as cardamom oil countered the diminished activity of catalase in hypercholesterolemic animals. Cardamom also enhanced the activity of heart superoxide dismutase in the hypercholesterolemic situation. The concentration of ascorbic acid in serum was significantly increased by dietary cardamom or its fractions in the hypercholesterolemic situation. CONCLUSION: This animal study has established the potential of cardamom oil in restoring the alteration in lipid homeostasis in conditions of hypercholesterolemia. The significant reduction in atherogenicity index by dietary intervention with cardamom powder and cardamom oil indicates the potential cardioprotective effect of cardamom."As taken from Nagashree S et al. 2017. J. Sci. Food Agric. 97(10), 3204-3210. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/27888503

5.3. Reproduction toxicity

Four groups of 10 virgin Crl CD rats were administered 0, 375, 750, or 1500 mg/kg bw of an essential oil (cardamom oil) known to contain greater than 65 % tertiary terpenoid alcohols with 5 1% alpha-terpineol acetate by mass. The test material was given by gavage once daily, 7 days prior to cohabitation, through cohabitation (maximum of 7 days), gestation, delivery, and a 4 day post-parturition period. The duration of the study was 39 days. Maternal indices monitored included twice-daily observation, measurement of body weights, food consumption, duration of gestation, and fertility parameters (mating and fertility index, gestation index, and number of offspring per litter). Offspring indices included daily observation, clinical signs, examination for gross external malformations, and measurement of body weight. Maternal observations included a non-statistically significant decrease in body weight gain and food consumption at 375 mg/kg/d. Mortality, clinical signs, a statistically significant decrease in body weight gain and food consumption, and gross lesions at necropsy were seen at 750 and 1500 mg/kg/d. The only effects on pups were a reduced body weight gain in pups at 750 and 1500 mg/kg/d and increased mortality at 1500 mg/kg/d. The authors concluded that there were no significant adverse effects in the dams or offspring at the 375 mg/kg/d dose. A maternal NOEL was reported to be less than 375 mg/kg/d based on reduced body weight gain and food consumption at 375 mg/kg/d and a developmental NOAEL was reported to be 375 mg/kg/d (Vollmuth et al. 1995). "Cardamom is a strong antioxidant plant, so it is called the queen of spices. In the present study, we explored the potentials of cardamom on developmental, learning ability and biochemical parameters of mice offspring. Thirty pregnant mice were allocated to three groups of ten animals in each. Groups Π and -¿ received pilsbury's Diet containing 10 and 20% of cardamom (w/w) respectively, whereas Group I used as control. Cardomom was administered from the first day of pregnancy and was continued until post-natal day 15 (PD 15) and thereafter the mothers were switched to plain pilsbury's Diet. During the weaning period, three pups in each litter were color marked from the others, and were subjected to various tests (Physical assessment such body weight and eye opening and hair appearance; the neuromaturation of reflexes like righting, rotating, and cliff avoidance reflexes; learning ability and memory retention; estimation of monoamines neurotransmitters like dopamine and serotonin, non-enzymatic oxidative stress such as TBARS and GSH in forebrain at different ages of pups). The results indicated that the body weight gain was declining significantly. Hair appearance and eyes opening were delayed significantly. Righting, rotating, and cliff avoidance reflexes were delayed in treated animals. Exposure to cardamom led to enhance learning and memory retention as compared to control. Monoamines (DA, 5-HT) and GSH were elevated, whereas TBARS was inhibited significantly. In conclusion, perinatal cardamom exposure enhanced learning and memory as compared to control. Cardamom and its benefit compounds were transported via placenta or/and milk during lactation. Cardamom needs more researches to investigate its benefits on other kinds of behavior." As taken from Abu-Taweel GM. 2018. Saudi J. Biol. Sci. 25(1), 186-193. PubMed, 2018 available at

https://www.ncbi.nlm.nih.gov/pubmed/29379379 “Background and Objectives: Lead exposure exerts extremely damaging effects over reproduction system. Elettaria cardamomum has several medicinal properties. This study was aimed to evaluate the protective effect of Elettaria cardamomum L. (dried fruit) hydroalcoholic extract on serum levels of gonadotropins and testosterone among lead acetate-induced adult male wistar rats. Methods: In this experimental study, 36 adult male wistar rats (220-250 gr) were randomly allocated equally into 6 groups. Animals in control group received 0.5 ml normal saline, while the other groups were; extract group 400 mg/kg, the group receiving lead acetate 500ppm in drinking water and experimental group receiving orally lead acetate 500ppm + extract group 200, 400 and 800 mg/kg. The duration of the test was 28 days. Treatment with the extract lasted for a week. In the end of examination, after anaesthetizing, blood samples were collected directly from heart and serum levels of testosterone, FSH and LH hormones were measured. Data were analyzed using SPSS software and one-way ANOVA P-values lower than 0.05 were considered to be significant. Results: The mean serum level of testosterone in experimental groups receiving lead acetate 500ppm + Elettaria cardamomum L. hydroalcoholic extract was significantly increased in all three doses and the mean serum levels of LH and FSH was significantly decreased in comparison to the group receiving lead acetate. The effectiveness was markedly dose-dependent. Conclusion: The present study showed that Elettaria cardamomum L. hydroalcoholic extract have significant effects on the serum levels of testosterone and gonadotropins. However, more precise studies are needed to investigate the involved mechanisms.” As taken from Elham R et al. 2017. Jorjani Biomedicine Journal 4(2), 21-33. Available at http://www.sid.ir/En/Journal/ViewPaper.aspx?ID=548374 “Cardamom is known as a plant with millions of benefits and it is known to contain aphrodisiac substances. The role of cardamom as an aphrodisiac need to be studied more deeply from the scientific point of view. The experimental object is a 3-month-old male white mice (Mus musculus albinus) with an average weight of about 30-35 grams. After being acclimatized for approximately 2 weeks, 35 mice were divided into 3 groups based on the dose of cardamom extract. At the end of the experiment, the mice will be sacrificed and then the testes were weighed and cauda epididymis were isolated to collect the sperm. Our findings suggested that the dose of administration has important role in affecting the weight of testes and bodies of mice. However, further studies on sperm profiles and optimal administration doses of cardamom extract are highly recommended.” As taken from Hartady T et al. 2019. ARSHI Vet. Lett. 3(1), 7-8.

5.4. Mutagenicity

In vivo No data

In vitro The Ames test was used to evaluate the mutagenicity of a number of neat complex flavor mixtures. Studies in which cardamom oleoresin was part of the test mixture include EMT960820 and EMT000307 (CD-ROM 1, JTI Submission, 2002). The results showed that these mixtures were not mutagenic.

No mutagenicity was seen when cardamom was tested in Salmonella typhimurium strains TA98, TA100, TA1535, TA1537 and TA1538. The use of S9 was not specified (Bersani et al. 1981).

A Japanese paper (with tabulated data in English) also reported no mutagenic activity in the same five strains of Salmonella typhimurium when tested at up to 5 mg/plate (Hachiya et al. 1985). [It is not clear whether or not S9 was used.]

A positive result was seen when cardamom oil was tested at a concentration of 2500 ng/plate in Salmonella typhimurium (strains not specified), in the presence of S9 (Anon, 1979).

In an assay similar to an Ames test, a positive result was seen when an alcoholic extract of cardamom was tested at concentrations of up to 50 mg/plate in Salmonella typhimurium strain TA98, streptomycin dependent #4, without S9. No mutagenicity was seen in Salmonella typhimurium strain TA98, streptomycin dependent #510 and the presence of S9 was not specified (Shashikanth & Hosono, 1986).

A positive result was seen when cardamom oil was tested at a concentration of 2500 ng/plate in Escherichia coli (strains not specified), in the absence of S9 (Anon, 1979).

A positive result was seen when cardamom oil was tested at a concentration of 19 mg/disc in Bacillus subtilis (strains not specified) and the cells were examined for DNA repair (DNA damage). The presence of S9 was not specified (Anon (1984). Food Hygiene Journal 25, 378). As taken from RTECS, 1997.

A Japanese paper (with tabulated data in English) reported a positive and weak positive result (without and with S9, respectively) in Bacillus subtilis M45/H17, when tested at up to 30 mg/disc (Hachiya et al. 1985).

5.5. Cytotoxicity

“BACKGROUND: The main objective of this study was the phytochemical characterization of four indigenous essential oils obtained from spices and their antibacterial activities against the multidrug resistant clinical and soil isolates prevalent in Pakistan, and ATCC reference strains. METHODS: Chemical composition of essential oils from four Pakistani spices cumin (Cuminum cyminum), cinnamon (Cinnamomum verum),cardamom (Amomum subulatum) and clove (Syzygium aromaticum) were analyzed on GC/MS. Their antibacterial activities were investigated by minimum inhibitory concentration (MIC) and Thin-Layer Chromatography-Bioautographic (TLC-Bioautographic) assays against pathogenic strains Salmonella typhi (D1 Vi-positive), Salmonella typhi (G7 Vi-negative), Salmonella paratyphi A, Escherichia coli (SS1), Staphylococcus aureus, Pseudomonas fluorescens and Bacillus licheniformis (ATCC 14580). The data were statistically analyzed by using Analysis of Variance (ANOVA) and Least Significant Difference (LSD) method to find out significant relationship of essential oils biological activities at $P<0.05$. RESULTS: Among all the tested essential oils, oil from the bark of C. verum showed best antibacterial activities against all selected bacterial strains in the MIC assay, especially with 2.9 mg/ml concentration against S. typhi G7 Vi-negative and P. fluorescens strains. TLC-bioautography confirmed the presence of biologically active anti-microbial components in all tested essential oils. P. fluorescens was found susceptible to C. verum essential oil while E. coli SS1 and S. aureus were resistant to C. verum and A. subulatum essential oils, respectively, as determined in bioautography assay. The GC/MS analysis revealed that essential oils of C. cyminum, C. verum, A. subulatum, and S. aromaticum contain 17.2% cuminaldehyde, 4.3% t-cinnamaldehyde, 5.2% eucalyptol and 0.73% eugenol, respectively. CONCLUSIONS: Most of the essential oils included in this study possessed good antibacterial activities against selected multi drug resistant clinical and soil bacterial strains. Cinnamaldehyde was identified as the most active antimicrobial component present in the cinnamon essential oil which acted as a strong inhibitory agent in MIC assay against the tested bacteria. The results indicate that essential oils from Pakistani spices can be pursued against multidrug resistant bacteria.” As taken from Naveed R et al. 2013. BMC Complement. Altern. Med. 13, 265. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24119438

"Amommum subulatum (Roxb.) or Cardamom extract is known to have anti-inflammatory and neuroprotective effects towards many gastrointestinal related problems. However, up till now different fractions of cardamom extract on fibroblasts with respect to potassium channel activity have not been investigated. Therefore, present study investigated the effects of different fractions of cardamom extract on potassium channels in non-tumor NIH3T3 cell line. Phytochemical analysis of hydroalcoholic, n-hexane, butane and ethyl acetate fractions of cardamom extracts were purified

and isolated by thin layer chromatography (TLC). 3T3 cells were cultured and incubated with hydroalcohol (1-2 µ/ml), n-hexane (1 µ/ml), butane (2 µ/ml) and ethyl acetate (1-2 µ/ml) for 5 hrs at 37°C. Modulation in potassium currents were recorded by whole-cell patch clamp method. The data showed two constituents Cineol (C10H18O) and Terpinyl acetate (C10H17OOCCH3) by TLC method. The present study shows that the constituents in n-hexane, hydro alcohol (1 µ/ml) and ethyl acetate (2 µ/ml) significantly increased ($p<0.01$) the potassium outward rectifying currents from NIH3T3 cells when compared to untreated controls cells. Whereas, butanol fraction (2 µ/ml) significantly decreased ($p<0.01$) the inward rectifying currents when compared to controls. Moreover hydroalcoholic and n-hexane fractions have increased the proliferation in 3T3 cell line. On the other hand butanol and ethyl acetate did not induce proliferation in 3T3 cells. Taken together, our data suggested that cardamom extract contains constituents that increased K+ currents, cell migration and proliferation and are involved in wound healing." As taken from Siddiqui S et al. 2017. Pak. J. Pharm. Sci. 30(6), 2211-2215. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29175791

“Spices are well known for their taste and flavor imparting properties. Green cardamom (Elletaria cardamomum), a herb spice belongs to family Zingiberaceae. In current study, GC-MS analysis of green cardamom essential oil (CEO) resulted in identification of twenty-six compounds with α-terpinyl acetate (38.4%), 1,8-cineole (28.71%), linalool acetate (8.42%), sabinene (5.21%), and linalool (3.97%) as major bioactive components. Present study also described the antimicrobial properties like zone of inhibition, minimum inhibitory concentration against microbial strains with special emphasis on quorum sensing inhibition. Disk diffusion assay showed that C. albicans and S. mutans were the most sensitive microorganisms followed by S. aureus, L. monocytogenes, B. cereus and S. typhimurium sensor strains, respectively. Whilst P. aeruginosa was found most resistant strain as CEO did not inhibited its growth. The minimum inhibitory concentration (MIC) values of CEO against tested strains were 10 ± 0.00 mg/mL against S. typhimurium, S. aureus and 5 ± 0.00 mg/mL against S. mutans, C. albicans strains, respectively. Regarding quorum sensing inhibition the tested concentrations 0.625 and 0.313 mg/mL of CEO inhibited violacein production with very little effect on growth of C. violaceum. Conclusively, study proved that quorum sensing inhibition values of CEO were much lower compared to MIC revealed values. Hence, cardamom bioactive constituents can effectively be used to develop novel antimicrobial drugs against conventional antibiotics.” As taken from Abdullah et al. 2017. J. Food Sci. Technol. 54(8), 2306-2315. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28740287

“Natural antimicrobials as well as essential oils (EOs) have gained interest to inhibit pathogenic microorganisms and to control food borne diseases. Campylobacter spp. are one of the most common causative agents of gastroenteritis. In this study, cardamom, cumin, and dill weed EOs were evaluated for their antibacterial activities against Campylobacter jejuni and Campylobacter coli by using agar-well diffusion and broth microdilution methods, along with the mechanisms of antimicrobial action. Chemical compositions of EOs were also tested by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). The results showed that cardamom and dill weed EOs possess greater antimicrobial activity than cumin with larger inhibition zones and lower minimum inhibitory concentrations. The permeability of cell membrane and cell membrane integrity were evaluated by determining relative electric conductivity and release of cell constituents into supernatant at 260 nm, respectively. Moreover, effect of EOs on the cell membrane of Campylobacter spp. was also investigated by measuring extracellular ATP concentration. Increase of relative electric conductivity, extracellular ATP concentration, and cell constituents' release after treatment with EOs demonstrated that tested EOs affected the membrane integrity of Campylobacter spp. The results supported high efficiency of cardamom, cumin, and dill weed EOs to inhibit Campylobacter spp. by impairing the bacterial cell membrane.” As taken from Mutlu-Ingok A and Karbancioglu-Guler F. 2017. Molecules 22(7), E1191. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28714890

“The essential oils from aromatic plants Rosmarinus officinalis, Mentha piperita, Schinus molle, Cinnamomum zeylanicum, Citrus sinensis, Pinus pinea, Lavandina abrial, Hyssopus officinalis,

Lippia alba, Cimbopogon nardus, Eletaria cardamomum and Aloysia polystachya are used in traditional medicine because their antibacterial and anti-inflammatory properties. However, there are no available reports of activity against Leishmania parasites. In the present study we evaluated the antileishmanial activity and cytotoxicity of essential oils extracted from these plants. The essential oils were solubilized in DMSO not exceeding 0.5%. The cytotoxicity was evaluated on U-937 macrophages using the 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) colorimetric method while the antileishmanial activity was evaluated on L. panamensis amastigotes by flow cytometry. The results are expressed as median Lethal Concentration (LC50) for cytotoxicity and median Effective Concentration (EC50) for effectiveness, both values calculated by Probit [1]. The most effective essential oil were M. piperita and S. molle with an EC50 < 3 and 15.2 mg/mL, respectively while E. cardamomun showed moderate antilaeishmanial activity (EC50 43.9 mg/mL). None of the essential oils was cytotoxic to the U-937 cells. This is the first report of antileishmanial activity to M. piperita, S. molle and E. cardamomun. Since the essential oils of these plants with high or moderate antileishmanial activity are traditionally used for management of skin lesions, the development of a formulation that combines the antileishmanial and anti-inflammatory properties could be a possible alternative for the management of uncomplicated cutaneous leishmaniasis." As taken from Robledo SM et al. 2017. PMIO 4(S 01), S1-S202.

"Spices have been traditionally used for prevention and cure of many diseases. Hence, this study was aimed to investigate the effects of aqueous and alcoholic extract of four different spices (asafoetida, ginger, cinnamon and cardamom) extracts on HeLa cell lines, human breast adenocarcinoma cell line (MCF) and HEP-G2 cancer cell lines through cell viability assay. Aqueous and alcohol extract (20 mg/ml) of asafoetida showed better Chemopreventive activity. Cell viability was 14% for aqueous extract whereas 9% for alcohol extract on HeLa cell line; 18% Cell viability for aqueous extract whereas 14% for alcohol extract on MCF-7 cell line; 14% Cell viability for aqueous extract whereas 9% for alcohol extract on HEP-G2 cell line. Therefore, these spices might be used for natural healing of the tumor." As taken from Mishra N and Behal KK. 2018. World Journal of Pharmaceutical Research 7(9), 625-631.

"Objective: The main objective of the study was to evaluate the cytotoxicity of selected essential oils on human skin, gastric, and brain cancer cell lines using microculture tetrazolium test. Materials and methods: Phytochemical analysis, as well as acute oral toxicity tests, was carried out in female albino mice with cardamom oil, lemon oil, and jasmine oil according to the Organization for Economic Co-operation and Development guidelines 425. Anticancer activities of the above test drugs were performed using human cancer cell lines. The studies were carried out at Skanda Life Sciences Pvt. Ltd., Bengaluru. Results: Phytochemical analysis has shown the presence of carbohydrates and flavonoids in cardamom oil. While lemon oil has shown the presence of carbohydrates, flavonoids, steroids, terpenoids, and tannins, jasmine oil has shown the presence of carbohydrates, alkaloids, flavonoids, steroids, terpenoids, and glycosides. Toxicity studies showed that cardamom oil, lemon oil, and jasmine oil were all found to be safe up to 2000 mg/kg body weight. Results have shown that lemon oil exhibited the strongest cytotoxicity toward three human cancer cell lines, namely skin cancer (A431), gastric cancer (MKN-45), and brain cancer (U-87 MG) cell lines, with higher IC50 values of 62.82 µg/ml, 220.9 µg/ml, and 440.1 µg/ml compared to standard. Jasmine oil exhibited the strongest cytotoxicity toward skin cancer and brain cancer cell lines, whereas cardamom oil has shown stronger cytotoxicity only toward skin cancer cell line but did not show any level of inhibition of growth of brain and gastric cancer cells. Conclusion: Our study reveals that lemon oil, jasmine oil, and cardamom oil possess potent antitumor activity compared to standard. At different concentrations, lemon oil has shown statistically significant (***P < 0.0001) anticancer activity toward all the three human cancer cell lines. While jasmine oil has shown statistically significant (***P < 0.0001) anticancer activity toward skin and brain cancer cell line, cardamom oil has also shown statistically significant (***P < 0.0001) anticancer activity but only toward skin cancer cell line." As taken from Manjunath C and Mahurkar N 2021. J. Can. Res. Ther. 17(1), 62-68. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/33723134/

“Cardamom (Elettaria cardamomum) is a traditional aromatic plant for which several pharmacological properties have been associated. In this study, the antibacterial activity of two cardamom extracts (fruit and seeds), rich in volatile compounds, against major periodontal pathogens was evaluated. Moreover, the ability of the extracts to exert anti-inflammatory activity was tested. Both cardamom fruit and seed extracts exerted an antibacterial effect against Aggregatibacter actinomycetemcomitans, Fusobacterium nucleatum, Porphyromonas gingivalis, and Prevotella intermedia (minimum inhibitory concentrations: 0.5% [v/v], 0.25%, 0.062%, 0.125%, respectively and minimum bactericidal concentrations: 1%, 0.25%, 0.062%, 0.25%, respectively). The cell membrane of P. gingivalis was disrupted by a treatment with cardamom extracts suggesting the bactericidal mode of action. The extracts also inhibited biofilm formation although it correlated with a growth reduction. Moreover, the cardamom extracts significantly decreased the secretion of IL-1β, TNF-α, and IL-8 by lipopolysaccharide-stimulated macrophages. Evidence were brought that the anti-inflammatory activity may result from inhibition of the NF-κB signaling pathway. This study is the first to provide evidence that cardamom fruit and seed extracts through their antibacterial and anti-inflammatory properties may be therapeutic agents of interest against periodontal infections.” As taken from Souissi M et al. 2020. Anaerobe 61, 102089. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/31430531/

“Elettaria cardamomum is cultivated in the Southern part of India showed great extinct of differences in their morphotypes and chemical compositions. In the present study, we have selected three varieties of Elettaria cardamomum “Valley Green, Palakuzhi, and ICRI”, to analyze the morphological perturbations, chemical compositions, and antimicrobial activities. The differences in the morphological character of cardamom varieties (Valley Green, Palakuzhi, and ICRI) were carried out on the basis of panicles, capsules shape, plant height, tiller, and seeds per capsule. The GC-MS analysis of the essential oils resulted in the identification of 27, 29, 30 compounds representing over 97.4%, 95.2%, and 98.8% of the Valley Green (VG), Palakuzhi (PAL), and ICRI fruit oils respectively. Monoterpene, α-terpinyl acetate varied from 35.4 to 47.5%, a major constituent while 1,8-cineole (22.8% to 27.4%) observed the second major compounds revealed in oils of these cultivars. Further, the antimicrobial activities of each essential oils were performed against Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Candida albicans, and Aspergillus niger. The maximum inhibition percentage against the microbes was observed in Valley Green essential oil as compared to oils of other varieties.” As taken from Alam A et al. 2019. Natural Product Communications 14(12), 1–7. Available at https://journals.sagepub.com/doi/pdf/10.1177/1934578X19892688

“Plants are a source of chemical compounds such as alkaloids, steroids, essential and fixed oils, which can be used against many diseases. Here, we evaluated the aqueous and solvent extract of four plants (Syzygium aromaticum, Elettaria cardamomum, Nigella sativa, Capsicum annuum), and assessed their antimicrobial activity against Escherichia coli using will diffusion method after 24 h. The oil crude extract of S. aromaticum showed the highest antimicrobial activity, while the oil crude extract of C. annuum showed the lowest activity. Our study revealed that the utilization of aqueous and solvent extract of some traditional plants against E. coli would be an effective way in the management of some environmental bacterial diseases.” As taken from Abdullah AA et al. 2019. GSC Biological and Pharmaceutical Sciences 9(3), 8-12. Available at https://gsconlinepress.com/journals/gscbps/node/1038 “Spice plants are known for their compounds that are useful as foods flavoring, food preservatives, and medicines. This due to the presence of secondary metabolite compounds in plants such as terpenoids, flavonoids, phenols, and saponins. These compounds are known to be potential to inhibit microorganism's growth causing decay in food and oxidation. The use of these sources for applications in the food sector is relatively safer and environmentally friendly than the use of antibiotics in general. This study was conducted to determine the antimicrobial and antioxidant activities from Coriandrum sativum L. (coriander) and Elettaria cardamomum (L.) Maton (cardamom). The essential oil extract from these plants was tested for phytochemical content qualitatively for terpenoid screening and by using Gas Chromatography-Mass Spectroscopy (GC/MS). Furthermore, the antioxidant activity from the oil

extracts was tested by DPPH method. Meanwhile, their ability to inhibit gram-positive bacteria Staphylococcus aureus and Gram-negative bacteria Escherichia coli were tested by paper disc method. The phytochemical characterization showed a positive result of terpenoid and GC/MS result showed dominant of monoterpenes compounds, such as α-pinene and ß-pinene. The DPPH results revealed that the essential oils have different antioxidant and antimicrobial potential, whereas Coriander tends to have a higher antimicrobial activity, while Cardamom superior in antioxidant activity. These results will become the basis for the development of potential essential oil with the best antimicrobial activity for food active packaging materials." As taken from Handayani W et al. 2019. Journal of Physics Conference Series 1317, 012092. Available at https://iopscience.iop.org/article/10.1088/1742-6596/1317/1/012092/meta

5.6. Carcinogenicity

"BACKGROUND: Cardamom (Elettaria cardamom), also known as "Queen of Spices", has been traditionally used as a culinary ingredient due to its pleasant aroma and taste. In addition to this role, studies on cardamom have demonstrated cancer chemopreventive potential in in vitro and in vivo systems. Nevertheless, the precise poly-pharmacological nature of naturally occurring chemo-preventive compounds in cardamom has still not been fully demystified. METHODS: In this study, an effort has been made to identify the proapoptopic, anti-inflammatory, anti-proliferative, anti-invasive and anti-angiogenic targets of Cardamom's bioactive principles (eucalyptol, alpha-pinene, beta-pinene, d-limonene and geraniol) by employing a dual reverse virtual screening protocol. Experimentally proven target information of the bioactive principles was annotated from bioassay databases and compared with the virtually screened set of targets to evaluate the reliability of the computational identification. To study the molecular interaction pattern of the anti-tumor action, molecular docking simulation was performed with Auto Dock Pyrx. Interaction studies of binding pose of eucalyptol with Caspase 3 were conducted to obtain an insight into the interacting amino acids and their inter-molecular bondings. RESULTS: A prioritized list of target proteins associated with multiple forms of cancer and ranked by their Fit Score (Pharm Mapper) and descending 3D score (Reverse Screen 3D) were obtained from the two independent inverse screening platforms. Molecular docking studies exploring the bioactive principle targeted action revealed that H- bonds and electrostatic interactions forms the chief contributing factor in inter-molecular interactions associated with anti-tumor activity. Eucalyptol binds to the Caspase 3 with a specific framework that is well-suited for nucleophilic attacks by polar residues inside the Caspase 3 catalytic site. CONCLUSION: This study revealed vital information about the poly-pharmacological anti-tumor mode-of-action of essential oils in cardamom. In addition, a probabilistic set of anti-tumor targets for cardamom was generated, which can be further confirmed by in vivo and in vitro experiments." As taken from Bhattacharjee B & Chatterjee J. 2013. Asian Pac. J. Cancer Prev. 14(6), 3735-42. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23886174

5.7. Irritation/immunotoxicity

Humans Cardamom oil (4% in petrolatum) did not produce irritation when applied to the skin of humans for 48 hours in a closed patch test (Opdyke, 1974).
Non-humans No irritation was seen when the undiluted oil was applied to the skin of rabbits (24 hour occlusion test) or mice (Opdyke, 1974).
Cardamom oil is one of a number of compounds assessed in an immunotoxicity screening study (Abstract). A rapid screening protocol incorporating key elements of the US National Toxicology Program's immunotoxicity tier testing strategy was used to evaluate the effects of 35 commonly used food flavouring ingredients on humoral and cell-mediated immune responses. The test compounds were administered intragastrically on a daily basis for 5 days at three dose levels to female CD-1 or $B6C3F_1$ mice, 6–8 wk old. A host resistance assay (Listeria monocytogenes bacterial challenge) was conducted to assess cell-mediated immunity. Humoral immunity was measured by the antibody plaque-forming cell (PFC) response to sheep erythrocytes. Body

weights, lymphoid organ weights and spleen cellularity were also measured. Cyclophosphamide (80 mg/kg) served as an immunosuppressive positive control agent. The results indicated that the majority of the flavouring ingredients tested did not modulate the cell-mediated or humoral immune response. However, at very high dose levels, two of the materials tested, peppermint oil and citral dimethyl acetal, did increase mortality rate and reduce survival time in the host resistance assay. Neither of these materials significantly altered the PFC response. This rapid, economical screening battery for potential immunotoxicants proved to be a useful means of evaluating a large number of structurally diverse compounds and mixtures to prioritize them for more definitive testing. As taken from Gaworski CL et al. Food Chem Toxicol. 1994 May;32(5):409-15. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/pubmed/8206439

Skin and respiratory tract sensitization Cardamom oil (4% in petrolatum) did not produce sensitisation in a maximisation test in 25 volunteers (Opdyke, 1974). A case is presented of a confectioner with a chronic hand dermatitis and positive patch test reactions to cardamom and certain terpenoid compounds present in the dried ripe seeds of cardamom. Cardamom is a popular traditional flavouring agent for baked goods and confectionery. Dermatitis from skin exposure to cardamom has to the best of our knowledge not been reported. We report one case of allergic contact dermatitis to cardamom elicited by terpenes present in the seeds (Mobacken & Fregert, 1975). Cardamom (powdered form used in foods, as opposed to cardamom seed oil) is reported to have caused sensitisation reactions, including itching, diarrhoea, wheezing and anaphylaxis, following oral ingestion (Hefle et al. 1996; Ohnuma et al. 1998).

“Nearly 80 essential oils (including 2 jasmine absolutes) have caused contact allergy. Fifty-five of these have been tested in consecutive patients suspected of contact dermatitis, and nine (laurel, turpentine, orange, tea tree, citronella, ylang-ylang, sandalwood, clove, and costus root) showed greater than 2% positive patch test reactions. Relevance data are generally missing or inadequate. Most reactions are caused by application of pure oils or high-concentration products. The clinical picture depends on the responsible product. Occupational contact dermatitis may occur in professionals performing massages. The (possible) allergens in essential oils are discussed. Several test allergens are available, but patients should preferably be tested with their own products. Co-reactivity with other essential oils and the fragrance mix is frequent, which may partly be explained by common ingredients. Patch test concentrations for essential oils are suggested.” As taken from de Groot AC and Schmidt E. 2016. Dermatitis 27(4), 170-5. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27427818

"This review presents the current trends in anaphylaxis management discussed at the fourth International Network for Online-Registration of Anaphylaxis (NORA) conference held in Berlin in April 2017. Current data from the anaphylaxis registry show that Hymenoptera venom, foods, and pharmaceutical drugs are still among the most frequent triggers of anaphylaxis. Rare triggers include chicory, cardamom, asparagus, and goji berries. A meta-analysis on recent trends in insect venom anaphylaxis demonstrated for the first time that, although data on the efficacy of insect venom immunotherapy is limited, the occurrence of severe reactions upon repeated sting events can be prevented and patients' quality of life improved. Molecular diagnostics of insect venom anaphylaxis have significantly improved diagnostic sensitivity and specificity. Self-treatment of anaphylaxis is of great importance. Recent data from the anaphylaxis registry show an increase (from 23% in 2012 to 29% in 2016) in the use of adrenaline as recommended in the guidelines. A

survey on the implementation of guidelines conducted among the centers reporting to the anaphylaxis registry highlights the extent to which the guideline has been perceived and implemented. Reports on a variety of cases in the anaphylaxis registry illustrate the diversity of this potentially life-threatening reaction. Component-resolved diagnostics can help to specify sensitization profiles in anaphylaxis, particularly in terms of the risk for severe reactions. Recent studies on anaphylaxis awareness show that training methods are effective; nevertheless, target groups and learning methods need to undergo further scientific investigation in coming years." As taken from Worm M et al. 2017. Allergo. J. Int. 26(8), 295-300. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29214141

“India has a rich diversity of medicinal plants and traditional knowledge on herbal medicine to treat the animals has both curative and preventive roles. Immunity of the animals affects the is production potential. Strengthening of non-specific immunity of the parturient animals can be used as an alternative approach to overcome the incidence of diseases in the peri and postpartum period where in these animals are more prone. One of the promising technologies is the use of immunomodulators to boost the immunity of animals during the transition period. Immunomodulators can be of natural or synthetic origin, which helps in boosting up the immunity for overcoming stress-related ailments, inhibition of reactive oxygen species generation and scavenging of free radicals to ensure the general wellbeing of animals. In the recent years, several different approaches have been examined to investigate the effect of various herb extracts and minerals as immunopotentiator separately. In literature many plants have been listed having immunomodulatory effect and some of them have been proved to have active principles with immunomodulatory, antioxidant, analgesic, antipyretic and antimicrobial properties. The present compilation deals with some of such medicinal plants like Anethum graveolens (Sowa), Elettaria cardamomum (Bari elaichi/Cardamom), Foeniculum vulgare (Saunf), Trachyspermum ammi (Ajwain), Zingiber officinale (Sundh) and Trigonella foenun- graceum etc.” As taken from Chandra S et al. 2017. Agricultural Reviews 38(4) , 297-303.

“Objective: In this study, we evaluated the anti-inflammatory effect of Elettaria cardamom oil and the underlying mechanism using in vivo models of inflammation. Methods: Male Sprague–Dawley rats, 4-6 weeks old, weighing 120-130 gms are used for the study. The anti-inflammatory study of E. cardamom oil was studied by injecting 0.1 ml of 1% carrageenan to the subplantar region of the right hind paw of rats. The development of acute inflammation was measured at the end of every 1st, 2nd, 3rd, 4th, 5th, and 6th h using plethysmometer. Results: As results from the above study, E. cardamom oil at a dose of 0.175 ml/kg was less significant than that of E. cardamom oil at a dose of 0.280 ml/kg when given orally. A $p<0.05$ shows a significant decrease in paw edema. It also reduced the levels of pro-inflammatory cytokines such as tumor necrosis factor α, interleukin (IL) 1, and IL 6 levels in the serum. The histopathology results also showed a significant reduction of congested blood vessels with no marked impression for inflammation. Conclusion: E. cardamom oil possesses anti-inflammatory activity in dose-dependent manner as they inhibit the levels of pro-inflammatory cytokines.” As,taken from Nithya S et al. 2018. Asian Journal of Pharmaceutical and Clinical Research 11(2), 207. Available at https://bit.ly/2UjpzAa

The genus Zingiberaceae has been widely used for phytotherapeutic purposes in traditional medicine throughout the world for its anti-inflammatory activity. Experimental studies have established that inflammation caused by chronic infections represents a risk factor for different forms of cancer. The objective of this study was focused on determining the anti-inflammatory capacity and cytotoxic activity of aqueous extracts of Elettaria cardamomum (cardamom) and Curcuma Longa (turmeric). The extracts were obtained by maceration and, through GC-MS/MS, a total of 11 different chemical components were determined in the aqueous extract of cardamom and 7 in the extract of turmeric. The main compounds found in cardamom and turmeric were α-terpinyl acetate (54.46%) and β-turmerone (33.45%), respectively. RT-qPCR results showed significantly lower gene expression levels of innate inflammatory cytokines (IL-6 and TNF-α) compared to the control (LPS). Also, it was observed that the extracts do not possess cytotoxic

activity against different cell lines, where E. cardamomum showed EC50 (µg/mL) of 473.84 (HeLa cells), 237.36 (J774A.1 cells), 257.51 (Vero E6 cells), and 431.16 (Balb/C peritoneal cells) and C. longa showed EC50 (µg/mL) of 351.17 (HeLa cells), 430.96 (J774A.1 cells), 396.24 (Vero E6 cells), and 362.86 (Balb/C peritoneal cells). The results of this research suggest that natural extracts of E. cardamomum and C. longa possess anti-inflammatory effects and no cytotoxic activity against HeLa, J774A.1, Vero E6, and Balb/C peritoneal cell lines. Finally, it was observed that the extracts also decreased nitric oxide (NO) production in peritoneal macrophages. As taken from Cardenas Garza GR et al. (2021) Available at https://pubmed.ncbi.nlm.nih.gov/34579443/

5.8. All other relevant types of toxicity

“Accumulating evidence suggests that free radical reactions play a key part in the development of degenerative diseases and that an antioxidant-rich diet is a major defense against these free radical reactions. In this study, we explore comparative antioxidant capacities of extracts of some commonly used in Indian spices (anise, cardamom, Ceylon cinnamon, and clove) along with their purified components (anethole, eucalyptol, cinnamaldehyde, and eugenol, respectively). Eugenol shows the highest 1,1-diphenyl-2-picrylhydrazyl, hydroxyl, and superoxide scavenging and reducing power activity in terms of weight; however, this was not found when compared in terms of equivalence. Extracts of the other three spices were found to be more potent antioxidants than their corresponding active components. Interestingly, clove extract, despite possessing the highest phenol and flavonoid content, is not the most potent radical scavenger. At low concentrations, both the crude extracts and their purified components (except for anethole and eugenol) have low hemolytic activity, but at higher concentrations purified components are more toxic than their respective crude extract. This study suggests that spices as a whole are more potent antioxidants than their purified active components, perhaps reflecting the synergism among different phytochemicals present in spice extracts.” As taken from Patra K et al. 2016. J. Environ. Pathol. Toxicol. Oncol. 35(4), 299-315. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27992311

“The growing number of Alzheimer's disease (AD) patients prompted us to seek effective natural resources for the prevention of AD. We focused on the inhibition of β-secretase, which is known to catalyze the production of senile plaque. Sixteen spices used in Asian countries were selected for the screening. Among the extracts tested, hexane extracts obtained from turmeric, cardamom, long pepper, cinnamon, Sichuan pepper, betel, white turmeric and aromatic ginger showed potent inhibitory activities. Their active principles were identified as sesquiterpenoids, monoterpenoids, fatty acid derivatives and phenylpropanoids using GC-MS analyses. The chemical structures and IC50 values of the compounds are disclosed. The results suggest that long-term consumption'of aromatic compounds from spices could be effective in the prevention of AD.” As taken from Matsumura S et al. 2016. Nat. Prod. Commun. 11(4), 507-10. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27396206

"In this research work, the antioxidant and metabolomic profiling of seven selected medicinally important herbs including Rauvolfia serpentina, Terminalia arjuna, Coriandrum sativum, Elettaria cardamom, Piper nigrum, Allium sativum, and Crataegus oxyacantha was performed. The in vivo cardioprotective potential of these medicinal plants was evaluated against surgically induced oxidative stress through left anterior descending coronary artery ligation (LADCA) in dogs. The antioxidant profiling of these plants was done through DPPH and DNA protection assay. The C. oxyacantha and T. arjuna showed maximum antioxidant potential, while the E. cardamom showed poor antioxidative strength even at its high concentration. Different concentrations of extracts of the said plants exhibited the protection of plasmid DNA against H2O2 damage as compared to the plasmid DNA merely treated with H2O2. The metabolomic profiling through LC-MS analysis of these antioxidants revealed the presence of active secondary metabolites responsible for their antioxidant potential. During in vivo analysis, blood samples of all treatment groups were drawn at different time intervals to analyze the cardiac and hemodynamic parameters. The results depicted

that the group pretreated with HC4 significantly sustained the level of CK-MB, SGOT, and LDH as well as hemodynamic parameters near to normal. The histopathological examination also confirmed the cardioprotective potential of HC4. Thus, the HC4 being safe and inexpensive cardioprotective herbal combination could be considered as an alternate of synthetic drugs." As taken from Afsheen N et al. 2018. Oxid. Med. Cell. Longev. 2018, 9819360. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29576858

“The current study was aimed at investigating the total antioxidant activity (TAC) of various fruits, vegetables, herbs and spices habitat in Pakistan. The ferric reducing ability of plasma (FRAP) assay was used to measure the TAC of various extracts (aqueous, ethanolic and aqueous-ethanolic). Following is the potency order for fruits (guava >strawberry >Pomegranate >apple >kinnow >melon >lemon >banana), vegetables (spinach >Cabbage (Purple) >Jalapeno >Radish >Brinjal >Bell Pepper >Lettuce >Carrot >Cabbage (White) >Onion >Potato >Tomato >Cucumber) and herbs/spices (clove >Rosemary >Thyme >Oregano >Cinnamon >Cumin >Kalonji >Paprika >Neem (Flower) >Fennel >Black Cardamom >Turmeric >Coriander >Ginger >Garlic). In conclusion, the guava, spinach and clove provide the best natural dietary option for treatment / prevention of oxidative stress and thus could alleviate several associated ailments.” As taken from Abid MA et al. 2017. Pak. J. Pharm. Sci. 30(6), 2147-2150. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29175783

"BACKGROUND: Spice consumption helps the treatment of diseases due to their antioxidant and anti-inflammatory contents. Cardamom is one of this spices; therefore, this study is designed to determine the effect of cardamom supplementation on serum lipids, glycemic indices, and blood pressure in pre-diabetic women. METHODS: Eighty overweight or obese pre-diabetic women were randomly allocated to two groups. The intervention group received 3 g of green cardamom and the placebo group received 3 g of rusk powder for 2 months. The physical activity level, dietary intake, anthropometric measurements, Blood pressure, fasting blood sugar (FBS), triglyceride (TG), total cholesterol (TC), low density lipoprotein (LDL-C), high density lipoprotein (HDL-C), insulin, body mass index (BMI), insulin resistance, and insulin sensitivity were measured before and after intervention. RESULTS: After intervention, mean TC ($p = 0.02$) and LDL-C ($p = 0.01$) significantly decreased and insulin sensitivity ($p = 0.03$) increased in the cardamom group. In the control group, mean HDL-C ($p = 0.02$) significantly decreased after the study. We observed no significant decrease in systolic and diastolic blood pressure, glycemic indices, and serum lipids values in the cardamom group compared to the placebo group. CONCLUSIONS: Green cardamom supplementation may have a protective effect on HDL-C level in pre-diabetic subjects. It improves some blood parameters in these subjects; however, its effects are not different from placebo." As taken from Fatemeh Y et al. 2017. J. Diabetes Metab. Disord. 16, 40. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29026804

“BACKGROUND: Cardamom is a well-known spice in Indian subcontinent, used in culinary and traditional medicine practices since ancient times. The current investigation was untaken to evaluate the potential benefit of cardamom powder supplementation in high carbohydrate high fat (HCHF) diet induced obese rats. METHOD: Male Wistar rats (28 rats) were divided into four different groups such as Control, Control + cardamom, HCHF, HCHF + cardamom. High carbohydrate and high fat (HCHF) diet was prepared in our laboratory. Oral glucose tolerance test, organs wet weight measurements and oxidative stress parameters analysis as well as liver marker enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) activities were assayed on the tissues collected from the rats. Plasma lipids profiles were also measured in all groups of animals. Moreover, histological staining was also performed to evaluate inflammatory cells infiltration and fibrosis in liver. RESULTS: The current investigation showed that, HCHF diet feeding in rats developed glucose intolerance and increased peritoneal fat deposition compared to control rats. Cardamom powder supplementation improved the glucose intolerance significantly ($p > 0.05$) and prevented the abdominal fat deposition in HCHF diet fed rats. HCHF diet feeding in rats also developed dyslipidemia, increased fat deposition and inflammation in liver compared to control rats. Cardamom powder supplementation significantly

prevented the rise of lipid parameters ($p > 0.05$) in HCHF diet fed rats. Histological assessments confirmed that HCHF diet increased the fat deposition and inflammatory cells infiltration in liver which was normalized by cardamom powder supplementation in HCHF diet fed rats. Furthermore, HCHF diet increased lipid peroxidation, decreased antioxidant enzymes activities and increased advanced protein oxidation product level significantly ($p > 0.05$) both in plasma and liver tissue which were modulated by cardamom powder supplementation in HCHF diet fed rats. HCHF diet feeding in rats also increased the ALT, AST and ALP enzyme activities in plasma which were also normalized by cardamom powder supplementation in HCHF diet fed rats. Moreover, cardamom powder supplementation ameliorated the fibrosis in liver of HCHF diet fed rats. CONCLUSION: This study suggests that, cardamom powder supplementation can prevent dyslipidemia, oxidative stress and hepatic damage in HCHF diet fed rats." As taken from Rahman MM et al. 2017. Lipids Health Dis. 16(1), 151. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28806968

"Spices are well known for their taste and flavor imparting properties. Green cardamom (Elletaria cardamomum), a herb spice belongs to family Zingiberaceae. In current study, GC-MS analysis of green cardamom essential oil (CEO) resulted in identification of twenty-six compounds with α-terpinyl acetate (38.4%), 1,8-cineole (28.71%), linalool acetate (8.42%), sabinene (5.21%), and linalool (3.97%) as major bioactive components. Present study also described the antimicrobial properties like zone of inhibition, minimum inhibitory concentration against microbial strains with special emphasis on quorum sensing inhibition. Disk diffusion assay showed that C. albicans and S. mutans were the most sensitive microorganisms followed by S. aureus, L. monocytogenes, B. cereus and S. typhimurium sensor strains, respectively. Whilst P. aeruginosa was found most resistant strain as CEO did not inhibited its growth. The minimum inhibitory concentration (MIC) values of CEO against tested strains were 10 ± 0.00 mg/mL against S. typhimurium, S. aureus and 5 ± 0.00 mg/mL against S. mutans, C. albicans strains, respectively. Regarding quorum sensing inhibition the tested concentrations 0.625 and 0.313 mg/mL of CEO inhibited violacein production with very little effect on growth of C. violaceum. Conclusively, study proved that quorum sensing inhibition values of CEO were much lower compared to MIC revealed values. Hence, cardamom bioactive constituents can effectively be used to develop novel antimicrobial drugs against conventional antibiotics." As taken from Abdullah et al. 2017. J. Food Sci. Technol. 54(8), 2306-2315. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28740287

"Natural antimicrobials as well as essential oils (EOs) have gained interest to inhibit pathogenic microorganisms and to control food borne diseases. Campylobacter spp. are one of the most common causative agents of gastroenteritis. In this study, cardamom, cumin, and dill weed EOs were evaluated for their antibacterial activities against Campylobacter jejuni and Campylobacter coli by using agar-well diffusion and broth microdilution methods, along with the mechanisms of antimicrobial action. Chemical compositions of EOs were also tested by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). The results showed that cardamom and dill weed EOs possess greater antimicrobial activity than cumin with larger inhibition zones and lower minimum inhibitory concentrations. The permeability of cell membrane and cell membrane integrity were evaluated by determining relative electric conductivity and release of cell constituents into supernatant at 260 nm, respectively. Moreover, effect of EOs on the cell membrane of Campylobacter spp. was also investigated by measuring extracellular ATP concentration. Increase of relative electric conductivity, extracellular ATP concentration, and cell constituents' release after treatment with EOs demonstrated that tested EOs affected the membrane integrity of Campylobacter spp. The results supported high efficiency of cardamom, cumin, and dill weed EOs to inhibit Campylobacter spp. by impairing the bacterial cell membrane." As taken from Mutlu-Ingok A and Karbancioglu-Guler F. 2017. Molecules 22(7), E1191. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28714890

"BACKGROUND: Several preclinical studies have shown that spices may decrease the risk of chronic diseases. However, it has been suggested that more clinical trials be carried out to strengthen this preclinical evidence. The purpose of the present study was to evaluate the effects of

cardamom (Elettaria cardamomum) supplementation on inflammation and oxidative stress in hyperlipidemic, overweight, and obese pre-diabetic women. METHODS: This randomized, placebo-controlled, double-blind clinical trial was conducted on 80 pre-diabetic subjects. They randomly received the cardamom supplement (n = 40, 3 g d-1) or identical inert placebo (n = 40) for 8 weeks. Serum concentrations of high-sensitivity C-reactive protein (hs-CRP), interleukin-6 (IL-6), tumour necrosis factor α, total antioxidant capacity, malondialdehyde (MDA), protein carbonyl, and erythrocyte superoxide dismutase and glutathione reductase activity were analyzed at the baseline and after intervention. RESULTS: After the adjustment of some covariates, cardamom supplementation significantly decreased serum hs-CRP (P = 0.02), hs-CRP:IL-6 ratio (P = 0.008), and MDA (P = 0.009) compared with the placebo group. CONCLUSION: Cardamom could improve some parameters of inflammation and oxidative stress in pre-diabetic subjects. Thus it may be useful in reducing complications associated with inflammation and oxidative stress in these patients." As taken from Kazemi S et al. 2017. J. Sci. Food Agric. 97(15), 5296-5301. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28480505

"The human body on exposure to high-altitude, undergoes many physiological challenges. The cardiopulmonary reserves are favoured against the digestive system. Hence, the efficiency of digestion is compromised to a great extent, which leads to anorexia, hypophagia, epigastralgia, dyspepsia, nausea, and peptic ulcers. The present study was focused on in vitro digestive influence of selected food ingredients viz. cardamom, carom, cumin, coriander, fennel, fenugreek, ginger, pepper, star anise, turmeric, papaya, orange, pineapple, liquorice, valerian, and tarragon on the activities of digestive enzymes of rat pancreas, duodenum, and small intestine. In-vitro antioxidant activities of the above food ingredients were also carried out with respect to their radical scavenging activity against DPPH·, NO·, and ferrous reducing antioxidant power. All the studied food ingredients showed a comparative range of free radical scavenging activity. Further, pineapple has shown enhanced enzymatic activity of pancreatic amylase, trypsin and chymotrypsin among the tested samples with 432, 252, and 86%, respectively. However, all food ingredients showed inhibitory effect towards maltase activity, while the sucrose activity was enhanced in tarragon compared to control. Almost all the selected food ingredients have been observed to have low glycemic index and low protein efficiency ratio except pineapple. The results suggested that ample merit in the use of pineapple extract can be carried forward for the formulation of highly digestible foods for extreme environmental conditions." As taken from Anusha MB et al. 2018. Journal of Food Science and Technology 55(5), 1913–1921. Available at https://link.springer.com/article/10.1007/s13197-018-3109-y

"Nowadays the use of new and safe antioxidant from plant, animal and microbial resources is increasing. The aim of this study was to investigate and compare the antioxidant properties and types of interaction (synergism and antagonism) of green tea and the Elettaria cardamomum extracts. Green tea and cardamom extract and BHT were prepared in 25, 50, 100, 150, 200 and 250 ?g/ml and combined extract in different combination to reach 50, 100, 150, 200 and 250 ?g/ml were prepared in different ratios (1: 1, 2: 1, 1: 2, 1: 3, 3: 1, 2: 2, 1: 4 and 4: 1). In this study, phenolic compound was evaluated by Folin-Ciocalteu's method. Their antioxidant activity was measured by four methods: DPPH free radical scavenging, FRAP assay, beta-carotene /linoleic and ability to prevent the oxidation of soybean oil. BHT was used as positive control for comparison. The results of the tests showed that, antioxidant properties of green tea extract was significantly more than from Elettaria cardamomum extract and BHT ($P<0.05$). In different ratio of combined extracts, free radical scavenging DPPH assay in all ratio, FRAP test in 8 ratio and beta-carotene-linoleic acid test in 5 ratios showed synergismtic effect. In the peroxide value assay, the chosen combination showed antagonism, although it was significantly ($p<0.05$) more effective than BHT in soybean oil stability." As taken from Arianfar A and Sardarodiyan M. 2018. Iranian Journal of Food Science and Technology 14(72), 25-36. Available at http://www.sid.ir/En/Journal/ViewPaper.aspx?ID=537458 "The presented article focuses on the in vitro inhibition of plant extracts on the human carbonic anhydrase isoforms (hCA I and hCAII), and paraoxonase-1 (PON1) activities. Five different plants (Alcea rosea, Foeniculum vulgare, Elettaria

cardamomum, Laurus azorica and Lavandula stoechas) were selected in this study. Methanol, ethanol, and water extracts of plants were prepared and the concentration-dependent inhibition degrees were found for hCA I and hCA II isozymes and hPON1 spectrophotometrically. Thus, IC50 (mg/mL) values were obtained for each extract. Methanolic extract of Elettaria cardamomum has the highest inhibitory effects (0.032 mg/mL). The water extracts of plants showed lower inhibitory impacts compared to the methanol and ethanol extracts." As taken from Kaya ED et al. 2019. Hacettepe J. Biol. & Chem. 47(1), 51-59.

6. Functional effects on

6.1. Broncho/pulmonary system

No data available to us at this time.

6.2. Cardiovascular system

Intravenous administration of the oil in doses of 5-20 mul/kg induced dose-dependent decreases in arterial blood pressure and heart rate in the rat. It did not depress isolated perfused rat heart (El Tahir et al. 1997). The results of this study demonstrated the ability of cardamom oil to depress the cardiovascular system in rats and to possess local anesthetic properties. [...] The observed effects seemed to be due to more than one component of the oil (El Tahir et al. 1997).

"BACKGROUND: Cardiovascular disease (CVD) is a spectrum of diseases involving the heart and blood vessels, and the first cause of mortality worldwide. Medicinal plants have been used for thousands of years to treat CVD. In Traditional Persian Medicine (TPM), there is a special focus on heart diseases. Avicenna, a Persian physician of the eleventh century compiled a book devoted to this field named "The treatise on cardiac drugs" which is a compendium of TPM knowledge on CVD. Avicenna mentioned 50 cardiovascular active plants and described their therapeutic effects in the treatment of CVDs. METHODS: Here, we perform a detailed search in scientific databases to verify the cardiovascular activities of the medicinal plants suggested by Avicenna. Also, we discussed cardiovascular activities of a number of the most important suggested plants as well as their efficacy in clinical studies. Major bioactive compounds identified from these plants are also discussed. RESULTS: Pharmacological studies have revealed that the majority of these plants are effective in cardiovascular health with various mechanisms. Among them, Crocus sativus L., Cinnamomum cassia (L.) J. Presl, Punica granatum L., Ocimum basilicum L., Elettaria cardamomum (L.) Maton, Melissa officinalis L. and Phyllanthus emblica L. have proved to be more effective. CONCLUSION: The above-mentioned plants can be rich sources for developing new and effective pharmaceuticals for the treatment of CVDs." As taken from Sobhani Z et al. 2017. Curr. Pharm. Des. 23(17), 2428-2443. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28215156

BACKGROUND: Cardamom (Elettaria cardamomum) is an aromatic seed spice grown extensively in India and used as a flavoring in sweets. In this study, the anti-hypercholesterolemic effect of cardamom was evaluated in Wistar rats by inducing hypercholesterolemia with a high-cholesterol diet for 8 weeks. Dietary interventions were made with (a) cardamom powder (50 g kg-1), (b) cardamom oil (3 g kg-1 , equivalent to 50 g kg-1 cardamom) and (c) de-oiled cardamom powder (50 g kg-1). RESULTS: A significant reduction in blood total cholesterol (31%) and low-density lipoprotein cholesterol (44%) was observed by oral administration of cardamom oil in hypercholesterolemic rats, accompanied by a marked decrease in serum triglycerides by 42%. The cholesterol content of cardiac muscle was beneficially lowered by 39% with administration of cardamom oil in hypercholesterolemic rats. Liver triglycerides were reduced by 33%. Incorporation of cardamom oil/powder in the diet did not alter feed consumption by rats. Compromised activities of hepatic antioxidant enzymes in the hypercholesterolemic situation were generally countered by dietary cardamom. Treatment with de-oiled cardamom as well as cardamom oil countered the

diminished activity of catalase in hypercholesterolemic animals. Cardamom also enhanced the activity of heart superoxide dismutase in the hypercholesterolemic situation. The concentration of ascorbic acid in serum was significantly increased by dietary cardamom or its fractions in the hypercholesterolemic situation. CONCLUSION: This animal study has established the potential of cardamom oil in restoring the alteration in lipid homeostasis in conditions of hypercholesterolemia. The significant reduction in atherogenicity index by dietary intervention with cardamom powder and cardamom oil indicates the potential cardioprotective effect of cardamom" As taken from Nagashree S et al. 2017. J. Sci. Food Agric. 97(10), 3204-3210. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/27888503

"BACKGROUND: Dietary factors play a key role in the development as well as prevention of certain human diseases, including cardiovascular diseases. Currently there has been an increase in global interest to identify medicinal plants that are pharmacologically effective and have low or no side effects for use in preventive medicine. Culinary herbs and spices are an important part of human nutrition in all the cultures of the world. There is a growing amount of literature concerning the potential benefits of these herbs and spices from a health perspective especially in conferring protection against cardiovascular diseases. OBJECTIVE: The objective of this review is to provide information on the recent scientific findings on some common spices that have a distinct place in folk medicine in several of the Asian countries as well as on their traditional uses for the role they can play in the management of heart diseases and which may be useful in defining cost effective and inexpensive interventions for the prevention and control of CVDs. METHOD: Systematic literature searches were carried out and the available information on various medicinal plants traditionally used for cardiovascular disorders was collected via electronic search (using Pubmed, SciFinder, Scirus, GoogleScholar, JCCC@INSTIRC and Web of Science) and a library search for articles published in peerreviewed journals. No restrictions regarding the language of publication were imposed. RESULTS: This article highlights the recent scientific findings on four common spices viz. Greater cardamom (Amomum subulatum Roxb.), Coriander (Coriandrum sativum L.), Turmeric (Curcuma longa L.) and Ginger (Zingiber officinale Roscoe), for the role they can play in the management of heart diseases. Although they have been used by many cultures since ancient times and have been known to exhibit several medicinal properties, current research shows that they can also be effectively used for the prevention and control of CVDs. CONCLUSION: Although scientific evidences supporting the benefits of spices in maintaining a healthy heart are available, more complete information is needed about the actual exposures to these dietary components that are required to bring about a response. The innumerable actions of spices that have been shown in in vitro experiments need to be demonstrated in more systematic, well-designed animal model studies. More rigorous clinical trials at the normally consumed levels are needed to determine long-term benefits as well as to assess adverse effects if any at higher concentrations, especially if consumed over longer periods. Once these extensive studies are carried out, it will be easy to define the appropriate intervention strategies utilizing these commonly used spices for achieving the maximum benefits on cardiovascular health without producing any ill-effects." As taken from Rastogi S et al. 2017. Curr. Pharm. Des.23(7), 989-998. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/27774899

"Herbal medicines with high amounts of phytochemicals have been shown to have beneficial effects on blood pressure (BP), endothelial function and anthropometric measures. This study aimed to determine the effect of herbal treatment on BP, endothelial function and anthropometric measures in patients with type 2 diabetes mellitus (T2DM). This clinical trial included 204 T2DM patients randomly assigned to four intervention groups receiving 3 g cinnamon, 3 g cardamom, 1 g saffron or 3 g ginger with three glasses of black tea, and one control group consuming only three glasses of tea without any herbals, for 8 weeks. Intercellular adhesion molecule-1 (ICAM-1), systolic and diastolic BP and anthropometric measures were collected at baseline and after 8 weeks. No significant difference was found between various medicinal plants in terms of influencing BP, serum soluble (s)ICAM-1 concentrations and anthropometric measures. However, in within-group comparison saffron and ginger intakes significantly reduced sICAM-1 concentrations

(340.9 ± 14.4 vs 339.69 ± 14.4 ng/ml, p = 0.01, and 391.78 ± 16.0 vs 390.97 ± 15.8 ng/ml, p = 0.009, respectively) and ginger intake affected systolic BP (143.06 ± 0.2 vs 142.07 ± 0.2 mmHg, p = 0.02). Although administration of these herbal medicines as supplementary remedies could affect BP and sICAM-1 concentrations, there was no significant difference between the plants in terms of influencing anthropometric measures, BP and endothelial function." As taken from Azimi P et al. 2016. Blood Press. 25(3), 133-40. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/26758574

"In this research work, the antioxidant and metabolomic profiling of seven selected medicinally important herbs including Rauvolfia serpentina, Terminalia arjuna, Coriandrum sativum, Elettaria cardamom, Piper nigrum, Allium sativum, and Crataegus oxyacantha was performed. The in vivo cardioprotective potential of these medicinal plants was evaluated against surgically induced oxidative stress through left anterior descending coronary artery ligation (LADCA) in dogs. The antioxidant profiling of these plants was done through DPPH and DNA protection assay. The C. oxyacantha and T. arjuna showed maximum antioxidant potential, while the E. cardamom showed poor antioxidative strength even at its high concentration. Different concentrations of extracts of the said plants exhibited the protection of plasmid DNA against H2O2 damage as compared to the plasmid DNA merely treated with H2O2. The metabolomic profiling through LC-MS analysis of these antioxidants revealed the presence of active secondary metabolites responsible for their antioxidant potential. During in vivo analysis, blood samples of all treatment groups were drawn at different time intervals to analyze the cardiac and hemodynamic parameters. The results depicted that the group pretreated with HC4 significantly sustained the level of CK-MB, SGOT, and LDH as well as hemodynamic parameters near to normal. The histopathological examination also confirmed the cardioprotective potential of HC4. Thus, the HC4 being safe and inexpensive cardioprotective herbal combination could be considered as an alternate of synthetic drugs." As taken from Afsheen N et al. 2018. Oxid. Med. Cell. Longev. 2018, 9819360. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29576858

"BACKGROUND: Spice consumption helps the treatment of diseases due to their antioxidant and anti-inflammatory contents. Cardamom is one of this spices; therefore, this study is designed to determine the effect of cardamom supplementation on serum lipids, glycemic indices, and blood pressure in pre-diabetic women. METHODS: Eighty overweight or obese pre-diabetic women were randomly allocated to two groups. The intervention group received 3 g of green cardamom and the placebo group received 3 g of rusk powder for 2 months. The physical activity level, dietary intake, anthropometric measurements, Blood pressure, fasting blood sugar (FBS), triglyceride (TG), total cholesterol (TC), low density lipoprotein (LDL-C), high density lipoprotein (HDL-C), insulin, body mass index (BMI), insulin resistance, and insulin sensitivity were measured before and after intervention. RESULTS: After intervention, mean TC (p = 0.02) and LDL-C (p = 0.01) significantly decreased and insulin sensitivity (p = 0.03) increased in the cardamom group. In the control group, mean HDL-C (p = 0.02) significantly decreased after the study. We observed no significant decrease in systolic and diastolic blood pressure, glycemic indices, and serum lipids values in the cardamom group compared to the placebo group. CONCLUSIONS: Green cardamom supplementation may have a protective effect on HDL-C level in pre-diabetic subjects. It improves some blood parameters in these subjects; however, its effects are not different from placebo." As taken from Fatemeh Y et al. 2017. J. Diabetes Metab. Disord. 16, 40. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29026804

"The plant of Elletteria cardamom was collected and phytochemical studies were made with different solvents using ethanol and water. The extracts showed different extractive values (2.0 and 9.0 respectively) and showed the presences of different bioactive compounds like Alkaloids, Saponins, flavonoids, Terpenes, Glycosides steroids. Based upon the literature this given us the positive signal which may induce cardio protective activity. Treatment with Ellettaria cardamom extract high dose 500mg/kg.b.w lowers the LDH levels, SGOT levels, SGPT levels, Total protein levels, Serum albumin levels, Alkaline phasphatase levels and Triglycerides in doxorubicin induced cardiac rats. Treatment with low dose 100mg/kg.b.w of Elletteria cardamom extract lowers the

Triglycerides levels in doxorubicin cardiac rats. Treatment with high dose 500mg/kg.b.w of Elletteria cardamom extract lowers the total cholesterol levels in doxorubicin cardiac rats. Treatment with high dose 500mg/kg.b.w of Elletteria cardamom extract improves the HDL Cholesterol levels in doxorubicin cardiac rats. Treatment with high dose 500mg/kg.b.w of Elletteria cardamom extract lowers the LDL Cholesterol levels in doxorubicin cardiac rats. Treatment with high dose 500mg/kg.b.w of Elletteria cardamom extract lowers the VLDL Cholesterol levels in doxorubicin cardiac rats. Treatment with Elletteria cardamom extract high dose 500mg/kg.b.w lowers the total chloride levels in doxorubicin induced cardiac rats." As taken from Shahidullah M et al. 2017. Indian Journal of Research in Pharmacy and Biotechnology 5(6), 366-370. Available at https://www.ijrpb.com/issues/ijrpb%205(6)%202.%20Shahidullah%20366-370.pdf

6.3. Nervous system

The depressant effects were significantly antagonized by treatment of the animals with cyproheptadine but not with mepyramine, ranitidine, hexamethonium, indomethacin, or cutting of the vagus nerves. Volatile oil did not affect electrically induced contraction of the cat nictitating membrane (El Tahir et al. 1997).
Large doses of volatile oil also antagonized stimulant effects of acetylcholine and nicotine on rabbit jejunum. Volatile oil doses of 0.01-0.04 mul/ml to isolated guinea pig ileum contracted the tissue. Exposure of the frog's sciatic nerve to oil in doses of 0.2-0.4 mul/ml suppressed the frog-limb withdrawal reflex suggesting a local anesthetic effect (El Tahir et al. 1997).
In addition the antispasmodic activity was determined on a rabbit intestine preparation using acetylcholine as agonist, the results proving that cardamom oil exerts its antispasmodic action through muscarinic receptor blockage (al-Zuhair et al. 1996).

"Elettaria cardamomum is an aromatic spice (cardamom) native to the humid Asian areas, which contains some compounds with a potential anticonvulsant activity. Various pharmacological properties such as anti-inflammatory, analgesic, antioxidant, and antimicrobial effects have been related to this plant. This research was conducted to examine the probable protective impact of the essential oil and methanolic extract of E. cardamomum against chemically (pentylentetrazole)- and electrically (maximal electroshock)-induced seizures in mice. In addition, neurotoxicity, acute lethality, and phytochemistry of the essential oil and methanolic extract were estimated. The TLC method showed the presence of kaempferol, rutin, and quercetin in the extract, and the concentration of quercetin in the extract was 0.5 µg/mL. The major compounds in the essential oil were 1,8-cineole (45.6%), α-terpinyl acetate (33.7%), sabinene (3.8%), 4-terpinen-4-ol (2.4%), and myrcene (2.2%), respectively. The extract and essential oil showed significant neurotoxicity in the rotarod test at the doses of 1.5 g/kg and 0.75 mL/kg, respectively. No mortalities were observed up to the doses of 2 g/kg and 0.75 mL/kg for the extract and essential oil. The essential oil was effective in both the pentylentetrazole and maximal electroshock models; however, the extract was only effective in the pentylentetrazole model. The study suggested that E. cardamomum methanolic extract had no significant lethality in mice. Both the essential oil and methanolic extract showed movement toxicity. Anticonvulsant effects of E. cardamomum were negligible against the seizures induced by pentylentetrazole and maximal electroshock." As taken from Masoumi-Ardakani Y et al.

2016. Planta Med. 82(17), 1482-1486. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27433883

“Post-traumatic stress disorder (PTSD) is a debilitating psychiatric condition which develops in 6-8% of the general population. Current standard pharmacological treatments for PTSD cannot be widely used due to having various side effects. Nowadays, various pharmacological properties have been related to Elettaria cardamomum L. (family of Zingiberaceae). The present study aims to evaluate the efficacy of E. cardamomum methanolic extract on anxiety-like behavior in a rat model of PTSD. Adult male Wistar rats (200-250gr) were used in this study. The rats underwent single prolonged stress (SPS) or control and intraperitoneally received either saline or different dosages (200, 400, and 800mg/kg) of E. cardamomum methanolic extract before and after stress sessions. Moreover, open field, elevated plus-maze, and rotarod tests were used to evaluate locomotion and anxiety-like behavior in the rats. Findings demonstrated that E. Cardamomum methanolic extract, particularly at the dose of 400mg/kg, significantly ($P<0.05$) improved anxiety-like behavior in a rat model of PTSD, as examined by the open field, elevated plus-maze, and rotarod tests. Administration of E. cardamomum methanolic extract after stress might help to prevent the formation of anxiety-like behavior in the animals. However, further studies are requiredto clarify the exact mechanisms involved.” As taken from Masoumi-Ardakani Y et al. 2017. Biomed. Pharmacother. 87, 489-495. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/28073098

“Cardamom is a strong antioxidant plant, so it is called the queen of spices. In the present study, we explored the potentials of cardamom on developmental, learning ability and biochemical parameters of mice offspring. Thirty pregnant mice were allocated to three groups of ten animals in each. Groups Π and -¿ received pilsbury's Diet containing 10 and 20% of cardamom (w/w) respectively, whereas Group I used as control. Cardomom was administered from the first day of pregnancy and was continued until post-natal day 15 (PD 15) and thereafter the mothers were switched to plain pilsbury's Diet. During the weaning period, three pups in each litter were color marked from the others, and were subjected to various tests (Physical assessment such body weight and eye opening and hair appearance; the neuromaturation of reflexes like righting, rotating, and cliff avoidance reflexes; learning ability and memory retention; estimation of monoamines neurotransmitters like dopamine and serotonin, non-enzymatic oxidative stress such as TBARS and GSH in forebrain at different ages of pups). The results indicated that the body weight gain was declining significantly. Hair appearance and eyes opening were delayed significantly. Righting, rotating, and cliff avoidance reflexes were delayed in treated animals. Exposure to cardamom led to enhance learning and memory retention as compared to control. Monoamines (DA, 5-HT) and GSH were elevated, whereas TBARS was inhibited significantly. In conclusion, perinatal cardamom exposure enhanced learning and memory as compared to control. Cardamom and its benefit compounds were transported via placenta or/and milk during lactation. Cardamom needs more researches to investigate its benefits on other kinds of behavior.” As taken from Abu-Taweel GM. 2018. Saudi J. Biol. Sci. 25(1), 186-193. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29379379

“PURPOSE: Recent studies suggested that the non-familiar form of Alzheimer's disease (AD) could be consequence of metabolic syndrome and neuroinflammation. Elettaria cardamomum extract (EC) has exhibited antidiabetic, antioxidant and anti-inflammatory properties. This research was conducted to evaluate the effects of EC on AD-like alterations in rats induced by high fructose and high fat diet coupled with a single small dose of STZ (25 mg/kg) (T2DM rats). METHODS: Phytochemical analysis was carried out. Behavioral tests, immunohistochemical examination, biochemical analysis and gene expression determination were performed in treated and controls rats. RESULTS: The majority of EC compounds were terpenoids. EC extract administration for 8 weeks attenuated AD-like alterations. It reversed a T2DM-induced decline in cognitive functions in passive avoidance task and Morris water maze test. It significantly lowered the elevated hippocampal level of AChE activity and caspase-3 activity, an indicator of degeneration in T2DM

rats Also, it reduced the accumulation of Aβ and p-tau in the brain of T2DM rats. Furthermore, it elevated the suppressed glutamate receptor expression (AMPA GluR1 subunit and NMDA receptor subunits NR1, NR2A, NR2B). EC treatment reduced hippocampal lipid peroxidation marker malondialdehyde (MDA) and augmented antioxidant defensive system, including superoxide dismutase (SOD) and reduced glutathione (GSH). Meanwhile, it lowered hippocampal TNFα, IL β1but not IL6 and reduced GSK-3β in brainT2D rats. CONCLUSION: EC treatment could ameliorate AD-like alterations in T2DM rats through activation of blunted insulin signal transduction in the brain, attenuation of associated oxidative stress and neuroinflammation." As taken from Gomaa AA et al. 2019. Cytokine 113, 405-416. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/30539783

"Acetylcholinesterase (AChE) is an enzyme involved in the progression of Alzheimer's disease (AD). Cardamom oil (CO) has been reported to have acetylcholinesterase inhibitory, antioxidant and anti-anxiety effects. Hence, we studied the effect of cardamom oil in aluminum chloride induced neurotoxicity in rats. AD like symptoms were induced in Wistar rats with aluminum chloride (100 mg/kg, p.o.). Cardamom oil was administered concomitantly by oral route at doses of 100 and 200 mg/kg for 42 days. Behavioral parameters like Morris water maze, elevated plus maze, passive avoidance test and locomotor activity were evaluated on day 21 and 42. AChE activity, oxidative stress parameters, histopathological studies and immunohistochemistry studies were carried out in hippocampus and cortex. Cardamom oil treatment showed significant improvement in behavioral parameters, inhibition of AChE activity ($p < 0.001$) and reduction in oxidative stress in the brain. Histopathological studies of hippocampus and cortex by hematoxylin & eosin (H. & E.) and congo red stain showed inhibition of neuronal damage and amyloid β plaque formation with cardamom oil treatment. Immunohistochemistry showed, CO treatment inhibited amyloid β expression and upregulated brain-derived neurotrophic factor (BDNF). The present study showed that, cardamom oil has neuroprotective effect in aluminum chloride induced neurotoxicity linked with inhibition of AChE activity and reduction in oxidative damage. This effect of cardamom oil may be useful in management of Alzheimer's disease." As taken from Auti ST and Kulkarni YA. 2019. Front. Neurol. 10, 399. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/31114535/

"In this article the depression is forcibly produced by sleep deprivation method and the analysis of the depression behavior is done by swimming behavior of rats. More on a recent paper suggested that females are more prone to depression then males. To resolve this Elletaria Cardamomum was used to analyze the effect of depression via sleep deprivation method. The aim of study was undertaken to evaluate the antidepressant effect of crude methanolic extract of seed of Elletaria cardamom at doses 200mg/kg and 400mg/kg using sleep deprivation test (imipramine (20mg/kg, body weight) was used as standard by taking the male and female rats for study. Effect of Elletaria cardamom dose (200mg/kg and 400mg/kg) were determined, the effect of dose were compared with control and standard group of animals. Data expressed as mean ± SEM (n=6) $p<0.05$, $*p<0.01$, $*p<0.03$,* $p<0.04$. Significant dose dependent decline in immobility time was observed in doses by swim test and it was observed, that female are more prone to depression elicited and Elletaria Cardamom extract exhibited effectual results." As taken from Singh M and Kumar S. 2019. J. Global Trends Pharm. Sci. 10(4), 6626-6631. Available at https://www.jgtps.com/admin/uploads/MbfxEg.pdf

"Abstract: Cardamomum which is known as Elettaria cardamomum, has been widely utilizing for thousands of years for various ailments and cooking purpose. This present study was aimed to evaluate the effect Elettaria cardamomum seeds on wound healing in Sprague Dawley rats using Excision Wound Model and analgesic activity in Albino mice using Tail Immersion Method. Extracts prepared by cold maceration method. The preliminary phytochemical screening of extract shows the presence of alkaloids, proteins, phenolic compounds, flavonoids, volatile oils and terpenoids. For Excision Wound Model, animals were divided in four groups of six rats each. Group I served as negative control, treated with simple ointment, group II treated with standard drug, Povidone iodine 10% w/w, group III treated with low dose (5%, w/w) extract and group IV were treated with high dose (10%, w/w) ofextract. All the treatments were done topically and were given once daily. The

wound healing effect was observed on 5th, 10th and 15th day. Furthermore, for Tail Immersion Method, mice were divided in four groups with six each. Group I served as normal control, treated with normal saline, group II as standard, and treated with Tramadol (20mg/kg), group III mice treated with ethanolic extract (200mg/kg) as low dose and group IV were treated with extract of (400mg/kg) as high dose. All the extracts and standard drug were given orally, and tail flick response time recorded for 30, 60, 90 and 120 minutes. The highly significant (***P<0.001) E.cardamomum ointment was observed in both 5%w/w and 10%w/w on 15th day when compared with negative control. Both 5%w/w and 10% E.cardamomum ointment revealed the effectiveness of improved wound healing. Besides that, for analgesic activity, ethanolic extract of E.cardamom(400mg/kg), high dose was highly significant (***P<0.001) whereby low dose (200mg/kg) extract showed less significant (*P<0.05) at 120 minutes. This study showed that ethanolic extract of Elettaria cardamomum seeds possess wound healing properties and has potential to treat pain, which may be due to presence of alkaloids, proteins, phenolic compounds, flavonoids, volatile oils and terpenoids." As taken from Krupavaram B et al. 2020. Current Trends in Biotechnology & Pharmacy 2020, Suppl., 108-109.

6.4. Other organ systems, dependent on the properties of the substance

Addition of oil to spontaneously contracting rabbit jejunum in small doses contracted the tissue but large doses relaxed it (El Tahir et al. 1997).
It was concluded that cardamom volatile oil-induced actions were due to more than one of its constituents that possessed local anesthetic coupled with serotoninergic and/or muscarinic agonist activities (El Tahir et al. 1997).
Cardamom seeds are widely used for flavouring purposes in food and as carminative. Little information has been reported on their pharmacological and toxicological properties or, for their volatile oil which constitutes about 5% of the seed's total weight. A comparative study of the anti-inflammatory activity of the oil extracted from commercial Elettaria cardamomum seeds, in doses of 175 and 280 microliters/kg and indomethacin in a dose of 30 mg/kg against acute carrageenan-induced planter oedema in male albino rats was performed, which proved to be marked. Moreover, investigation of the analgesic activity using p-benzoquinone as a chemical stimulus proved that a dose of 233 microliters/kg of the oil produced 50% protection against the writhing (stretching syndrome) induced by intraperitoneal administration of a 0.02% solution of p-benzoquinone in mice (al-Zuhair et al. 1996).
Cardamom, the fruits of Elettaria cardamomum Maton. (Zingiberaceae) commonly known as "Heel khurd" is used in Unani system of medicine to treat gastrointestinal disorders. A crude methanolic extract (TM), essential oil (EO), petroleum ether soluble (PS) and insoluble (PI) fractions of methanolic extract, were studied in rats at doses of 100-500, 12.5-50, 12.5-150 and 450 mg/kg, respectively for their ability to inhibit the gastric lesions induced by aspirin, ethanol and pylorous ligature. In addition their effects on wall mucus and gastric acid output were recorded. All fractions (TM, EO, PS, PI) significantly inhibited gastric lesions induced by ethanol and aspirin but not those induced by pylorus ligation. TM proved to be active reducing lesions by about 70% in the EtOH-induced ulcer model at 500 mg/kg. The PS fraction reduced the lesions by 50% at 50 and 100mg/kg (no dose response was observed) with similar effect than the PI fraction at 450 mg/kg. In the aspirin-induced gastric ulcer, the best gastroprotective effect was found in the PS fraction, which inhibited lesions by nearly 100% at 12.5mg/kg (Jamal et al. 2006).
The in vitro and in vivo effect of pretreatment by cardamom oil, a crude drug extract, in ethanol/water vehicles on the transdermal delivery of indomethacin was investigated. The cyclic monoterpene components in cardamom oil were also determined and quantified in this study. The permeation of indomethacin was significantly enhanced after pretreatment of cardamom oil both in the in vitro and in vivo studies. The result of various pre-treatment periods showed that the indomethacin flux decreased as the length of the pretreatment increased. Both natural cardamom oil and a cyclic monoterpene mixture composed of the components of the oil showed similar enhancement on indomethacin permeation, indicating cyclic monoterpenes are the predominant

components altering the barrier property of stratum corneum. The results also showed that three minor components in cardamom oil (alpha-pinene, 6.5%; beta-pinene, 4.8%; alpha-terpineol, 0.4%) had a synergistic effect with 1,8-cineole (59.3%) and d-limonene (29.0%) to enhance the permeation of indomethacin (Huang et al. 1999).
The influence of essential oils from naturally occurring plant dietary items such as cardamom, celery seed, cumin seed, coriander, ginger, nutmeg, and zanthoxylum on the activities of hepatic carcinogen-metabolizing enzymes (cytochrome P450, aryl hydrocarbon hydroxylase, and glutathione S-transferase) and acid-soluble sulfhydryl level was investigated in Swiss albino mice. Each oil was fed by gavage at 10 microliters/day for 14 days, and then the animals were sacrificed and their hepatic enzyme activities and sulfhydryl levels were evaluated. Only nutmeg and zanthoxylum oils induced cytochrome P450 level significantly ($P<0.05$), whereas cardamom oil caused a significant reduction in its activity ($P<0.05$). Furthermore, aryl hydrocarbon hydroxylase activity was significantly elevated only by treatment with ginger oil ($P<0.01$), whereas nutmeg oil caused a significant reduction in its activity ($P<0.01$). The remaining oils did not significantly alter the level of cytochrome P450 and aryl hydrocarbon hydroxylase activity. Glutathione S-transferase activity was significantly elevated in all experimental groups ($P<0.1$-$P<0.001$) compared with controls. The acid-soluble sulfhydryl was significantly elevated only by the essential oils of cardamom ($P<0.05$), nutmeg ($P<0.05$), and zanthoxylum ($P<0.01$). Our observations suggest that intake of essential oils affects the host enzymes associated with activation and detoxication of xenobiotic compounds, including chemical carcinogens and mutagens (Banerjee et al. 1994). Many smokers relapse during cessation attempts due to increases in negative affect. Previous research has shown that chewing confectionary chewing gum appears to lessen the severity of acute nicotine withdrawal symptoms and help individuals who are trying to reduce smoking in part due to the flavor of the gum chewed. The current study compared the effects of three flavored gums to a No Gum Control during 48-hour cessation periods for young dependent smokers. Forty-nine smokers participated in three experimental conditions (peppermint, vanilla, and baked apple cardamom flavored gum) as well as a No Gum Control across four weeks while abstaining from smoking for 48-hours each week. Compared to the No Gum Control, participants in the Gum conditions reported lower levels of anxiety, dysphoria, and tension. Vanilla and baked apple cardamom flavored gum resulted in lower levels of negative affect while peppermint flavored gum was not different from the No Gum Control. These findings indicate that some flavors of gum are effective in reducing the negative affect associated with nicotine withdrawal and may serve as a valuable tool in helping smokers quit (Cohen et al. 2010).

“BACKGROUND: Cardamom (Elettaria cardamomum) is an aromatic seed spice grown extensively in India and used as a flavoring in sweets. In this study, the anti-hypercholesterolemic effect of cardamom was evaluated in Wistar rats by inducing hypercholesterolemia with a high-cholesterol diet for 8 weeks. Dietary interventions were made with (a) cardamom powder (50 g kg-1), (b) cardamom oil (3 g kg-1 , equivalent to 50 g kg-1 cardamom) and (c) de-oiled cardamom powder (50 g kg-1). RESULTS: A significant reduction in blood total cholesterol (31%) and low-density lipoprotein cholesterol (44%) was observed by oral administration of cardamom oil in hypercholesterolemic rats, accompanied by a marked decrease in serum triglycerides by 42%. The cholesterol content of cardiac muscle was beneficially lowered by 39% with administration of cardamom oil in hypercholesterolemic rats. Liver triglycerides were reduced by 33%. Incorporation

of cardamom oil/powder in the diet did not alter feed consumption by rats. Compromised activities of hepatic antioxidant enzymes in the hypercholesterolemic situation were generally countered by dietary cardamom. Treatment with de-oiled cardamom as well as cardamom oil countered the diminished activity of catalase in hypercholesterolemic animals. Cardamom also enhanced the activity of heart superoxide dismutase in the hypercholesterolemic situation. The concentration of ascorbic acid in serum was significantly increased by dietary cardamom or its fractions in the hypercholesterolemic situation. CONCLUSION: This animal study has established the potential of cardamom oil in restoring the alteration in lipid homeostasis in conditions of hypercholesterolemia. The significant reduction in atherogenicity index by dietary intervention with cardamom powder and cardamom oil indicates the potential cardioprotective effect of cardamom." As taken from Nagashree S et al. 2017. J. Sci. Food Agric. 97(10), 3204-3210. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/27888503

"BACKGROUND: Medicinal herb-derived drug development has become important in the relief of liver pathology. Amomun cardamomum is traditionally used therapeutically in Korea to treat various human ailments including dyspepsia, hiccupping, and vomiting. We investigated to assess the protective effect of A. cardamomum on carbon tetrachloride (CCl4)-induced liver damage through antioxidant activity in hepatic tissues of Sprague-Dawley rats. METHODS: Antioxidant properties of different fractions from A. cardamomum from ethanol extracts were evaluated by an in vitro free radical scavenging systems. The protective effect of the ethyl acetate fraction from A. cardamomum (EAAC) against CCl4-induced cytotoxicity was determined by a cell viability assay using HepG2 hepatocarcinoma cells. In vivo study, the influence of EAAC concentrations of 100 and 200 mg/kg following CCl4-induced hepatic injury was assessed. Serum levels of glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), and alkaline phosphatase (ALP) were determined, as was lipid peroxidation (malondialdehyde, MDA). Effect of EAAC on liver detoxification enzymes including superoxide dismutase (SOD), total glutathione (GSH), and glutathione S-transferase (GST) activity was measured in rat liver homogenates. Liver cytochrome P450 (CYP2E1) expression level was determined by quantification of mRNA. RESULTS: Phytochemical analysis of A. cardamomum indicated that EAAC was enriched in total polyphenol and total flavonoid. Most of the tannins were confined to the hexane fraction. Hepatoprotective properties of EAAC were evident, with significantly reduced serum levels of GOT, GPT, and ALP compared with the control group. Improved hepatic antioxidant status was evident by increased SOD, GSH, and GST enzymes in rat liver tissue. Liver lipid peroxidation induced by CCl4 was apparent by increased intracellular MDA level. EAAC suppressed lipid peroxidation as evidenced by the significant decrease in MDA production. Expression of CYP2E1 was also significantly decreased at the higher concentration of EAAC, indicating the hepatoprotective efficacy of EAAC on acute liver damage. CONCLUSION: These results indicated that EAAC has a significant hepatoprotective activity on CCl4-induced acute hepatic injury in rats, which might be derived from its antioxidant properties and CYP2E1 downregulation." As taken from Lim DW et al. (2016). BMC Complement. Altern. Med. 16, 155. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27246748

"Amommum subulatum (Roxb.) or Cardamom extract is known to have anti-inflammatory and neuroprotective effects towards many gastrointestinal related problems. However, up till now different fractions of cardamom extract on fibroblasts with respect to potassium channel activity have not been investigated. Therefore, present study investigated the effects of different fractions of cardamom extract on potassium channels in non-tumor NIH3T3 cell line. Phytochemical analysis of hydroalcoholic, n-hexane, butane and ethyl acetate fractions of cardamom extracts were purified and isolated by thin layer chromatography (TLC). 3T3 cells were cultured and incubated with hydroalcohol (1-2 µ/ml), n-hexane (1 µ/ml), butane (2 µ/ml) and ethyl acetate (1-2 µ/ml) for 5 hrs at 37°C. Modulation in potassium currents were recorded by whole-cell patch clamp method. The data showed two constituents Cineol (C10H18O) and Terpinyl acetate (C10H17OOCCH3) by TLC method. The present study shows that the constituents in n-hexane, hydro alcohol (1 µ/ml) and

ethyl acetate (2 µ/ml) significantly increased ($p<0.01$) the potassium outward rectifying currents from NIH3T3 cells when compared to untreated controls cells. Whereas, butanol fraction (2 µ/ml) significantly decreased ($p<0.01$) the inward rectifying currents when compared to controls. Moreover hydroalcoholic and n-hexane fractions have increased the proliferation in 3T3 cell line. On the other hand butanol and ethyl acetate did not induce proliferation in 3T3 cells. Taken together, our data suggested that cardamom extract contains constituents that increased K+ currents, cell migration and proliferation and are involved in wound healing." As taken from Siddiqui S et al. 2017. Pak. J. Pharm. Sci. 30(6), 2211-2215. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29175791

“BACKGROUND: Cardamom is a well-known spice in Indian subcontinent, used in culinary and traditional medicine practices since ancient times. The current investigation was untaken to evaluate the potential benefit of cardamom powder supplementation in high carbohydrate high fat (HCHF) diet induced obese rats. METHOD: Male Wistar rats (28 rats) were divided into four different groups such as Control, Control + cardamom, HCHF, HCHF + cardamom. High carbohydrate and high fat (HCHF) diet was prepared in our laboratory. Oral glucose tolerance test, organs wet weight measurements and oxidative stress parameters analysis as well as liver marker enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) activities were assayed on the tissues collected from the rats. Plasma lipids profiles were also measured in all groups of animals. Moreover, histological staining was also performed to evaluate inflammatory cells infiltration and fibrosis in liver. RESULTS: The current investigation showed that, HCHF diet feeding in rats developed glucose intolerance and increased peritoneal fat deposition compared to control rats. Cardamom powder supplementation improved the glucose intolerance significantly ($p > 0.05$) and prevented the abdominal fat deposition in HCHF diet fed rats. HCHF diet feeding in rats also developed dyslipidemia, increased fat deposition and inflammation in liver compared to control rats. Cardamom powder supplementation significantly prevented the rise of lipid parameters ($p > 0.05$) in HCHF diet fed rats. Histological assessments confirmed that HCHF diet increased the fat deposition and inflammatory cells infiltration in liver which was normalized by cardamom powder supplementation in HCHF diet fed rats. Furthermore, HCHF diet increased lipid peroxidation, decreased antioxidant enzymes activities and increased advanced protein oxidation product level significantly ($p > 0.05$) both in plasma and liver tissue which were modulated by cardamom powder supplementation in HCHF diet fed rats. HCHF diet feeding in rats also increased the ALT, AST and ALP enzyme activities in plasma which were also normalized by cardamom powder supplementation in HCHF diet fed rats. Moreover, cardamom powder supplementation ameliorated the fibrosis in liver of HCHF diet fed rats. CONCLUSION: This study suggests that, cardamom powder supplementation can prevent dyslipidemia, oxidative stress and hepatic damage in HCHF diet fed rats.” As taken from Rahman MM et al. 2017. Lipids Health Dis. 16(1), 151. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28806968

“The human body on exposure to high-altitude, undergoes many physiological challenges. The cardiopulmonary reserves are favoured against the digestive system. Hence, the efficiency of digestion is compromised to a great extent, which leads to anorexia, hypophagia, epigastralgia, dyspepsia, nausea, and peptic ulcers. The present study was focused on in vitro digestive influence of selected food ingredients viz. cardamom, carom, cumin, coriander, fennel, fenugreek, ginger, pepper, star anise, turmeric, papaya, orange, pineapple, liquorice, valerian, and tarragon on the activities of digestive enzymes of rat pancreas, duodenum, and small intestine. In-vitro antioxidant activities of the above food ingredients were also carried out with respect to their radical scavenging activity against DPPH·, NO·, and ferrous reducing antioxidant power. All the studied food ingredients showed a comparative range of free radical scavenging activity. Further, pineapple has shown enhanced enzymatic activity of pancreatic amylase, trypsin and chymotrypsin among the tested samples with 432, 252, and 86%, respectively. However, all food ingredients showed inhibitory effect towards maltase activity, while the sucrose activity was enhanced in tarragon compared to control. Almost all the selected food ingredients have been observed to have low glycemic index and low protein efficiency ratio except pineapple. The results suggested that ample

merit in the use of pineapple extract can be carried forward for the formulation of highly digestible foods for extreme environmental conditions." As taken from Anusha MB et al. 2018. Journal of Food Science and Technology 55(5), 1913–1921. Available at https://link.springer.com/article/10.1007/s13197-018-3109-y

Feeding of poly-herbal like, Anethole (Carminative, stimulant, stomachic, mildly diuretic, galactagogue, antimicrobial, anti-inflammatory, antihypercholesterolemic and antihyperlipidemic activities), Asparagus racemosus (enhanced milk production), Elettaria cardamomum (analgesic, anti-inflammatory, anti-microbial, antioxidant and anti-spasmodic activity), Foeniculum vulgare (galactagogue, carminative, Hepatoprotective, Antioxidant, Free radical scavenging, superoxide anion radical scavenging, hydrogen peroxide scavenging, metal chelating, anti-inflammatory, antifungal, antibacterial, acaricidal activity), Trachyspermum ammi (carminative, laxative, stomachic and anthelmintic), Trigonella foenum-graecum (Immunomodulatory, antioxidant, antidiabetic, anti-inflammatory and anti-pyretic) and Zingiber officinale (antioxidant, anti-inflammatory and anti-pyretic) during transition period, may be helpful in reduction of peripartum stress by improving immune status and, beneficial effect on productive and reproductive performance of the animals. As taken from Chandra S et al. 2017. Agricultural Reviews. 38(4): 297-303,

"The gastroprotective and therapeutic effects of cardamom was examined using aspirin-induced gastric ulcers. Male albino rats were separated into healthy control and peptic ulcer-induced groups. The latter group was treated with cardamom extract before or after ulcer induction to investigate its protective and therapeutic effects. Another group was treated with Omeprazole. Rats were sacrificed; the gastric tissue was examined for ulcer index and score, pH and total acidity measurements. Moreover, we studied antiinflammatory and antioxidant biomarkers. Results demonstrated that cardamom exerts an ameliorative effect, shown by a decrease in the gastric ulcer index and score. The extract restores the free radical-scavenging enzymes and reduces inflammatory markers. Pepsin and putrescine levels decreased significantly, while hydroxyproline levels increased in groups treated with cardamom extract, either as a protective or therapeutic agent. Cardamom is therapeutically valuable for enhancing and hastening gastric ulcer remediation as it is capable of inhibiting aspirin-induced damage." As taken from Hamza AH et al. 2019. Medical Science 23(97), 395-403. Available at http://www.discoveryjournals.org/medicalscience/current_issue/v23/n97/A23.pdf

7. Addiction

JTI is not aware of any information that demonstrates that this ingredient has any addictive effect.

8. Burnt ingredient toxicity

This ingredient was considered as part of an overall safety assessment of ingredients added to tobacco in the manufacture of cigarettes. An expert panel of toxicologists reviewed the open literature and internal toxicology data of 5 tobacco companies to evaluate a composite list of ingredients used in the manufacture of cigarettes. The conclusion of this report was that these ingredients did not increase the inherent biological activity of tobacco cigarettes, and are considered to be acceptable under conditions of intended use (Doull et al., 1994 & 1998). Tobacco smoke condensates from cigarettes containing Cardamom oil (8000-66-6) and an additive free, reference cigarettes were tested in a battery of in vitro and/or in vivo test(s). Within the sensitivity and specificity of the bioassay(s) the activity of the condensate was not changed by the addition of Cardamom oil (8000-66-6). Table below provides tested level(s) and specific endpoint(s).

Endpoint	Tested level (ppm)	Reference
Smoke chemistry	2	Carmines, 2002 & Rustemeier et al., 2002
	94 (seed oil)	Baker et al., 2004a

	13 39	JTI KB Study Report(s)
	3	Roemer et al, 2014
In vitro genotoxicity	2	Carmines, 2002 & Roemer et al., 2002
	94 (seed oil)	Baker et al., 2004c
	13	Renne et al., 2006
	13 39	JTI KB Study Report(s)
	29.9	fGLH Study Report (2010)
	3	Roemer et al, 2014
In vitro cytotoxicity	2	Carmines, 2002 & Roemer et al., 2002
	94 (seed oil)	Baker et al., 2004c
	39	JTI KB Study Report(s)
	29.9	fGLH Study Report (2010)
	3	Roemer et al, 2014
Inhalation study	7.5	Gaworski et al., 1998
	2	Carmines, 2002 & Vanscheeuwijck et al., 2002
	94 (seed oil)	Baker et al., 2004c
	13	Renne et al., 2006
	13 39	JTI KB Study Report(s)
	3	Schramke et al, 2014
Skin painting	8	Gaworski et al., 1999
	13 39	JTI KB Study Report(s)
In vivo genotoxicity	3	Schramke et al, 2014

9. Heated/vapor emissions toxicity

Aerosol from an electronic nicotine delivery system (ENDS) that creates a vapor by heating an e-liquid containing Cardamom oil and/or seed oil was tested in a battery of in vitro and/or in vivo test(s). Under the test conditions and within the sensitivity and specificity of the bioassay(s), no mutagenic, genotoxic or cytotoxic responses were observed when exposed to Aerosol Collected Matter (ACM) and/or aerosol Gas Vapor Phase (GVP) and no adverse findings from a 90-day in vivo repeat-dose inhalation toxicity study were observed after exposure to the aerosol even when exposure concentrations were the maximal amount that could be achieved with the specific product(s). These results are in contrast to those observed with combustible cigarette which showed mutagenic, genotoxic, cytotoxic and adverse effects upon exposure. The table below provides the highest tested level(s) and specific endpoint(s):

Endpoint	**Tested level (ppm)**	**Reference**
Aerosol chemistry	50	Logic (2019a) Labstat International Inc. (2021)
In vitro genotoxicity	50	Logic (2019a) Labstat International Inc. (2022)

In vitro cytotoxicity	50	Logic (2019a) Labstat International Inc. (2022)
In vivo genotoxicity	50	Logic (2019a)
Inhalation study	50	Logic (2019a)

Aerosol from an electronic nicotine delivery system (ENDS) product that creates a vapor by heating an e-liquid; the vapor then passes through a capsule containing tobacco granules, containing Cardamom oil and/or seed oil was tested in a battery of in vitro and/or in vivo test(s). Under the test conditions and within the sensitivity and specificity of the bioassay(s), no mutagenic, genotoxic or cytotoxic responses were observed when exposed to Aerosol Collected Matter (ACM) and/or aerosol Gas Vapor Phase (GVP) and no adverse findings from a 90-day in vivo repeat-dose inhalation toxicity study were observed after exposure to the aerosol even when exposure concentrations were the maximal amount that could be achieved with the specific product(s). These results are in contrast to those observed with combustible cigarette which showed mutagenic, genotoxic, cytotoxic and adverse effects upon exposure. The table below provides tested level(s) and specific endpoint(s):

Endpoint	Tested level	Reference
Aerosol chemistry	0.0990 mg/(tobacco portion; 310 mg)	Logic (2019b)
In vitro genotoxicity	0.0990 mg/(tobacco portion; 310 mg)	Logic (2019b)
In vitro cytotoxicity	0.0990 mg/(tobacco portion; 310 mg)	Logic (2019b)
In vivo genotoxicity	0.0990 mg/(tobacco portion; 310 mg)	Logic (2019b)
Inhalation study	0.0990 mg/(tobacco portion; 310 mg)	Logic (2019b)

Aerosol from heated tobacco stick(s) containing Cardamom extract, oil and/or seed oil was tested in aerosol chemistry and a battery of in vitro test(s). Under the test conditions and within the sensitivity and specificity of the bioassay(s), the activity of the total particulate matter (TPM) and/or gas vapor phase (GVP) were not increased by the addition of this ingredient when compared to TPM and/or GVP from reference combustible cigarettes. The table below provides the highest tested level(s) and specific endpoint(s):

Endpoint	Tested level (mg/stick)	Reference
Aerosol chemistry	0.040	Labstat International Inc. (2020a) Labstat International Inc. (2021a) JTI Heated Tobacco Stick Study Report(s)
In vitro genotoxicity	0.040	Labstat International Inc. (2020b) Labstat International Inc. (2021b) JTI Heated Tobacco Stick Study Report(s)
In vitro cytotoxicity	0.040	Labstat International Inc. (2020b) Labstat International Inc. (2021b) JTI Heated Tobacco Stick Study Report(s)

10. Ecotoxicity

10.1. Environmental fate

EPISuite provides the following data for cardamom oil (8000-66-6):
Henrys Law Constant (25 deg C) [HENRYWIN v3.20]:

Bond Method :	2.04E-004 atm-m3/mole (2.06E+001 Pa-m3/mole)
Group Method:	1.31E-004 atm-m3/mole (1.33E+001 Pa-m3/mole)
Exper Database:	1.10E-04 atm-m3/mole (1.11E+001 Pa-m3/mole)
Henrys LC [via VP/WSol estimate using User-Entered or Estimated values]:	HLC: 9.534E-004 atm-m3/mole (9.660E+001 Pa-m3/mole) VP: 1.56 mm Hg (source: MPBPVP) WS: 332 mg/L (source: WSKOWWIN)

Log Octanol-Air Partition Coefficient (25 deg C) [KOAWIN v1.10]:

Log Kow used:	2.74 (exp database)
Log Kaw used:	-2.347 (exp database)
Log Koa (KOAWIN v1.10 estimate):	5.087
Log Koa (experimental database):	None

Probability of Rapid Biodegradation (BIOWIN v4.10):

Biowin1 (Linear Model): Biowin2 (Non-Linear Model) : Biowin3 (Ultimate Survey Model): Biowin4 (Primary Survey Model) : Biowin5 (MITI Linear Model) : Biowin6 (MITI Non-Linear Model): Biowin7 (Anaerobic Linear Model):	-0.0411 0.0023 2.4254 (weeks-months) 3.3086 (days-weeks) 0.4825 0.4105 -0.7689
Ready Biodegradability Prediction:	NO

Hydrocarbon Biodegradation (BioHCwin v1.01):

Structure incompatible with current estimation method!

Sorption to aerosols (25 Dec C)[AEROWIN v1.00]:

Vapor pressure (liquid/subcooled):	253 Pa (1.9 mm Hg)
Log Koa (Koawin est):	5.087
Kp (particle/gas partition coef. (m3/ug)): Mackay model: Octanol/air (Koa) model:	1.18E-008 3E-008

Fraction sorbed to airborne particulates (phi):

Junge-Pankow model:	4.28E-007
Mackay model:	9.47E-007
Octanol/air (Koa) model:	2.4E-006

Atmospheric Oxidation (25 deg C) [AopWin v1.92]: Hydroxyl Radicals Reaction:

OVERALL OH Rate Constant =	22.5684 E-12 cm3/molecule-sec
Half-Life =	0.474 Days (12-hr day; 1.5E6 OH/cm3)
Half-Life =	5.687 Hrs
Ozone Reaction:	No Ozone Reaction Estimation
Fraction sorbed to airborne particulates (phi): 6.88E-007 (Junge-Pankow, Mackay avg) 2.4E-006 (Koa method) Note: the sorbed fraction may be resistant to atmospheric oxidation	

Soil Adsorption Coefficient (KOCWIN v2.00):

Koc :	221.2 L/kg (MCI method)
Log Koc:	2.345 (MCI method)
Koc :	223.9 L/kg (Kow method)
Log Koc:	2.350 (Kow method)

Aqueous Base/Acid-Catalyzed Hydrolysis (25 deg C) [HYDROWIN v2.00]: Rate constants can NOT be estimated for this structure!
Volatilization from Water: Henry LC: 0.00011 atm-m3/mole (Henry experimental database)

Half-Life from Model River:	7.878 hours
Half-Life from Model Lake:	190.1 hours (7.92 days)

Removal In Wastewater Treatment:

Total removal:	9.05 percent
Total biodegradation:	0.11 percent

Total sludge adsorption:	3.76 percent
Total to Air:	5.19 percent

(using 10000 hr Bio P,A,S)
Level III Fugacity Model:

	Mass Amount (percent)	Half-Life (hr)	Emissions (kg/hr)
Air	1.55	23.1	1000
Water	17.9	900	1000
Soil	80.3	1.8e+003	1000
Sediment	0.23	8.1e+003	0

Persistence Time: 802 hr

The Ecological Categorization Results from the Canadian Domestic Substances List simply state that cardamom oils (CAS RN 8000-66-6) are of uncertain persistence in the environment.

Data accessed March 2017 on the OECD website.

10.2. Aquatic toxicity

The Ecological Categorization Results from the Canadian Domestic Substances List simply state that cardamom oils (CAS RN 8000-66-6) are not inherently toxic to aquatic organisms and are of low ecotoxicological concern.

Data accessed March 2017 on the OECD website.

The New Zealand EPA consider cardamom oils (8000-66-6) to be “very ecotoxic in the aquatic environment” to fish, crustacea and algae (NZ EPA CCID).

ECOSAR version 1.11 reports the following aquatic toxicity data for CAS RN 8000-66-6:

Values used to Generate ECOSAR Profile: Log Kow: 3.133 (EPISuite Kowwin v1.68 Estimate) Wat Sol: 3500 (mg/L, PhysProp DB exp value)

ECOSAR v1.11 Class-specific Estimations

Neutral Organics

ECOSAR Class	**Organism**	**Duration**	**End Pt**	**Predicted mg/L (ppm)**
Neutral Organics :	Fish	96-hr	LC50	12.165
Neutral Organics :	Daphnid	48-hr	LC50	7.669
Neutral Organics :	Green Algae	96-hr	EC50	8.805
Neutral Organics :	Fish		ChV	1.345

Neutral Organics :	Daphnid		ChV	1.001
Neutral Organics :	Green Algae		ChV	2.911
Neutral Organics :	Fish (SW)	96-hr	LC50	15.419
Neutral Organics :	Mysid	96-hr	LC50	5.318
Neutral Organics :	Fish (SW)		ChV	3.343
Neutral Organics :	Mysid (SW)		ChV	0.329

10.3. Sediment toxicity

No data available to us at this time.

10.4. Terrestrial toxicity

ECOSAR version 1.11 reports the following terrestrial toxicity data for CAS RN 8000-66-6:

Values used to Generate ECOSAR Profile: Log Kow: 3.133 (EPISuite Kowwin v1.68 Estimate) Wat Sol: 3500 (mg/L, PhysProp DB exp value)

ECOSAR v1.11 Class-specific Estimations

Neutral Organics

ECOSAR Class	Organism	Duration	End Pt	Predicted mg/L (ppm)
Neutral Organics :	Earthworm	14-day	LC50	204.609

10.5. All other relevant types of ecotoxicity

EPISuite provides the following data for cardamom oil (8000-66-6): **Bioaccumulation Estimates (BCFBAF v3.01):**

Log BCF from regression-based method:	1.475 (BCF = 29.84 L/kg wet-wt)
Log Biotransformation Half-life (HL):	0.0700 days (HL = 1.175 days)
Log BCF Arnot-Gobas method (upper trophic):	1.720 (BCF = 52.45)
Log BAF Arnot-Gobas method (upper trophic):	1.720 (BAF = 52.45)
log Kow used:	2.74 (expkow database)

The Ecological Categorization Results from the Canadian Domestic Substances List simply state that cardamom oils (CAS RN 8000-66-6) are of uncertain bioaccumulative potential in the environment.

Data accessed March 2017 on the OECD website.

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FEMA GRAS assessment of natural flavor complexes: Lavender, Guaiac Coriander-derived and related flavoring ingredients

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A B S T R A C T

In 2015, the Expert Panel of the Flavor and Extract Manufacturers Association (FEMA) initiated a program for the re-evaluation of the safety of over 250 natural flavor complexes (NFCs) used as flavor ingredients. This publication, fifth in the series, evaluates the safety of NFCs containing linalool and/or other characteristic mono- and sesquiterpenoid tertiary alcohols and esters using the safety evaluation procedure published by the FEMA Expert Panel in 2005 and updated in 2018. The procedure relies on a complete chemical characterization of the NFC intended for commerce and organization of the chemical constituents of each NFC into well-defined congeneric groups. The safety of each NFC is evaluated using the well-established and conservative threshold of toxicological concern (TTC) concept in addition to data on absorption, metabolism and toxicology of both the constituent congeneric groups and the NFCs. Sixteen NFCs, derived from the *Lavandula, Aniba, Elettaria, Daucus, Salvia, Coriandrum, Ribes, Guaiacum/Bulnesia, Citrus, Pogostemon, Melaleuca* and *Michelia* genera, were affirmed as generally recognized as safe (GRAS) under their conditions of intended use as flavor ingredients based on an evaluation of each NFC and the constituents and congeneric groups therein.

1. Introduction

The Expert Panel of the Flavor and Extract Manufacturers Association (FEMA), formed in 1960, has been the primary, independent body evaluating the safety of flavoring ingredients for use in human foods in the United States. Flavor ingredients are evaluated for "generally recognized as safe" (GRAS) status for intended use consistent with the 1958 Food Additive Amendment to the Federal Food Drug and Cosmetic Act (Hallagan and Hall, 1995, 2009; Hallagan et al., 2020). Flavoring ingredients can be pure chemically defined compounds or complex mixtures, known as natural flavor complexes (NFCs). To date, the FEMA Expert Panel has determined that over 2,700 flavoring ingredients have met the GRAS criteria for their intended uses.

The FEMA Expert Panel published its first list of GRAS flavoring ingredients that included both chemically defined and NFC flavoring ingredients in 1965 (Hall and Oser, 1965). A key part of the FEMA GRAS program is the re-evaluation of GRAS flavoring ingredients. The FEMA Expert Panel has completed two re-evaluations of FEMA GRAS chemically defined flavor ingredients and in 2015 expanded the re-evaluation program to encompass FEMA GRAS NFCs. For the safety evaluation of NFCs, the FEMA Expert Panel developed a scientifically-based procedure based on the chemical composition of the NFC (Smith et al., 2005). This procedure was reviewed and updated in 2018 (Cohen et al., 2018a). Because the constituents of NFCs are typically derived from common biochemical pathways, the constituents can be organized into a finite number of well-established chemical groupings called congeneric groups. For the safety evaluation of each NFC, information is gathered

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Abbreviations

BfR	German Bundesinstitut für Risikobewertung
CA	Chromosomal Aberration
CF	Correction Factor
CFR	Code of Federal Regulations
CG	Congeneric Group
CHO	Chinese Hamster Ovary (cells)
DTC	Decision Tree Class
EFFA	European Flavour Association
EFSA	European Food Safety Authority
ERS/USDA	Economic Research Service/United States Department of Agriculture
FAO	Food and Agriculture Organization of the United Nations
FCC	Food Chemicals Codex
FDA	Food and Drug Administration
FEMA	Flavor and Extract Manufacturers Association
FID	Flame Ionization Detector
GC-MS	Gas Chromatography-Mass Spectrometry
GLP	Good Laboratory Practice
GRAS	Generally Recognized as Safe
HPBL	Human Peripheral Blood Lymphocytes
IFEAT	International Federation of Essential Oils and Aroma Trades
IOFI	International Organization of the Flavor Industry
JFFMA	Japan Fragrance and Flavor Materials Association
LC-MS	Liquid Chromatography-Mass Spectrometry
LOAEL	Lowest-Observed-Adverse-Effect-Level
MoS	Margin of Safety
NFC	Natural Flavoring Complex
NOAEL	No Observed Adverse Effect Level
NTP	National Toxicology Program
OECD	Organization for Economic Co-Operation and Development
PCI	Per Capita Intake
TD50	Dose giving a 50% tumor incidence
TDI	Tolerable Daily Intake
TTC	Threshold of Toxicological Concern
UDS	Unscheduled DNA Synthesis
US-EPA	United States Environmental Protection Agency
WHO	World Health Organization

on the estimated intake, metabolism and toxicology for each constituent congeneric group. The Threshold of Toxicological Concern (TTC) approach is applied to evaluate the estimated intake of each constituent congeneric group (Kroes et al., 2000; Munro et al., 1996). In addition, the potential toxicity of the unidentified constituent fraction is also evaluated in the updated procedure.

Beginning in 2015, the FEMA Expert Panel has issued a series of calls for data requesting detailed chemical analyses for over 250 NFCs with FEMA GRAS status. Members from the International Organization of the Flavor Industry (IOFI), including FEMA, the Japan Fragrance and Flavor Materials Association (JFFMA) and the European Flavour Association (EFFA), in addition to the International Federation of Essential Oils and Aroma Trades (IFEAT) have provided information in response to these data requests. NFC flavoring ingredients are often derived from botanical plants that are also sources of familiar foods and spices. Due to the large number of NFCs to be evaluated, the NFCs were parsed into groups based on their constituent congeneric group profile. The congeneric groups used for NFC analysis by the FEMA Expert Panel are provided in an appendix to the safety evaluation procedure (Cohen et al., 2018a) The first group of NFCs reviewed by the FEMA Expert Panel were derived from the *Citrus* genus and included orange, lemon, lime and grapefruit-derived NFCs (Cohen et al., 2019). The second group of NFCs evaluated were several mint, dill, caraway and buchu-derived NFCs for which Group 10 (Alicyclic ketones, secondary alcohols and related esters) constituents were a major fraction of their composition profile (Cohen et al., 2020). The Panel's third publication outlined the safety evaluation of cinnamon and cassia-derived NFCs whose composition profiles contained Group 16 (Cinnamyl alcohol, cinnamaldehyde, cinnamic acid and related esters) constituents (Rietjens et al., 2020). In its 4th publication in the series, the safety of eugenol-rich clove, cinnamon leaf and West Indian bay leaf-derived NFCs was evaluated (Gooderham et al., 2020). This publication, the fifth in the series, continues the re-evaluations by the FEMA Expert Panel on a set of NFCs which are characterized by the presence of Group 12 (Aliphatic and aromatic tertiary alcohols and related esters) constituents such as linalool, α-terpineol and patchouli alcohol. These NFCs, listed in Table 1, include essential oils and extracts derived from botanicals of the *Lavandula, Aniba, Elettaria, Daucus, Salvia, Coriandrum, Ribes, Guaiacum/Bulnesia, Citrus, Pogostemon, Melaleuca* and *Michelia* genera that are used as flavoring ingredients.

2. History of food use

Lavender, spike lavender, lavandin and related perennial flowering plants in the *Lavandula* genus are herbs of the *Lamiaceae* family native to the Mediterranean region. Southern France is one of the largest past and present producers of lavender oil (Guenther, 1949) and Bulgaria and China are now also major producers (Giray, 2018). Considered hardy herbs, the different species of *Lavandula* are now cultivated around the world, including in the United States, Australia, Russia and several European countries (Guenther, 1949; Lis-Balchin, 2002a). Lavandin, a hybrid between true lavender and spike lavender, has a flowering period between that of true lavender (August) and spike lavender (September) during which it is harvested for use (Guenther, 1949). Lavender gets its name from the Latin *lavare* meaning "to wash" due to its use by ancient Romans to perfume bath water (Castle and Lis-Balchin, 2002). Users of lavender in Victorian England alternatively derived the name from the Latin *livere* meaning bluish (Festing, 1989). Lavender is used as a culinary herb in foods and beverages and is a component in 'Herbes de Provence' blends that are popular for the flavoring of savory foods (Grieve, 1970; Kehler and Schooley, 2006; Laget, 2005). Clary sage, another plant of the *Lamiaceae* family, has historically been used as a culinary herb, a substitute for hops in brewing and as a flavoring for wines, particularly wines originating from the Rhine region of Germany (Grieve, 1970; Guenther, 1949). Another member of the *Lamiaceae* family, the patchouly plant, is the source of an essential oil with distinctive aromatic and flavoring properties. Patchouly is cultivated extensively in Malaysia, Indonesia and other tropical climates and its leaves were used in traditional medicine (Murugan and Livingstone, 2010; Swamy and Sinniah, 2015). Patchouly oil, produced from the steam distillation of the leaves of the plant, is used as both a perfumery and a flavor ingredient (Guenther, 1949; van Beek and Joulain, 2018).

In addition to lavender, flowers from other botanicals are sources for several other NFCs used for flavoring food. The fragrant flowers of the bitter orange tree (*Citrus aurantium* L) can be distilled to collect its essential oil, known as neroli oil, or can be extracted sequentially with a non-polar solvent and ethanol to yield an absolute, both of which are used as flavor ingredients. In addition, orange blossom water from the distillation of neroli oil is commonly used as an ingredient in Moroccan cuisine. The fruit of this *Citrus* species is acidic in character and less palatable than sweet oranges, but the fruit peels are used in the preparation of marmalades and the essential oil from the peel is used to flavor

Table 1
NFCs evaluated by the Expert Panel.

Name	FEMA No.	Estimated Intake (μg/person/day)[a]	Most recent surveyed annual volume (kg)[b]
Bois De Rose Oil (*Aniba rosaeodore* Ducke)	2156	6	63
Cardamom Seed Oil (*Elettaria cardamomum* (L.) Maton)	2241	340	3,310
Carrot Oil (*Daucus carota* L.)	2244	24	240
Clary Oil (*Salvia sclarea* L.)	2321	11	110
Coriander Seed Oil (*Coriandrum sativum* L.)	2334	1,190	11,500
Currant Buds Black Absolute (*Ribes nigrum* L.)	2346	12	120
Guaiac Wood Extract (*Guaiacum officinale* L.; *G. sanctum* L.; *Bulnesia sarmienti* Lorentz)	2533	1	11[c]
Guaiac Wood Oil (Guaiacum officinale L.; G. sanctum L.; *Bulnesia sarmienti* Lorentz)	2534	150	1440
Lavandin Oil (Hybrids between *Lavandula offincinalis* Chaix *and L. latifolia* Viil.)	2618	1,010	9,780
Lavender Absolute (*Lavandula officinalis* Chaix)	2620	0.01	0.1
Lavender Oil (*Lavandula officinalis* Chaix)	2622	810	7,840
Orange Blossoms Absolute (*Citrus aurantium* L.)	2818	1	11
Patchouly Oil (*Pogostemon cablin* Benth. and *P. heyneanus* Benth.)	2838	220	2,120
Spike Lavender Oil (*Lavandula latifolia* Vill. (L. spica DC.))	3033	4	43[d]
Tea Tree Oil (*Melaleuca alternifolia*)	3902	330	32,300
Michelia Alba Oil (*Michelia alba* D.C.)	3950	2	18

[a] For high volume materials (greater than 22,700 kg/year), the PCI *per capita* is shown. For materials with a lower surveyed volume (less than 22,700 kg/year, PCI × 10 ("eaters only') calculation is shown.

[b] Harman, C.L., Murray, I.J., 2018.2015 Poundage and Technical Effects Survey. Flavor and Extract Manufacturers Association, Washington, DC, USA.

[c] Gavin, C.L., Williams, M.C. and Hallagan, J.B., 2008.2005 Poundage and Technical Effects Survey. Flavor and Extract Manufacturers Association of the United States (FEMA), Washington, DC, USA.

[d] Source: Harman, C.L., Lipman, M.D. and Hallagan, J.B. 2013. Flavor and Extract Manufacturers Association of the United States (FEMA) 2010 Poundage and Technical Effects Survey, Washington DC, USA.

food (Boelens and Oporto, 1991). Another example of the use of flowers to flavor food are the flowers of the *Michelia alba* tree. These flowers are distilled to produce an essential oil that is commonly used to flavor teas (Cohen et al., 2019; Ueyama et al., 1992). Blackcurrant is grown in the colder regions of Europe and its fruits are harvested for use in liqueurs such as crème de cassis, the preparation of juice concentrates and purees (Duponcel, 2007). The flower buds of the blackcurrant are extracted to produce an absolute that imparts a blackcurrant-like flavor in the flavoring of foods and alcoholic and non-alcoholic beverages (Fenaroli, 1975; Wytenhove, 1984).

Carrots are a well-recognized root vegetable of the *Apiaceae* family. Like other members of this family, such as coriander, dill and fennel, the carrot plant forms umbels which are groups of flowers that produce seeds. Historically, wild carrot varieties were found in Europe, Asia and Africa and carrot seeds were used as a traditional medicine by cultures that lived around the Mediterranean basin and carrot seed oil has a history of use in the preparation of alcoholic liquors in France (Guenther, 1950). Cultivation of carrot for harvesting of its roots began approximately five thousand years ago in the Iranian Plateau and in the Persian Empire (Stolarczyk and Janick, 2011) and continues to the present time.

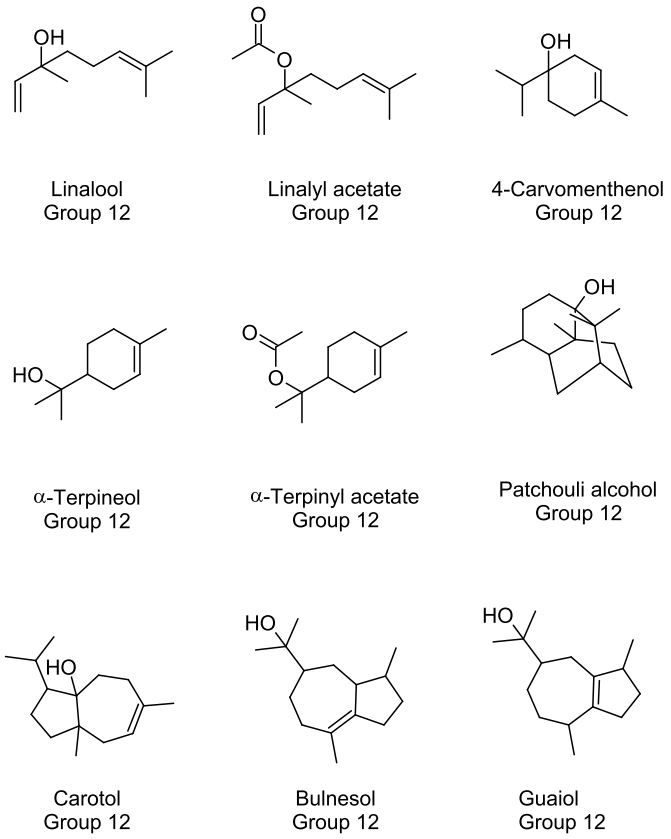


Fig. 1. Structures of commonly found Group 12 (Aliphatic and aromatic tertiary alcohols) constituents in the NFCs.

Another member of the *Apiaceae* family, coriander, also has a long history of food use, dating back to ancient India and Greece (Nadeem et al., 2013). In the Middle Ages, coriander was used as flavoring for meats and soups (Guenther, 1950). It is used in alcoholic beverages and is particularly important to the production of gin. While mainly cultivated in the eastern hemisphere, the herb was first introduced to North America by British colonists, and it later spread to South America (Guenther, 1950). In the United States, "coriander' typically refers to the seed of the plant that is used as a spice while the green leaves are commonly known as cilantro, while in Europe, the seed of the plant is known as coriander seed and the herby leaves are commonly known as coriander leaves.

A native of Asia, cardamom, a member of the *Zingiberaceae* or ginger family, was introduced to western civilizations via the spice trade. There are several varieties of cardamom that have historically been used as spices, but cardamom oil is derived from the species *Elettaria cardamomum*, or "true cardamom". Cardamom pods are the fruit of the plant and each pod encases brown and black seeds. It is an essential ingredient in many Indian dishes including garam masala and chai tea, and is frequently used in baked goods in Scandinavia (Korikanthimathum et al., 2001).

Several NFCs listed in Table 1 are derived from woody plants. Tea tree oil, produced by the distillation of the terminal branches and leaves of the plant, has historically been used as both flavoring and traditional medicine. In his voyage to Australia in the 18th century, Captain James Cook noted a shrub from which the leaves were used by his crew to brew tea. This species can be cultivated in sub-tropical areas of the world, including in its native Australia, as well as the United States, Zimbabwe, New Zealand, China and India (Colton and Murtagh, 1999; Southwell, 1999). *Bulnesia sarmietoi*, the tree species from which guaiac wood oil is derived, is native to South America, specifically the Chaco region of

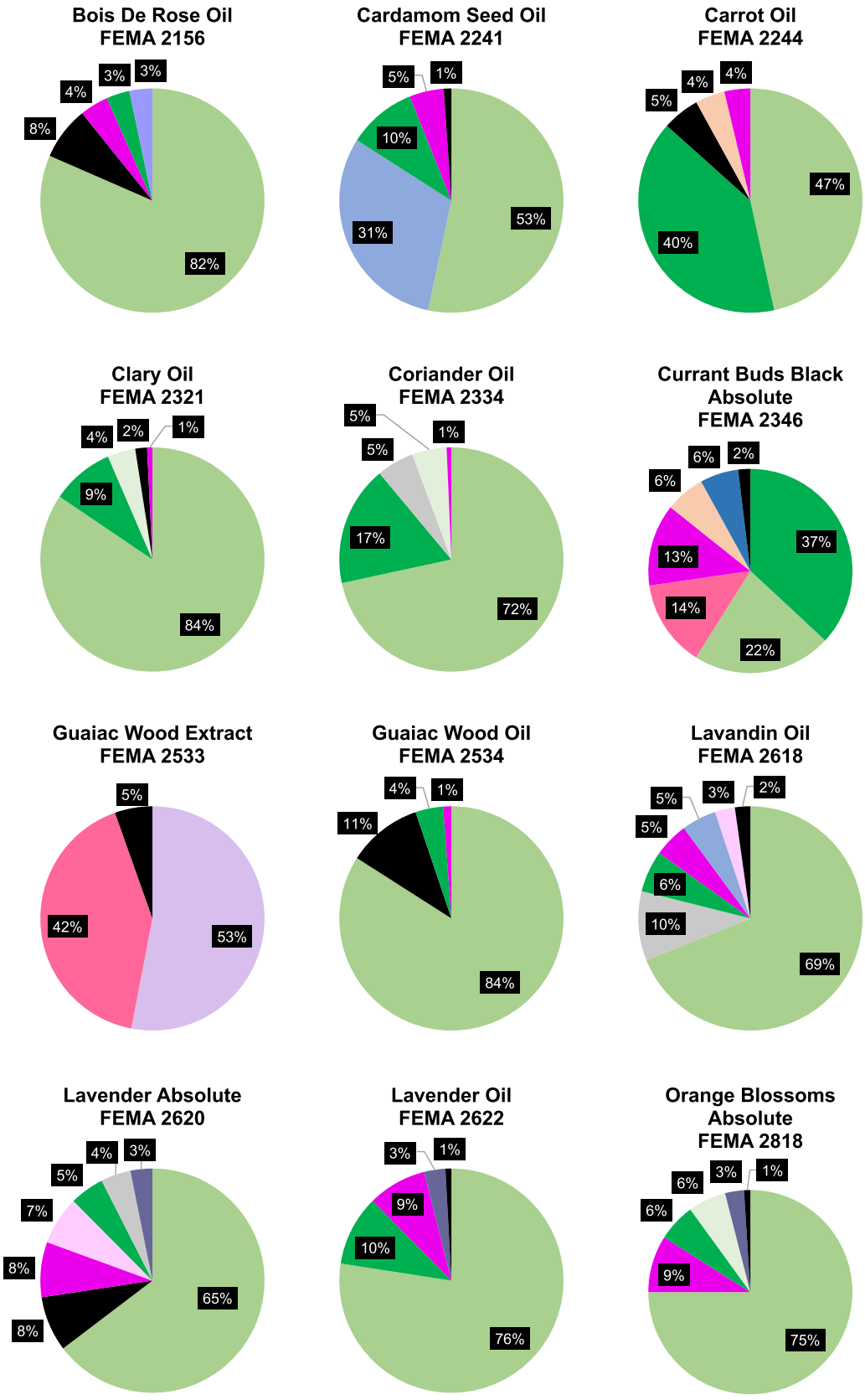


Fig. 2. Constituent congeneric group profiles for the NFCs under consideration.

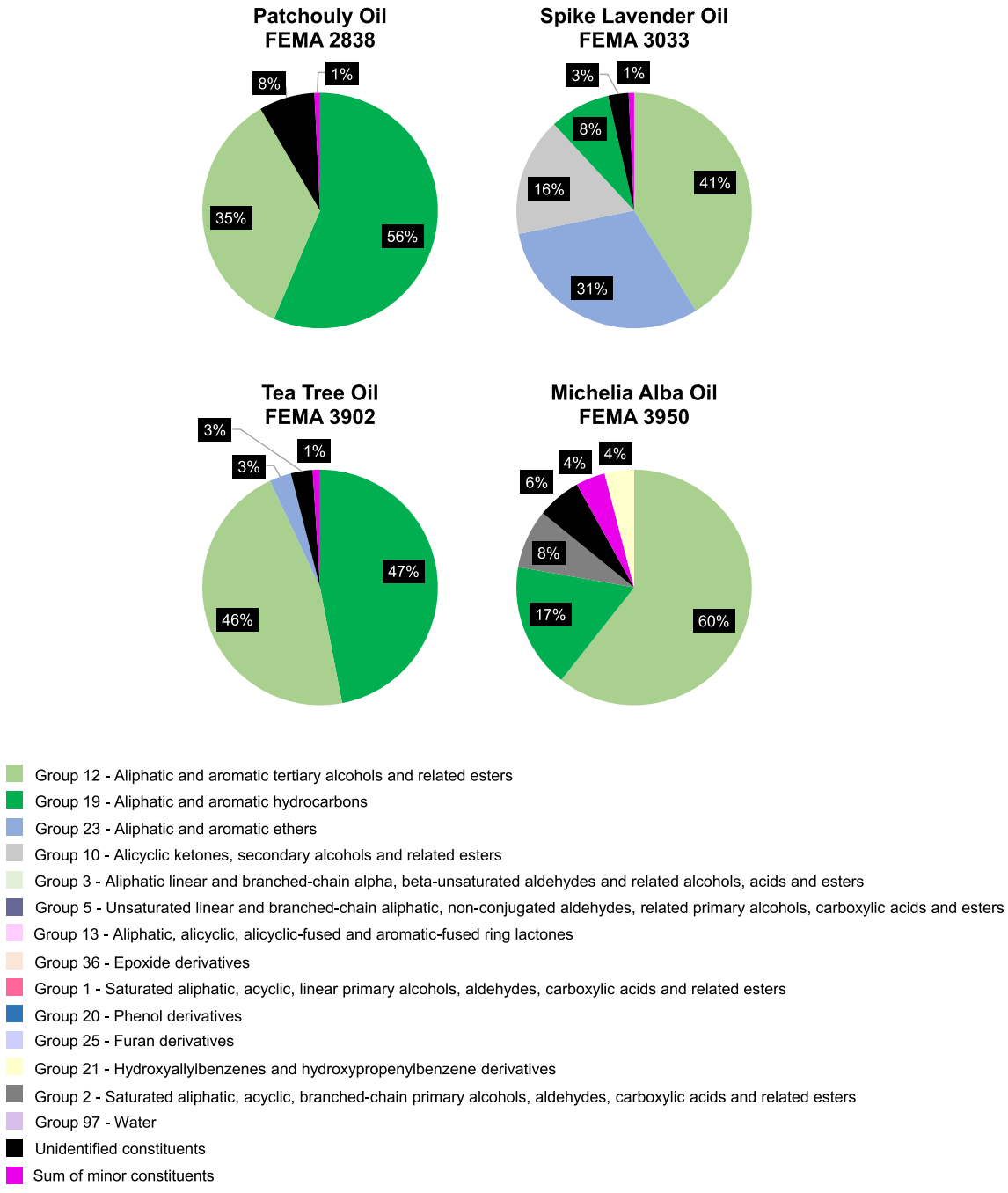


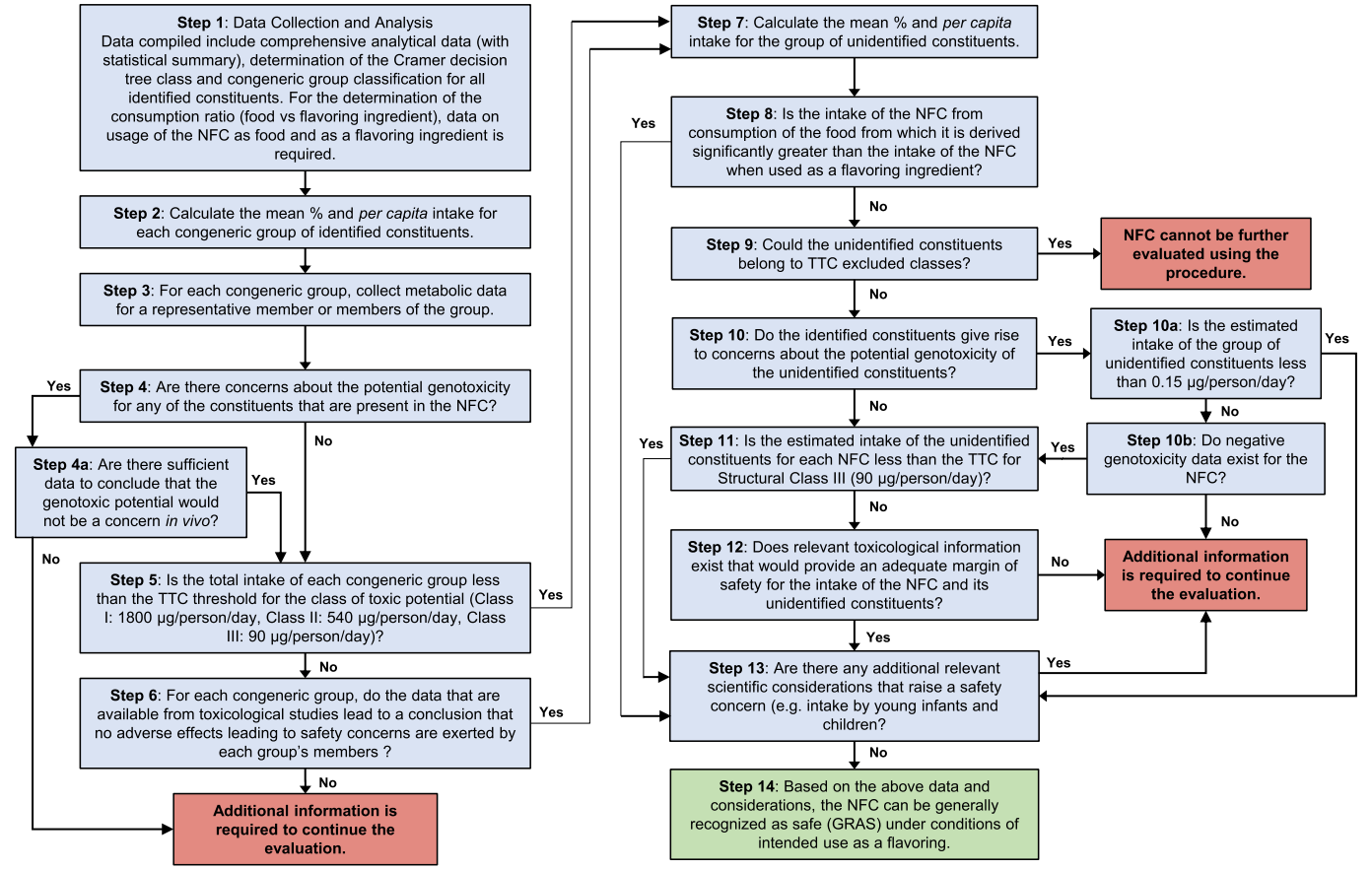
Fig. 2. (*continued*).

Argentina and Paraguay (Rodilla et al., 2011). The wood of the *Bulnesia* tree is very similar to that of the *Guaiacum* species, which is less available due to over-harvesting. The heartwood of rosewood trees, *Aniba rosaeodore,* is the source of bois de rose oil. (FAO, 1995; Fenaroli, 1975). *Aniba rosaeodore* originates in Brazil and is now cultivated in other regions.

3. Current usage

The NFCs listed in Table 1 are used in a variety of foods including beverages (both alcoholic and non-alcoholic), condiments, gravies, confectionary and others. Within this group of NFCs, Tea Tree Oil (FEMA 3902) has the highest annual reported volume of 32,300 kg. In contrast, NFCs of the *Lavandula* genus have more moderate annual usage ranging from 0.1 kg for Lavender Absolute (FEMA 2620) to 9780 kg for Lavandin Oil (FEMA 2618). Patchouly Oil (FEMA 2838), which is known for its distinctive aroma, had a reported use of 2120 kg in 2015. NFCs such as Currant Buds Black Absolute (FEMA 2346), Bois De Rose Oil (FEMA 2156), Carrot Oil (FEMA 2244), Clary Oil (FEMA 2321), *Michelia Alba* Oil (FEMA 3950), Guaiac Wood Extract (FEMA 2533), Guaiac Wood Oil (FEMA 2534) and Orange Blossoms Absolute (FEMA 2818) show low to moderate usage with annual volumes ranging from 11 to 1440 kg.

Both Coriander Seed Oil (FEMA 2334) and Cardamom Seed Oil



This scheme presents a summary of the revised procedure for the evaluation of NFCs to give an overall structural view. When applying the procedure, the full procedure described in the manuscript should be followed.

Fig. 3. Procedure for the safety evaluation of NFCs (Cohen et al., 2018a).

(FEMA 2241) are derived from botanicals commonly used as spices/food and have annual volumes of 11,500 and 3310 kg, respectively. The Economic Research Service (ERS) of the United States Department of Agriculture (USDA) compiles data on the yearly import of spices and reports that 5,850,000 kg of coriander seed was imported into the USA in 2015 (ERS/USDA, 2019). The essential oil content of coriander seed has been reported to be 0.3% (Nejad Ebrahimi et al., 2010), resulting in an estimated consumption of 17,550 kg of coriander seed oil from coriander seed in the USA. Because import data on cardamom seed are aggregated with other spices, a similar estimation of intake of the essential oil from the whole seed used as a spice cannot be made.

4. Manufacturing methodology

Species of the *Lavandula* genus, including lavender and lavandin, are cultivated from seed and are typically harvested following flowering (Denny, 2002). For the preparation of lavender essential oil, the freshly cut flowering tops, containing the ripe flowers and adjacent stems, are subsequently steam distilled, collecting the essential oil (Denny, 2002; Di Sotto et al., 2011; Lis-Balchin, 2002b). For another *Lavandula* species, spike lavender, the oil can also be prepared from the dried flowering tops of the plants (Boelens, 1986). Steam distillation in the fields limits handling and prevents exposure of the oils to air, which can lead to evaporation and loss of product (Denny, 2002). The essential oil of the fragrant flowers of the *Michelia alba* tree, cultivated in Southeast Asia, is also extracted by steam distillation (Pensuk et al., 2007).

Several other NFCs listed in Table 1 are prepared using distillation technology. Clary plants are harvested at an early maturation period, in which the plants are mechanically cut, and the flower heads, stems and select leaves are immediately chopped and collected in a tub. The contents of the collection tub are steam distilled to obtain the oil (Lawrence, 1994). The dried leaves of patchouly and of tea trees are both steam distillated to yield their respective oils (Southwell, 1999; Surburg and Panten, 2006). While carrot oil can be obtained from aerial parts of the plant after flowering, the flowering umbels are not used commercially for oil production (Jasicka-Misiak et al., 2004; Lawrence, 2003; Tawil et al., 2015). The seeds obtained from the umbels are crushed and steam distilled to extract the oil (Surburg and Panten, 2006). Cardamom seed oil is distilled from the seeds of the plant that are removed from the outer hull of the cardamom pod and crushed shortly before distillation (Menon and Sreekumar, 1994). Similarly, the partially dried fruits (seeds) of coriander are ground just prior to steam distillation or hydrodistillation to yield coriander seed oil (Anitescu et al., 1997; Fenaroli, 1975). Guaiac wood extract and guaiac wood oil, which come from either *Guaiacum* or *Bulnesia sarmientoi* trees, are derived from chipped wood or sawdust through solvent extraction and distillation, respectively (Rodilla et al., 2011). Bois de rose oil is similarly obtained from steam distillation of the chipped heartwood from *Aniba rosaeodora* variations (Farooqi and Sreeramu, 2004; Fenaroli, 1975; Ohashi et al., 1997).

Absolutes prepared from the flowers or buds of *L. officinalis*, *C. aurantium and Ribes nigrum* are also valuable flavoring ingredients. A "concrete" is prepared by the extraction of botanical material with a non-polar solvent such as hexane, toluene or petroleum ether (Surburg and Panten, 2006). Following this extraction, the solvent is removed resulting in a waxy material known as a concrete. Absolutes are

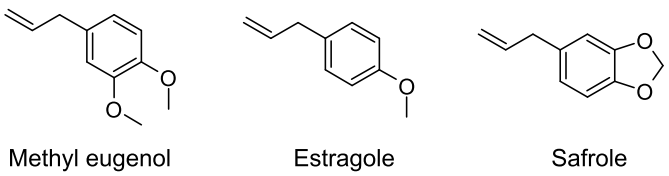


Fig. 4. Structures of methyl eugenol, estragole and safrole.

prepared from the concrete by mixing the concrete with ethanol, heating the solution, followed by a cooling step, filtering of the mixture to remove waxes and finally evaporating off the ethanol (Fenaroli, 1975; Lis-Balchin, 2002b). While botanically derived concretes and absolutes are used more commonly as perfumery ingredients, a few, such as lavender absolute, orange blossoms absolute and black currant buds absolute also have been historically used as flavor ingredients. Absolutes from orange blossoms and black currant buds are prepared from the carefully harvested flowers or dormant leaf buds, respectively, using this process (Boelens and Oporto, 1991; Fenaroli, 1975; Lawrence, 1997; Surburg and Panten, 2006).

5. Chemical composition

The compositions of the NFCs presented in Table 1 were determined by gas chromatography using mass spectrometry (GC-MS) to identify volatile constituents and a flame ionization (FID) or other general detector for quantitation. Identified and unidentified GC peaks were reported as the percent area of the chromatogram. For each NFC, the constituent data were collected and analyzed (Appendix A). In Appendix A, the constituents present at greater or equal to 1% are listed by their respective congeneric groups. The sum of the minor constituents is reported for each congeneric group and minor constituents (less than 1%) reported for other congeneric groups are summed and reported on the last line of the constituent table. The chemical structure of some common constituents of these NFCs are shown in Fig. 1.

The constituent profile for each NFC, summarized in the pie charts shown in Fig. 2, all show a large percentage of Group 12 (Aliphatic and aromatic tertiary alcohols and related esters) and Group 19 (Aliphatic and aromatic hydrocarbons) constituents, except for Guaiac Wood Extract which is an aqueous ethanolic solution. The primary Group 12 constituents for Coriander Seed Oil (FEMA 2334), *Michelia Alba* Oil (FEMA 3950), Clary Oil (FEMA 2321), Orange Blossoms Absolute (FEMA 2818) and the *Lavandula*-derived NFCs were linalool and linalyl acetate. Other Group 12 constituents include carotol in Carrot Oil (FEMA 2244), guaiol and bulnesol in Guaiac Wood Oil (FEMA 2534), patchouli alcohol in Patchouly Oil (FEMA 2838) and 4-carvomenthenol in Tea Tree Oil (FEMA 3902). Other congeneric groups represented in the constituent profiles of the NFCs under consideration include Group 23 (Aliphatic and aromatic ethers), Group 10 (Alicyclic ketones, secondary alcohols and related esters), Group 1 (Saturated aliphatic, acyclic, linear primary alcohols, aldehydes, carboxylic acids and related esters) and Group 3 (Aliphatic linear and branched-chain α,β-unsaturated aldehydes and related alcohols acids and esters).

6. Safety evaluation

The safety evaluation for NFCs was first described in two publications (Smith et al., 2004, 2005) and has been recently updated (Cohen et al., 2018a). The updated procedure is summarized in Fig. 3. Briefly, the NFC passes through a 14-step process: Step 1 requires the gathering of data and assesses the consumption of the NFC as a flavor relative to intake from the natural source when consumed as food; Steps 2 through 6 evaluate the exposure and potential toxicity of the identified constituents by application of the Threshold of Toxicological Concern (TTC) approach (Kroes et al., 2000)[1] and scientific data on metabolism and toxicity for each congeneric group; Steps 7-12 address the potential toxicity, including genotoxicity of the unidentified constituents; Step 13 evaluates the overall safety along with considerations of safety for use by children, given their lower body weights; Step 14 makes a determination of GRAS status. Below, the safety evaluation is presented in which each step of the procedure, as stated in Cohen et al. (2018a) and provided in italics, is considered and answered for the NFCs under consideration.

Step 1

To conduct a safety evaluation of an NFC, the Panel requires that comprehensive analytical data are provided. The analytical methodologies employed should reflect the expected composition of the NFC and provide data that identify, to the greatest extent possible, the constituents of the NFC and the levels (%) at which they are present. It is anticipated that GC-MS and LC-MS would be used for characterization of most NFCs, and that the chromatographic peaks based on peak area of total ion current will be almost completely identified. The percentage of unknowns should be low enough to not raise a safety concern. Other appropriate methods (e.g., Karl Fischer titration, amino acid analysis, etc.) should be employed as necessary. The analytical parameters should be submitted for each type of analysis, including the method of quantitation for both identified and unidentified constituents and libraries, databases and methodology employed for the identification of analytes. The Panel requires data from multiple batches to understand the inherent variability of the NFC.

a. Consumption of foods from which the NFCs are derived

Calculate the per capita daily intake (PCI) of the NFC based on the annual volume added to food.

*For NFCs with a reported volume of use greater than 22,*700 kg *(50,*000 lbs*), the intake may be calculated by assuming that consumption of the NFC is spread among the entire population, on a case-by-case basis. In these cases, the PCI is calculated as follows:*

$$PCI\ (\mu g\ /\ person\ /\ day) = \frac{annual\ volume\ in\ kg\ \times 10^9}{population\ \times\ CF\ \times\ 365\ days}$$

where:

*The annual volume of use of NFCs currently used as flavorings for food is reported in flavor industry surveys (*Gavin et al., 2008; Harman et al., 2013, 2018; Lucas et al., 1999*). A correction factor (CF) is used in the calculation to correct for possible incompleteness of the annual volume survey. For flavorings, including NFCs, that are undergoing GRAS re-evaluation, the CF, currently 0.8, is established based on the response rate from the most*

[1] In Step 5, the estimated intake for each congeneric group of the NFC is compared to the TTC threshold for the structural class of the group. TTC thresholds were determined for structural classes I, II and III based on the 5th percentiles of the NOAEL of each class with an additional 100-fold uncertainty factor, providing a highly conservative threshold for each class (Cramer et al., 1978; Munro et al., 1996; Kroes et al., 2000).

recently reported flavor industry volume-of-use surveys.

For new flavorings undergoing an initial GRAS evaluation, the anticipated volume is used and a correction factor of 0.6 is applied which is a conservative assumption that only 60% of the total anticipated volume is reported.

*For NFCs with a reported volume of use less than 22,*700 kg *(50,*000 lbs*), the eaters' population intake assumes that consumption of the NFC is distributed among only 10% of the entire population. In these cases, the per capita intake for assuming a 10% "eaters only" population (PCI × 10) is calculated as follows:*

$$\underset{(\mu g/person/day)}{Intake\ of\ congeneric\ group} = \frac{Mean\%\ congeneric\ group \times Intake\ of\ NFC\ (\mu g/person/day)}{100}$$

$$PCI \times 10\ (\mu g\ /\ person\ /\ day) = \frac{annual\ volume\ in\ kg \times 10^9}{population \times CF \times 365\ days} \times 10$$

*If applicable, estimate the intake resulting from consumption of the commonly consumed food from which the NFC is derived. The aspect of food use is particularly important. It determines whether intake of the NFC occurs predominantly from the food of which it is derived, or from the NFC itself when it is added as a flavoring ingredient (*Stofberg and Grundschober, 1987*).*[2] *At this step, if the conditions of use*[3] *for the NFC result in levels that differ from intake of the same constituents in the food source, it should be reported.*

Although several botanicals from which the NFCs in this set are derived have historically been used as spices or ingredients in food, quantitative data on their usage are generally not available, except for coriander seeds. For coriander seeds, the United States Department of Agriculture's Economic Research Service reports that 5,850,000 kg was imported into the USA in 2015 (ERS/USDA, 2019). Coriander seeds have an average essential oil content of 0.3% (Nejad Ebrahimi et al., 2010) resulting in an estimated 17,550 kg of coriander seed oil consumed from the consumption of coriander seed as a spice in the USA in 2015. This annual usage is higher than the 11,500 kg annual usage reported for Coriander Seed Oil (FEMA 2334) used as flavoring, reported in FEMA's 2015 survey (Harman et al., 2018).

Table 2
Natural occurrence and estimated intake of methyl eugenol, estragole or safrole from Guaiac Wood Extract (FEMA 2533), Tea Tree Oil (FEMA 3902) and *Michelia Alba* Oil (FEMA 3950).

NFC FEMA No.	NFC Description	Constituent of Concern	Mean %	Estimated Intake (μg/person/day)
2533	Guaiac Wood Extract	Estragole	0.001	0.001
2533	Guaiac Wood Extract	Methyl eugenol	0.001	0.001
3902	Tea Tree Oil	Methyl eugenol	0.01	0.03
3950	*Michelia Alba* Oil	Methyl eugenol	3	0.06
3950	*Michelia Alba* Oil	Estragole	0.3	0.006
3950	*Michelia Alba* Oil	Safrole	0.05	0.001

[2] See Stofberg and Grundschober (1987) for data on the consumption of NFCs from commonly consumed foods.

[3] The focus throughout this evaluation sequence is on the intake of the constituents of the NFC. To the extent that processing conditions, for example, alter the intake of constituents, those conditions of use need to be noted, and their consequences evaluated in arriving at the safety judgments that are the purpose of this procedure.

b. Identification of all known constituents and assignment of Cramer Decision Tree Class

*In this step, the results of the complete chemical analyses for each NFC are examined, and where appropriate for each constituent the Cramer Decision Tree Class (DTC) is determined (*Cramer et al., 1978*).*

All constituents identified in each NFC were sorted by congeneric group and a summary report for each NFC is provided in Appendix A.

Congeneric groups are recorded in order from highest to lowest mean %, with only mean % greater than or equal to 1% of the total NFC reported. Minor constituent percentages (<1% of the total NFC) are summed for the listed congeneric groups and the total mean % of each congeneric group is shown.

c. Assignment of the constituents to Congeneric groups; assignment of congeneric group DTC

In this step, the identified constituents are sorted by their structural features into congeneric groups. Each congeneric group should be expected, based on established data, to exhibit consistently similar rates and pathways of absorption, distribution, metabolism and excretion, and common toxicological endpoints (e.g. benzyl acetate, benzaldehyde, and benzoic acid are expected to have similar toxicological properties).

Assign a decision tree structural class to each congeneric group. Within a congeneric group, when there are multiple decision tree structural classes for individual constituents, the class of highest toxicological concern is assigned to the group. In cases where constituents do not belong to a congeneric group, potential safety concerns would be addressed in Step 13.

Proceed to Step 2.

For each NFC, the DTC for each identified congeneric group was determined and reported in Appendix A.

Step 2

Determine (a) the mean percentage (%) of each congeneric group in NFCs, and (b) the daily per capita intake[4] *of each congeneric group. (a) is calculated by summing the mean percentage of each of the constituents within a congeneric group, and (b) is calculated from consumption of the NFC and the mean percentage.*

Calculation of PCI for each constituent congeneric group of the NFC

where:

The mean % is the mean percentage % of the congeneric group.

The intake of NFC (μg/person/day) is calculated using the PCI × 10 or PCI equation as appropriate.

Proceed to Step 3.

The summary report for each NFC, provided in Appendix A, provides the subtotal mean % and estimated intake values (PCI × 10 or PCI, where appropriate) for each constituent congeneric group.

Step 3

For each congeneric group, collect metabolic data for a representative member or members of the group. Step 3 is critical in assessing whether the

[4] See Smith et al. (2005) for a discussion on the use of PCI × 10 for exposure calculations in the procedure.

Table 3
Data on Group 12 and 19 constituents for NFCs where the estimated intake of the congeneric group exceeds the relevant TTC.

Name (FEMA No.)	DTC[a]	Estimated Intake of CG (μg/p/day)	Estimated Intake of CG (mg/kg bw/day)	NOAEL (mg/kg bw/day)	MoS[b]
Congeneric Group 12 - Aliphatic and aromatic tertiary alcohols and related esters					
Lavandin Oil (FEMA 2618)	II	690	0.01	50	>4,300
Lavender Oil (FEMA 2622)	III	610	0.01	50	>4,900
Tea Tree Oil (FEMA 3902)	III	120	0.002	50	>25,000
Congeneric Group 19 - Aliphatic and aromatic hydrocarbons					
Patchouly Oil (FEMA 2838)	III	120	0.002	41	>20,000

[a] The DTC for each congeneric group is determined to be the most conservative DTC of the constituents reported in the respective group. Although CG12 is reported for both Lavandin Oil (FEMA 2618) and Tea Tree Oil (FEMA 3902), the reported CG12 constituents and their respective DTCs are different for these two NFCs.

[b] The MoS for Group 12 constituents is based the NOAEL determined from an 84-day study in which linalool was administered to rats of both sexes in the diet at 50 mg/kg bw/day (Oser, 1958). The MoS for Group 19 constituents in Patchouly Oil is based on a NOAEL of 41 mg/kg bw/day for patchouli oil, determined for both sexes, in the repeated dose portion of an OECD-compliant combined 28-day dietary and reproductive/developmental toxicity study in rats (Liwska, 2013b).

metabolism of the members of each congeneric group would require additional considerations in step 13 of the procedure.

Proceed to Step 4.

Appendix A lists the identified constituent congeneric groups for each NFC. A recent FEMA Expert Panel publication outlined the use of metabolic data in the safety evaluation of flavoring substances and provided a summary of the expected metabolism for each congeneric group (Smith et al., 2018). For the congeneric groups present in these NFCs, data exist on the constituents of the group or related compounds to conclude that the members of these respective congeneric groups are expected to be metabolized to innocuous products. Safety assessments, including metabolic data, for flavoring ingredients of several of the congeneric groups represented in the NFCs under consideration have been published by the FEMA Expert Panel, including assessments for Group 12 (Aliphatic and aromatic tertiary alcohols and related esters), Group 19 (Aliphatic and aromatic hydrocarbons) and Group 10 (Alicyclic ketones, secondary alcohols and related esters) flavoring ingredients (Adams et al., 1996, 2011; Marnett et al., 2014). In addition, the Panel has also published evaluations of other groups or individual constituents (Adams et al., 2004; Adams et al., 2005a, b, c; Adams et al., 2002; Adams et al., 1997; Adams et al., 2008; Adams et al., 1998; Adams et al., 2007; Newberne et al., 1999).

Step 4

Are there concerns about potential genotoxicity for any of the constituents that are present in the NFC?

If Yes, proceed to Step 4a.

If No, proceed to Step 5.

With the exception of Guaiac Wood Extract (FEMA 2533), Tea Tree Oil (FEMA 3902) and *Michelia Alba* Oil (FEMA 3950), the identified constituents of the NFCs do not present a genotoxic concern. In its review of *in vitro* and *in vivo* genotoxicity studies for Group 12 (Aliphatic and aromatic tertiary alcohols and related esters) flavoring ingredients, the primary congeneric group constituent of the NFCs under consideration, the FEMA Expert Panel determined a lack of genotoxic potential for these and related compounds (Marnett et al., 2014). A lack of genotoxic potential was also determined for the other major constituent groups reported in the NFCs under consideration, Group 19 (Aliphatic and aromatic hydrocarbons) and Group 10 (Alicyclic ketones, secondary alcohols and related esters) flavoring ingredients (Adams et al., 1996, 2011). More recent genotoxicity studies on Group 12 constituents and the NFCs are summarized in Table 6 and described later under "Biochemical and Toxicological Supporting Information Relevant to the Safety Evaluation" section of this manuscript. A review of the minor constituent profile of Bois de Rose Oil (FEMA 2156), Cardamom Seed Oil (FEMA 2241), Carrot Oil (FEMA 2244), Clary Oil (FEMA 2321), Coriander Seed Oil (FEMA 2334), Currant Buds Black Absolute (FEMA 2346), Guaiac Wood Oil (FEMA 2534), Lavandin Oil (FEMA 2618), Lavender Absolute (FEMA 2620), Lavender Oil (FEMA 2622), Orange Blossoms Absolute (FEMA 2818), Patchouly Oil (FEMA 2838), and Spike Lavender Oil (FEMA 3033) also indicates no genotoxic concern for the congeneric groups presented. These NFCs proceed to Step 5.

Guaiac Wood Extract (FEMA 2533), Tea Tree Oil (FEMA 3902) and *Michelia Alba* Oil (FEMA 3950), contain Group 21 (Hydroxy- and alkoxy-substituted propenyl benzenes) constituents methyl eugenol, estragole and safrole which have an allylalkoxybenzene structural motif (see Fig. 4), raising a genotoxicity concern (Rietjens et al., 2014a). All three can be found naturally occurring in *Michelia Alba Oil* (FEMA 3950) at concentrations of 3, 0.3 and 0.05%, respectively, estragole and methyl eugenol are reported in Guaiac Wood Extract (FEMA 2533) (0.001%), while only methyl eugenol is found naturally to occur in Tea Tree Oil (FEMA 3902) at a low concentration (0.01%). The natural occurrence and estimated intakes for the constituents of concern in these NFCs are shown in Table 2. Guaiac Wood Extract (FEMA 2533), Tea Tree Oil (FEMA 3902) and *Michelia Alba* Oil (FEMA 3950) proceed to Step 4a.

Step 4a

Are there sufficient data to conclude that the genotoxic potential would not be a concern in vivo?

If Yes, proceed to Step 5.

If No, additional information is required to continue the evaluation.

Table 4
Estimated Intake of unidentified constituents.

Name	FEMA No.	Estimated Intake(μg/person/day)
Bois De Rose Oil	2156	0.5
Cardamom Seed Oil	2241	3
Carrot Oil	2244	1
Clary Oil	2321	0.2
Coriander Seed Oil	2334	0
Currant Buds Black Absolute	2346	0.2
Guaiac Wood Extract	2533	0.05
Guaiac Wood Oil	2534	16
Lavandin Oil	2618	20
Lavender Absolute	2620	0.0008
Lavender Oil	2622	8
Orange Blossoms Absolute	2818	0.07
Patchouly Oil	2838	17
Spike Lavender Oil	3033	0.1
Tea Tree Oil	3902	9
Michelia Alba Oil	3950	0.1

The structures of methyl eugenol, estragole and safrole (see Fig. 4) share a motif of a benzene ring substituted with an alkoxy group located *para* to a 2-propenyl substituent. Cytochrome P450s catalyze the formation of 1′-hydroxy metabolites of these allylalkoxybenzene compounds which may be sulfated by a sulfotransferase. The subsequent elimination of sulfate creates a DNA reactive species (Daimon et al., 1997; Herrmann et al., 2012, 2014; Jeurissen et al., 2004, 2007; Phillips

et al., 1984; Randerath et al., 1984; Rietjens et al., 2005, 2014b; Ueng et al., 2004; Wiseman et al., 1987). Rodent studies have indicated that estragole, safrole and methyl eugenol are hepatocarcinogens at high dose levels (Abbott et al., 1961; Homburger et al., 1965; Homburger et al., 1962; Long et al., 1963; Miller et al., 1983; NTP, 2000).

The direct addition of safrole to food is prohibited in the USA (21 CFR §189.180) and the addition of safrole, estragole and methyl eugenol as such to food is prohibited in the European Union and limits have been set for the presence of each in finished food categories (European Commission, 2008). In 2018, the FEMA Expert Panel removed methyl eugenol from the FEMA GRAS list, citing the need for additional data to clarify the relevance of DNA adducts formed by methyl eugenol in humans (Cohen et al., 2018b). Later, in October 2018, FDA's food additive regulations were amended to no longer authorize the use of methyl eugenol as synthetic flavoring substances and adjuvants for use in food (83 Fed. Reg. 50490.October 9, 2018) in response to a food additive petition. The FDA explained that it had based its decision "as a matter of law" on the "extraordinarily rigid" Delaney Clause of the Federal Food, Drug, and Cosmetic Act and further noted that based on the data evaluated, that "it is unlikely that consumption of methyl eugenol presents a risk to the public health from use as a flavoring substance" (83 Fed. Reg. 50490.October 9, 2018).

Estragole, methyl eugenol and safrole, however, are naturally occurring constituents in common culinary herbs and spices such as basil, tarragon, allspice, cinnamon, anise, nutmeg and mace as well as Guaiac Wood Extract (FEMA 2533), Tea Tree Oil (FEMA 3902) and *Michelia Alba* Oil (FEMA 3950). Regarding the natural occurrence of methyl eugenol in herbs, spices and their essential oils and extracts, the FEMA Expert Panel stated, "that these flavorings continue to meet the criteria for FEMA GRAS under their conditions of intended use as flavorings" (Cohen et al., 2018b). In its decision to amend the food additive regulations permitting the addition of synthetic methyl eugenol to food, the FDA states "… there is nothing in the data FDA has reviewed in responding to the pending food additive petition that causes FDA concern about the safety of foods that contain natural counterparts or extracts from such foods" (83 Fed. Reg. 50490.October 9, 2018). Similarly, the European Union has established maximum levels for estragole, methyl eugenol and safrole in finished foods that have been flavored with flavorings and food ingredients in which these constituents occur naturally (European Commission, 2008).

As presented in Table 2, the estimated intakes of methyl eugenol, estragole and safrole from the consumption of Guaiac Wood Extract (FEMA 2533), Tea Tree Oil (FEMA 3902) and *Michelia Alba* Oil (FEMA 3950) are low, ranging from 0.001 to 0.06 μg/person/day. These values are below the TTC of 0.15 μg/person/day for compounds with structural alerts for genotoxicity as originally stated by Kroes et al. in 2004 (Kroes et al., 2004). This value was determined based on an analysis of the dose-response data for carcinogenic compounds, provided by the Gold database on carcinogens presenting the dose giving a 50% tumor incidence (TD_{50}) (Gold et al., 1984; Kroes et al., 2004). By linear extrapolation of these TD_{50} data to a 1 in 10^6 tumor incidence, an exposure level or TTC at which the lifetime risk of cancer was 1 in 10^6 was determined to be 0.15 μg/person/day (Kroes et al., 2004). In a recent EFSA/WHO review of the TTC approach, a 0.15 μg/person/day threshold was proposed and considered sufficiently protective for compounds with structural alerts for genotoxicity with the exclusion of high potency carcinogens (the Cohort of Concern) specified by Kroes and co-workers (EFSA/WHO, 2016; Kroes et al., 2004; Nohmi, 2018). Because the estimated intake for each of the constituents of concern for Guaiac Wood Extract (FEMA 2533), *Michelia Alb*a Oil (FEMA 3950) and Tea Tree Oil (FEMA 3902) listed in Table 2 is below the 0.15 μg/person/day TTC for compounds with structural alerts for genotoxicity, the constituents do not raise a safety concern and these NFCs proceed to Step 5.

Table 5
Estimated Intake and mean % of coumarin in NFCs.

FEMA No.	NFC	Mean %	Estimated Intake of Coumarin (μg/person/day)
2618	Lavandin Oil	3	30
2620	Lavender Absolute	4	0.0004
2622	Lavender Oil	0.01	0.08

Step 5

Is the total intake of the congeneric group less than the TTC for the class of toxic potential assigned to the group (i.e., Class I: 1800 μg*/person/day, Class II:* 540 μg*/person/day, Class III:* 90 μg*/person/day) (*Kroes et al., 2000; Munro et al., 1996*)? For congeneric groups that contain members of different structural classes, the class of highest toxicological concern is selected.*

If Yes, proceed to Step 7.

If No, proceed to Step 6.

The estimated intakes for all reported congeneric groups present in Bois De Rose Oil (FEMA 2156), Cardamom Seed Oil (FEMA 2241), Carrot Oil (FEMA 2244), Clary Oil (FEMA 2321), Coriander Seed Oil (FEMA 2334), Currant Buds Black Absolute (FEMA 2346), Guaiac Wood Extract (FEMA 2533), Guaiac Wood Oil (FEMA 2534), Lavender Absolute (FEMA 2620), Orange Blossoms Absolute (FEMA 2818), Spike Lavender Oil (FEMA 3033) and *Michelia Alba* Oil (FEMA 3950) are below the TTC for their respective structural classes. These NFCs proceed to Step 7. The remaining NFCs, Lavandin Oil (FEMA 2618), Lavender Oil (FEMA 2622), Patchouly Oil (FEMA 2838) and Tea Tree Oil (FEMA 3902), each have one congeneric group for which the estimated intake exceeds the relevant TTC (see Table 3), and the evaluation of these NFCs proceeds to Step 6.

Step 6

For each congeneric group, do the data that are available from toxicological studies lead to a conclusion that no adverse effects leading to safety concerns are exerted by each group's members?

This question can commonly be answered by considering the database of relevant metabolic and toxicological data that exist for a representative member or members of the congeneric group, or the NFC itself. A comprehensive safety evaluation of the congeneric group and a sufficient margin of safety (MoS) based on the data available is to be determined on a case-by-case basis. Examples of factors that contribute to the determination of a safety margin include 1) species differences, 2) inter-individual variation, 3) the extent of natural occurrence of each of the constituents of the congeneric group throughout the food supply, 4) the nature and concentration of constituents in related botanical genera and species. Although natural occurrence is no guarantee of safety, if exposure to the intentionally added constituent is trivial compared to intake of the constituent from consumption of food, then this should be taken into consideration in the safety evaluation (Kroes et al., 2000).

If Yes, proceed to Step 7.

If No, additional information is required to continue the evaluation.

For Lavandin Oil (FEMA 2618), Lavender Oil (FEMA 2622) and Tea Tree Oil (FEMA 3902), the margin of safety (MoS) is calculated for Group 12 (Aliphatic and aromatic tertiary alcohols and related esters) constituents and shown in Table 3. The MoS for Group 12 constituents is based on the NOAEL derived from an 84-day study in which linalool was administered to rats of both sexes in the diet at 50 mg/kg bw/day (Oser, 1958). With the calculation of adequate MoS values for the NFCs with estimated intakes above the TTC for Congeneric Group 12, these NFCs proceed to Step 7.

The estimated intake of congeneric Group 19 (Aliphatic and aromatic hydrocarbons) exceeds the TTC in Patchouly Oil (FEMA 2838). A

review of toxicological studies was conducted for the GRAS re-affirmation of flavoring materials of this group (Adams et al., 2011) and more recently for the GRAS affirmation of *Citrus*-derived NFCs (Cohen et al., 2019). For Patchouly Oil (FEMA 2838), an adequate MoS was calculated based on a NOAEL of 41 mg/kg bw/day, determined for both sexes, in the repeated dose portion of an OECD-compliant combined 28-day dietary and reproductive/developmental toxicity study for patchouli oil in rats (Liwska, 2013b), as shown in Table 3. With the determination of an adequate MoS, this NFC proceeds to Step 7.

Step 7

Calculate the mean percentage (%) for the group of unidentified constituents of unknown structure in each NFC (as noted in Step 1) and determine the daily per capita intake (PCI or PCI × 10) for this group.

Proceed to Step 8.

The daily *per capita* intakes for the group of unidentified constituents reported for each NFC are listed below in Table 4 and in Appendix A.

Step 8

Using the data from Step 1, is the intake of the NFC from consumption of the food[5] from which it is derived significantly greater than the intake of the NFC when used as a flavoring ingredient?

If Yes, proceed to Step 13.

If No, proceed to Step 9.

No. For the NFCs under consideration, except for Coriander Seed Oil (FEMA 2334), consumption as food/spice cannot be determined or is unlikely and therefore all the NFCs proceed to Step 9. In the case of Coriander Seed Oil (FEMA 2334), it is estimated that consumption of coriander seed oil from food is less than two times greater than the volume of Coriander Seed Oil (FEMA 2334). Based on this ratio, the consumption of coriander seed oil from food cannot be considered significantly higher than consumption as added flavoring and as a result, Coriander Seed Oil (FEMA 2334) also proceeds to Step 9.

Step 9

Could the unidentified constituents belong to TTC-excluded classes?[6] The excluded classes are defined as high potency carcinogens, certain inorganic substances, metals and organometallics, certain proteins, steroids known or predicted bio-accumulators, nanomaterials, and radioactive materials (EFSA, 2016; Kroes et al., 2004).

If Yes, the NFC is not appropriate for consideration via this procedure.

If No, proceed to Step 10.

No. As previously discussed, this group of NFCs are collected from various flowers, seeds, leaves and woody plant fibers by either steam distillation of solvent extraction. The oils are primarily composed of low molecular weight monoterpenoid and sesquiterpenoid alcohols, esters and hydrocarbons. Based on the identified constituents, production methods and current literature, it is not expected that the unidentified constituents would belong to TTC-excluded classes. Proceed to Step 10.

Step 10

Do the identified constituents give rise to concerns about the potential genotoxicity of the unidentified constituents?

If Yes, proceed to Step 10a.

If No, proceed to Step 11.

For the NFCs listed in Table 4, with the exception of Guaiac Wood Extract (FEMA 2533), *Michelia Alba* Oil (FEMA 3950) and Tea Tree Oil (FEMA 3902), the identified constituent profile does not give rise to concern about the potential genotoxicity of the unidentified constituents. These NFCs are primarily composed of linalool, linalyl acetate, 4-carvomenthenol and other Group 12 constituents, Group 19 (Aliphatic and aromatic hydrocarbons) constituents and other terpenoid pathway products that lack genotoxic potential (Adams et al., 2011; Marnett et al., 2014). The unidentified constituents are likely to belong to these groups and to not exhibit genotoxic potential. A review of available genotoxicity studies on the NFCs are presented later in this manuscript. These studies reported no evidence of genotoxic potential for these NFCs. These NFCs proceed to Step 11.

In Step 4, the occurrence of genotoxins estragole, methyl eugenol and safrole were reported in small amounts in *Michelia Alba* Oil (FEMA 3950), estragole and methyl eugenol were reported in small amounts in Guaiac Wood Extract (FEMA 2533), and a small amount of methyl eugenol was reported in Tea Tree Oil (FEMA 3902). The intake for these constituents was estimated to be less than the TTC of 0.15 μg/person/day for compounds with a structural alert for genotoxicity and thus do not raise a safety concern. Allylalkoxybenzene compounds such as estragole, methyl eugenol, safrole, myristicin and elemicin are represented in current mass spectral libraries and are readily detected and identified by GC-MS instruments. Consequently, these compounds will only be part of the unidentified fraction when they occur at concentrations below the limit of detection. For this reason, in addition to a lack of other reports of the occurrence of allylalkoxybenzenes in Guaiac Wood Extract (FEMA 2533), *Michelia Alba* Oil (FEMA 3950) and Tea Tree Oil (FEMA 3902), the FEMA Expert Panel determined that these compounds are unlikely to be present in the unidentified constituent fraction and that there is not a genotoxic concern for the unidentified constituents. Proceed to Step 11.

Step 10a

*Is the estimated intake of the group of unidentified constituents less than 0.*15 μg*/person/day (Koster* et al.*, 2011*; *Rulis, 1989)? A TTC of 0.*15 μg*/person/day has been proposed for potentially genotoxic substances that are not from the TTC-excluded classes (Kroes* et al.*, 2004).*

If Yes, proceed to Step 13.

If No, proceed to Step 10b.

Not required.

Step 10b

Do negative genotoxicity data exist for the NFC?

If Yes, proceed to Step 11.

If No, retain for further evaluation, which would include the collecting of data from appropriate genotoxicity tests, obtaining further analytical data to reduce the fraction of unidentified constituents, and/or considering toxicity data for other NFCs having a similar composition. When additional data are available, the NFC could be reconsidered for further evaluation.

Not required.

Step 11

Is the estimated intake of the unidentified constituents (calculated in Step

[5] Provided the intake of the unidentified constituents is greater from consumption of the food itself, the intake of unidentified constituents from the added NFC is considered trivial.

[6] This can be based on arguments including: Expert judgement; Nature of the identified ingredients; Knowledge on the production/extraction process (see also Koster et al. (2011) and EFSA (2016)).

Table 6
Summary of genotoxicity study results.

Name of Substance Tested	Test Type (System)	Doses Tested	Results	Reference
a. Tertiary Alcohol Constituents				
Linalool	Reverse mutation in *S. typhimurium* and *E. coli*[a]	1.6–1580 μg/plate	Negative[a]	Slonina (2019)
Linalool	Reverse mutation in *S. typhimurium* and *E. coli*[a]	90–170 μg/plate	Negative[a]	Di Sotto et al. (2008)
Linalool	Reverse mutation in *S. typhimurium*[a]	0.23–1.8 mg/plate	Negative[a]	Beric et al. (2008)
Linalool	*In vitro* micronucleus in human lymphocytes	0.5–300 μL/mL	Negative	Di Sotto et al. (2011)
Linalool	*In vitro* chromosomal aberration in Chinese hamster ovary cells[a]	100–400 nL/mL[b] 100–500 nL/mL[c]	Negative[a]	Galloway (1983)
Linalool	Forward mutation in L5178Y mouse lymphoma cells[a]	12.5–500 μL/mL (test 1) 25–399 μL/mL (test 2)	Negative[a]	Cifone (1994)
Linalool	*In vivo* comet assay – forebrain tissue and peripheral blood of mice	10, 50, 100 and 200 mg/kg i.p.	Negative	Coelho et al. (2013)
Linalyl acetate	Reverse mutation in *S. typhimurium* and *E. coli*[a]	1.7–9000 μg/plate	Negative[a] (*S. typhimurium*) Positive at conc. greater than 3200 μg/plate[a] (*E. coli*)	Di Sotto et al. (2008)
Linalyl acetate	Reverse mutation in *S. typhimurium*[a]	20–5000 μg/plate (test 1) 20–2000 μg/plate (test 2) 3–50 μg/plate (test 3)	Negative[a]	ECHA (2019a)
Linalyl acetate	*In vitro* micronucleus in human lymphocytes	0.5–300 μL/mL	Positive	Di Sotto et al. (2011)
4-Carvomenthenol	Reverse mutation in *S. typhimurium*[a]	16–5000 μg/plate (test 1, 2)	Negative[a]	Scheerbaum (2001)
4-Carvomenthenol	*In vitro* micronucleus in human lymphocytes[a]	10–90 μg/mL[a,e] 0.5–1540 μg/mL[b,f]	Negative[a]	Roy, 2015
α-Terpineol	Reverse mutation in *S. typhimurium* and *E. coli*[a]	1.58–5000 μg/plate (test 1, 2)	Negative[a]	Rao (2019)
α-Terpineol	Reverse mutation in *S. typhimurium*[a]	10–1000 μg/mL	Negative[a]	Seifried et al. (2006)
α-Terpineol	Forward mutation in L5178Y mouse lymphoma cells[a]	0.14–0.65 μg/mL	Negative[a]	Seifried et al. (2006)
Terpineol (isomeric mixture)	Reverse mutation in *S. typhimurium and E. coli*[a]	9.77–1250 μg/plate	Negative[a]	ECHA (2013b)
Terpineol (isomeric mixture)	*In vitro* chromosomal aberration in human lymphocytes[a]	350, 425, 450 μg/mL[c,e] 300–650 μg/mL[b,e] 75, 200, 225 μg/mL[b,f]	Negative[a]	ECHA (2010)
Terpineol (isomeric mixture)	*In vitro* chromosomal aberration in Chinese hamster lung cells[a]	100–400 μg/mL[c,e] 100–500 μg/mL[b,e] 100–400 μg/mL[b,f]	Negative[a]	ECHA (2013a)
Terpinyl acetate (isomeric mixture)	*In vitro* micronucleus in human lymphocytes[a]	103–300 μg/mL[c,e] 49.4–175 μg/mL[b,e] 17.2–83.7 μg/mL[b,f]	Negative[a]	Bhalli (2015)
α-Terpinyl acetate	Reverse mutation in *S. typhimurium* and *E. coli*[a]	21–5000 μg/plate (test 1) 3.13–250 μg/plate (test 1)	Negative[a]	van den Wijngaard (2012)
α-Terpinyl acetate	Reverse mutation in *S. typhimurium* and *E. coli*[a]	5–5000 μg/plate (test 1) 1.6–5000 μg/plate (test 2)	Negative[a]	Bhalli (2014a)
α-Terpinyl acetate	*In vitro* micronucleus in human lymphocytes[a]	96.9–225 μg/mL[a,e] (test 1) 27.9–80 μg/mL[b,f] (test 1) 50–225 μg/mL[c,e] (test 2)	Negative[a]	Bhalli (2014b)
Patchouli alcohol	Reverse mutation in *S. typhimurium*[a] and *E. coli*[c]	1.6–500 μg/plate[a] (*S. typhimurium*) 16–5000 μg/plate[c] (*E. coli*)	Negative[a]	Bhalli (2014c)
Patchouli alcohol	*In vitro* micronucleus in human lymphocytes[a]	113–550 μg/mL[a,e] (test 1) 20.3–150 μg/mL[a,e] (test 2,3) 39.5–192 μg/mL[b,f]	Negative[a]	Bhalli (2014d)
a. Natural Flavor Complexes				
Bois de rose oil	Reverse mutation in *S. typhimurium* and *E. coli*[a]	1.6–5000 μg/plate (test 1) 5–1600 μg/plate (test 2)	Negative[a]	Mee (2017)
Bois de rose oil	*In vitro* micronucleus in human lymphocytes[a]	466.5–620.9 μg/mL[c,e] 491.1–568.5 μg/mL[b,e] 117.1–263.4 μg/mL[b,f]	Negative[a]	Clare (2017)
Cardamom oil	Reverse mutation in *S. typhimurium*[a]	0.005–2.5 μL/plate	Negative[a]	(DeGraff, 1983b; Heck et al., 1989)
Cardamom oil	Reverse mutation in *S. typhimurium*[a]	0.04–2.34 μL/plate	Negative[a]	Brusick (1982)
Cardamom oil	Forward mutation in L5178Y mouse lymphoma cells[a]	Up to 112 μg/mL[b] Up to 233 μg/mL[c]	Negative[a]	(Cifone, 1982; Heck et al., 1989)
Cardamom oil	Unscheduled DNA synthesis in rat hepatocytes	50.4 mg/mL[d]	Negative	Heck et al. (1989)
Clary oil	Reverse mutation in *S. typhimurium* and *E. coli*[a]	1.6–5000 μg/plate (test 1) 5–1600 μg/plate (test 2)	Negative[a]	Mee (2016a)
Clary oil	Reverse mutation in *S. typhimurium*[a]	5000 μg/plate[d]	Negative[a]	Heck et al. (1989)
Clary oil	*In vitro* micronucleus in human lymphocytes	263.4–888.9 μg/mL[c,e] 34.68–117.1 μg/mL[b,e] 59.97–117.1 μg/mL[b,f]	Negative[a]	Mee (2016b)
Clary oil	Unscheduled DNA synthesis in rat hepatocytes	101 μg/mL[d]	Negative	Heck et al. (1989)
Clary oil	Rec assay in *B. subtilis*	10, 30 μg/disk	Negative	Zani et al. (1991)

(*continued on next page*)

Table 6 (*continued*)

Name of Substance Tested	Test Type (System)	Doses Tested	Results	Reference
Coriander seed oil	Reverse mutation in *S. typhimurium*[a]	0.01–5 μL/plate	Negative[a]	(DeGraff, 1983a; Heck et al., 1989)
Coriander seed oil	Reverse mutation in *S. typhimurium*[a]	2, 7 mg/plate	Negative[a]	Marcus and Lichtenstein (1982)
Coriander seed oil	Unscheduled DNA synthesis in rat hepatocytes	300 μg/mL[d]	Negative	Heck et al. (1989)
Coriander seed oil	Forward mutation in L5178Y mouse lymphoma cells[a]	10–160 nL/mL[b] 50–300 nL/mL[c]	Negative[a]	(Cifone, 1983; Heck et al., 1989)
Coriander seed oil	*In vitro* chromosomal aberration in Chinese hamster fibroblasts	0.125 mg/mL[d]	Negative	(Ishidate Jr. et al., 1984)
Coriander seed oil	Rec assay in *B. subtilis*	8 mg/disk	Positive[b] Negative[c]	Ueno et al. (1984)
Guaiac wood oil	Reverse mutation in *S. typhimurium* and *E. coli*[a]	1.6–5000 μg/plate (test 1) 5–1600 μg/plate (test 2)	Negative[a]	Mee (2016b)
Guaiac wood oil	*In vitro* micronucleus in human lymphocytes[a]	117.1–395.1 μg/mL[c,e] 84.84–102.7 μg/mL[b,e] 63.74–77.13 μg/mL[b,f]	Negative[a]	Mee (2016c)
Lavender oil	Reverse mutation in *S. typhimurium*	4.4, 8.8 ng/plate	Positive	Sivaswamy et al. (1991)
Lavender oil	Reverse mutation in *S. typhimurium* and *E. coli*[a]	1.5–5000 μg/plate (test 1) 5–5000 μg/plate (test 2)	Negative[a]	Dakoulas (2014)
Lavender oil	Reverse mutation in *S. typhimurium* and *E. coli*[a]	Up to 2780 μg/plate[a] (*S. typhimurium*) Up to 2500 μg/plate[a] (*E. coli*)	Negative[a]	Evandri et al. (2005)
Lavender oil	Reverse mutation in *S. typhimurium*[a]	87, 177, 443 μg/plate	Negative	De Martino et al. (2009)
Lavender oil	*In vitro* micronucleus in human lymphocytes	0.5–300 μg/mL	Positive[d]	Di Sotto et al. (2011)
Lavender oil	*In vitro* micronucleus in human lymphocytes[a]	50–450 μg/mL[c,e] 10–150 μg/mL[b,e] 10–125 μg/mL[b,f]	Negative[a]	Roy (2015b)
Patchouly oil	Reverse mutation in *S. typhimurium*[a]	0.5–50 μg/plate (test 1, 2)	Negative[a]	Jones (1988)
Patchouly oil	*In vitro* chromosomal aberration in Chinese hamster ovary cells[a]	1.6–50 μg/mL[a] (test 1) 12.5–75 μg/mL[c] (test 2) 7.5–60 μg/mL[c] (test 3)	Negative[a]	Brooker (1989)
Patchouly oil	*In vitro* chromosomal aberration in Chinese hamster ovary cells[a]	6–60 μg/mL[b] 50–90 μg/mL[c]	Negative[a]	Song (2009)
Patchouly oil	Forward mutation in L5178Y mouse lymphoma cells[a]	20–275 μg/mL[c,e] 0.5–50 μg/mL[b,e] 6–36 μg/mL[b,f]	Negative[a]	Kirby (2009)
Tea tree oil	Reverse mutation in *S. typhimurium* and *E. coli*[a]	Up to 2000 μg/plate	Negative[a]	Evandri et al. (2005)
Tea tree oil	Reverse mutation in *S. typhimurium*[a]	Up to 5000 μg/mL	Negative[a]	Fletcher et al. (2005)
Tea tree oil	*In vitro* micronucleus in human lymphocytes[a]	95, 182, 365 μg/mL	Negative[a]	Pereira et al. (2014)
Tea tree oil	*In vitro* chromosomal aberration in human lymphocytes[a]	95, 182, 365 μg/mL	Negative[a]	Pereira et al. (2014)

[a] In the absence and presence of an exogenous metabolic activation system.
[b] In the absence of S9.
[c] In the presence of S9.
[d] Highest inactive dose tested or lowest active dose tested.
[e] 3h or 4 h treatment.
[f] 24 h treatment.

Table 7
NFCs affirmed FEMA GRAS

FEMA No.	Name
2156	Bois De Rose Oil (*Aniba rosaeodore* Ducke)
2241	Cardamom Seed Oil (*Elettaria cardamomum* (L.) Maton)
2244	Carrot Oil (*Daucus carota* L.)
2321	Clary Oil (*Salvia sclarea* L.)
2334	Coriander Seed Oil (*Coriandrum sativum* L.)
2346	Currant Buds Black Absolute (*Ribes nigrum* L.)
2533	Guaiac Wood Extract (*Guaiacum officinale* L.; *G. sanctum* L.; *Bulnesia sarmienti* Lorentz)
2534	Guaiac Wood Oil (*Guaiacum officinale* L.; *G. sanctum* L.; *Bulnesia sarmienti* Lorentz)
2618	Lavandin Oil (Hybrids between *Lavandula officinalis* Chaix *and L. latifolia* Viil.)
2620	Lavender Absolute (*Lavandula officinalis* Chaix)
2622	Lavender Oil (*Lavandula officinalis* Chaix)
2818	Orange Blossom Absolute (*Citrus aurantium* L.)
2838	Patchouly Oil (*Pogostemon cablin* Benth. and *P. heyneanus* Benth.)
3033	Spike Lavender Oil (*Lavandula latifolia* Vill. (*L. spica* DC.)
3902	Tea Tree Oil (*Melaleuca alternifolia*)
3950	*Michelia Alba* Oil (*Michelia alba* D.C.)

*7) less than the TTC (*Kroes et al., 2000; Munro et al., 1996*) for Structural Class III (*90 μg*/person/day)?*[7]

If Yes, proceed to Step 13.

If No, proceed to Step 12.

Yes, as shown in Table 4, the estimated intake of the fraction of unidentified constituents for each of NFC under consideration does not exceed the TTC for Structural Class III, 90 μg/person/day. These NFCs

[7] The human exposure threshold of 90 μg/person/day is determined from a database of NOAELs obtained from 448 subchronic and chronic studies of substances of the highest toxic potential (Structural Class III) mainly herbicides, pesticides and pharmacologically active substances (Munro et al., 1996). The 5th percentile NOAEL (lowest 5%) was determined to be 0.15 mg/kg bw/day which upon incorporation of a 100-fold safety factor for a 60 kg person yielded a human exposure threshold of the 90 μg/person/day. However, no flavoring substance or food additive in this structural class exhibited a NOAEL less than 25 mg/kg bw/d. Therefore the 90 μg/person/day threshold is an extremely conservative threshold for the types of substances expected in natural flavoring complexes. Additional data on other specific toxic endpoints (*e.g.,* neurotoxicity, reproductive and endocrine disruption) support the use of this threshold value (Kroes et al., 2000).

proceed to Step 13.

Step 12

Does relevant toxicological information exist that would provide an adequate margin of safety for the intake of the NFC and its unidentified constituents?

This question may be addressed by considering data for the NFC or an NFC with similar composition. It may have to be considered further on a case-by-case basis, particularly for NFCs with primarily non-volatile constituents.

If Yes, proceed to Step 13.

If No, perform appropriate toxicity tests or obtain further analytical data to reduce the fraction of unidentified constituents. Resubmit for further evaluation.

Not required.

Step 13

Are there any additional relevant scientific considerations that raise a safety concern (e.g. intake by young infants and children)?

If Yes, acquire and evaluate additional data required to address the concern before proceeding to Step 14.

If No, proceed to Step 14.

Small percentages of naturally occurring coumarin have been identified in Lavandin Oil (FEMA 2618), Lavender Absolute (FEMA 2620) and Lavender Oil (FEMA 2622). The intake of coumarin for each of these NFCs is presented in Table 5. In 1954, the US Food and Drug Administration prohibited coumarin, a naturally occurring constituent of tonka beans as well as *Cinnamomum cassia*, from use as an added flavor in foods (21 CFR 189.130). This restriction was implemented following the observation of hepatotoxic effects in dietary feeding studies of coumarin conducted in rats and dogs (Hazleton et al., 1956). Since the publication of the 1956 study, additional studies were performed and reported that investigate the hepatotoxicity observed in experimental animals, the metabolic pathways of coumarin and whether study findings in rodents are relevant to humans. Based on this work, which is also briefly summarized by the FEMA Expert Panel in a recent manuscript on *Cinnamomum* and *Myroxylon*-derived NFCs (Rietjens et al., 2020) the European Food Safety Authority (EFSA) has determined that coumarin is not an *in vivo* genotoxicant and that a threshold exists for the toxicity for coumarin (EFSA, 2004, 2008). Concurrently, EFSA established (and later maintained) a tolerable daily intake (TDI) of 0.1 mg/kg bw based on a NOAEL of 10 mg/kg bw/day for coumarin determined from a two-year feeding study in dogs and a safety factor of 100, in consideration of the potentially more vulnerable CYP2A6-deficient subpopulation that cannot metabolize coumarin efficiently (EFSA, 2004, 2008). In an expert opinion report commissioned by the German Federal Institute for Drugs and Medical Devices, the German Bundesinstitut für Risikobewertung (BfR, Federal Institute for Risk Assessment) concurred with EFSA's opinion that coumarin-induced hepatotoxicity occurs by a non-genotoxic mechanism and has a threshold. The risk assessment by the BfR was based on the lowest hepatotoxic exposure of coumarin reported in humans, 25 mg/day, and an uncertainty factor of 5 to derive an intake level at which no adverse effects would be observed, even in sensitive populations (Abraham et al., 2010; Bergmann, 1999). Using these parameters, a safe level of 5 mg/person/day was established and a rounded TDI of 0.1 mg/kg bw for a 60 kg adult was determined (Abraham et al., 2010). As presented in Table 5, the estimated intake of coumarin found naturally Lavandin Oil (FEMA 2618), Lavender Absolute (FEMA 2620) and Lavender Oil (FEMA 2622) is substantially below the TDI established by both EFSA and the German BfR. The TDI of 0.1 mg/kg bw is equivalent to an intake of 6000 μg/person/day for a 60 kg adult and 2000 μg/person/day for a 20 kg child. Therefore, the levels of coumarin in these NFCs do not raise a safety concern.

In addition, two furocoumarins, bergaptene and psoralen, were reported in Orange Blossom Absolute (FEMA 2818) at mean % values of 0.3 and 0.1%, respectively. Furocoumarins are a well-known group of natural food constituents known to occur in *Citrus* peel oils and foods, such as parsnips, carrots, parsley and celery (Dolan et al., 2010). Furocoumarins have both phototoxic and photomutagenic properties following exposure to UV light and thus the use of furocoumarin-containing materials in skin care and cosmetic products is regulated (Cosmetic Ingredient Review Expert Panel, 2016; Scientific Committee on Consumer Products, 2005). In consideration of the limited information on the typical intake of furocoumarins from food and their potential effects, regulatory bodies have not regulated dietary exposure to furocoumarin content from food. Opinions published by regulatory groups on the dietary exposure to furocoumarin were reviewed by the FEMA Expert Panel in its review of over 50 *Citrus* NFCs (Cohen et al., 2019). The Panel concurs with these opinions and concludes that the potential additional safety concerns arising from the extremely low level of furocoumarins present in *Citrus*-derived NFCs such as Orange Blossom Absolute (FEMA 2818) used as flavor ingredients does not present a safety concern under conditions of intended use.

A further evaluation to consider possible exposure of children and infants, given their lower body weights and the potential for differences in toxicokinetics and toxicodynamics as compared to adults, was conducted for each NFC evaluated. With the exception of Group 12 constituents of Lavandin Oil (FEMA 2618), Lavender Oil (FEMA 2622) and Patchouly Oil (FEMA 2838) and Group 19 constituents of Tea Tree Oil (FEMA 3902), the estimated intakes are substantially below the corresponding TTC for their respective groups, with none close to the TTC. For the congeneric groups that exceed the TTC in Lavandin Oil (FEMA 2618), Lavender Oil (FEMA 2622), Patchouly Oil (FEMA 2838) and Tea Tree Oil (FEMA 3902) listed in Table 3, adequate margins of safety were established that are protective at lower body weights. Table 4 lists the intake of the unknown constituent fraction, of which, none are close to the TTC value for Class III. Intakes well below the TTC for compounds with a structural alert for genotoxicity result from the reported low naturally occurring concentrations of methyl eugenol, estragole and safrole in *Michelia Alba* Oil (FEMA 3950) of estragole and methyl eugenol in Guaiac Wood Extract (FEMA 2533) and of methyl eugenol in Tea Tree Oil (FEMA 3902) as presented in Step 4a. Together, these results indicate the approach to be protective for consumption by children.

Step 14

Based on the above data and considerations, the NFC can be generally recognized as safe (GRAS) under conditions of intended use as a flavoring ingredient.

Yes. Based on the above assessment, the FEMA Expert Panel concluded that the current FEMA GRAS NFCs listed in Table 7 are affirmed as GRAS under conditions of intended use as flavoring substances.

7. Biochemical and Toxicological Supporting Information Relevant to the Safety Evaluation

Group 12 (Aliphatic and aromatic tertiary alcohols and related esters) dominates the constituent profiles of the NFCs described here. The FEMA Expert Panel has reviewed the safety of flavoring ingredients of this group (Marnett et al., 2014) as well as other dominant consitutent groups: Group 19 (Aliphatic and aromatic hydrocarbons) and Group 10 (Alicyclic ketones, secondary alcohols and related esters) (Adams et al., 1996, 2011). In addition, the Panel has also published evaluations of other groups or individual constituents (Adams et al., 2004; Adams et al., 2005a, b, c; Adams et al., 2002; Adams et al., 1997; Adams et al., 2008; Adams et al., 1998; Adams et al., 2007; Newberne et al., 1999).

The additional information presented here includes studies on the NFCs themselves, studies on the principal constituents of these NFCs and newly available studies on constituents not considered within the

reviews mentioned above. Studies concerning genotoxicity are summarized in Table 6.

7.1. Tertiary alcohol constituents

7.1.1. Linalool

In an OECD-compliant Ames assay, mutagenicity was not observed when linalool was tested at concentrations up to 1580 μg/plate in *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 and *Escherichia coli* WP2 *uvrA* both in the presence and absence of an S9 metabolic activation system derived from the liver of phenobarbital and benzoflavone treated rats (Slonina, 2019). In a bacterial reverse mutation assay in *S. typhimurium* strains TA98 and TA100 and *E. coli* WP2 *uvrA*, linalool was non-mutagenic when tested at concentrations up to 170 μg/plate, with and without rat liver S9 obtained from phenobarbital/β-naphthoflavone-treated rats (Di Sotto et al., 2008). An additional mutagenicity assay tested linalool in *S. typhimurium* strains TA98, TA100 and TA102 in the presence and absence of S9 activation. When tested up to 1.8 mg/plate, linalool was negative for inducing revertant mutant colonies (Beric et al., 2008). Linalool was also negative in a human lymphocyte micronucleus assay, where micronucleus induction was not observed when linalool was incubated with human peripheral lymphocytes at concentrations ranging from 0.5 to 300 μg/mL (Di Sotto et al., 2011). In a chromosomal aberration (CA) assay in Chinese hamster ovary (CHO) cells, linalool did not induce chromosomal aberrations in CHO cells treated with up to 340 μg/mL of linalool in the presence of S9 and up to 430 μg/mL in the absence of S9, which was derived from the liver of rats treated with Aroclor 1254 (Galloway, 1983). When tested up to an overall maximum of 500 μg/mL, linalool did not cause forward mutations in mouse lymphoma L5178Y cells incubated with and without exogenous metabolic activation by S9 obtained from the liver of Aroclor 1254-treated rats (Cifone, 1994). An *in vivo* comet assay study in mice found no DNA damage in the forebrain tissue and peripheral blood sampled following the administration of a single 10, 50, 100 or 200 mg/kg dose of linalool by intraperitoneal injection (Coelho et al., 2013). In summary, linalool was negative for all measured endpoints.

In a short-term dietary study, a 1:1 mixture of linalool and citronellol, resulting in an average daily intake of 50 mg/kg bw/day of each, was incorporated into the diet of male and female rats treated for 12 weeks. A slight decrease in body weight gain was observed in the male rats but was concluded by the study author not to be of biological relevance (Oser, 1958). This study was used to calculate a MoS for the intake of Group 12 (Aliphatic and aromatic tertiary alcohols and related esters) constituents for Lavadin Oil (FEMA 2618), Lavender Oil (FEMA 2622) and Tea Tree Oil (FEMA 3902) in Step 6 of the safety evaluation.

In a reproductive/developmental toxicity study, there were no deaths or signs of gross toxicity in female Sprague-Dawley rats administered linalool at doses of 0 (control), 250, 500 or 1000 mg/kg bw/day for 11 days after confirmed gestation (Politano et al., 2008). There were no treatment-related deaths or gross signs of toxicity in the maternal animals. There were also no adverse developmental effects noted in the offspring. The no-observed-adverse-effect level (NOAEL) for maternal rats was determined to be 500 mg/kg bw/day due to treatment-related changes in motor function and fur stained with urine at the highest dose. The NOAEL for the development of offspring was determined to be 1000 mg/kg bw/day (Politano et al., 2008).

7.1.2. Linalyl acetate

In a bacterial reverse mutation assay, linalyl acetate did not increase the number of revertant mutants in either of the *S. typhimurium* strains TA98 and TA100 but did induce statistically significant, concentration-dependent increases in revertant colonies in *E. coli* WP2 *uvrA* up to the highest dose tested, 9000 μg/plate, with and without S9 activation (Di Sotto et al., 2008). A statistically significant increase in revertant colonies was only observed at concentrations at and above 3200 μg/plate. The two highest concentrations tested, 6400 and 9000 μg/plate, exceed the recommended 5000 μg/plate upper limit for this assay in the OECD guideline (Di Sotto and Mazzanti, 2016; OECD, 1997). The same authors, in a separate study, reported that linalyl acetate also yielded a significant concentration-dependent induction of micronuclei in a human lymphocyte micronucleus assay at concentrations from 10 to 300 μg/mL (Di Sotto et al., 2011). This study was considered further in a fragrance safety assessment (Api et al., 2015), to which a response from the study authors was received (Di Sotto and Mazzanti, 2016). The conditions of the *in vitro* micronucleus study on linalyl acetate did not comply with OECD testing guidelines since the test substance exposure period of 72 h greatly exceeded the OECD suggested 3- to 6 h exposure time to detect clastogens and aneugens (OECD, 2014). The authors noted that the study was performed according to a protocol for the cytokinesis-block micronucleus assay with only minor modifications (Di Sotto and Mazzanti, 2016; Fenech, 2007). The positive outcomes of these studies are inconsistent with previously reviewed studies on linalyl acetate (Marnett et al., 2014) that reported negative mutagenicity in *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 both in the presence and absence of metabolic activation (Heck et al., 1989), lack of measurable DNA damage in the *Bacillus subtilis* rec assay (Oda et al., 1978), lack of induction of chromosomal aberrations in human peripheral blood lymphocytes (Bertens, 2000) and lack of unscheduled DNA synthesis (UDS) in rat hepatocytes (Heck et al., 1989). In addition, in a recent report, linalyl acetate was reported not to be mutagenic in *S. typhimurium* strains TA98, TA100, TA1535 and TA1538 at concentrations up to 5000 μg/plate in the presence and absence of Aroclor 1254-induced rat liver S9 (ECHA, 2019a). In addition to these studies, linalyl acetate is expected to be hydrolyzed to linalool upon oral consumption, which has been shown to be non-genotoxic. In summary, the weight-of-evidence indicates that linalyl acetate is not genotoxic despite the positive result reported in a non-OECD-compliant *in vitro* micronucleus study.

7.1.3. 4-Carvomenthenol

In an OECD-compliant Ames assay, mutagenicity was not observed when 4-carvomenthenol was tested at concentrations between 16 and 5000 μg/plate in *S. typhimurium* strains TA97a, TA98, TA100, TA102 and TA1535 both in the presence and absence of an S9 metabolic activation system derived from the liver of phenobarbital/*β*-naphthoflavone-treated rats. Cytotoxicity was noted at 1600 μg/plate (Scheerbaum, 2001). In an OECD-compliant *in vitro* micronucleus assay, human peripheral blood lymphocytes were treated with 4-carvomenthenol for 4 and 24 h, with and without metabolic activation. Up to the maximum tested concentration of 1540 μg/mL, 4-carvomenthenol tested negative for the induction of micronuclei (Roy, 2015). In conclusion, two guideline studies on 4-carvomenthenol were both found to be negative, leading to the conclusion that the substance is not of genotoxic concern.

In a 28-day toxicity study, 4-carvomenthenol was administered by oral gavage to male Sprague-Dawley rats at 400 mg/kg bw/day to investigate its nephrotoxic potential. The study concluded that 4-carvomenthenol did not induce any treatment-related renal changes (Schilcher and Leuschner, 1997).

7.1.4. α-Terpineol

A recently conducted OECD-compliant reverse mutation assay tested α-terpineol in *S. typhimurium* TA98, TA100, TA1535 and TA1537 and *E. coli* WP2 *uvr*A. α-Terpineol did not increase the frequency of revertants when tested up to 5000 μg/plate in the presence and absence of an S9 metabolic activation system prepared from phenobarbital/5,6-benzoflavone-treated rats. Therefore, α-terpineol was considered negative for genotoxicity under the conditions tested (Rao, 2019). In a second reverse mutation assay using *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538, α-terpineol was not mutagenic at concentrations of 10–1000 μg/plate in the presence and absence of S9 from Aroclor 1254-treated male Syrian hamsters or Sprague-Dawley rats

(Seifried et al., 2006). α-Terpineol was non-mutagenic in a mouse lymphoma L5178Y forward mutation assay when tested at ranges of 0.14–0.38 μg/mL or 0.17–0.56 μg/mL in the absence or presence of liver S9 from male rats treated with Aroclor 1254, respectively (Seifried et al., 2006).

7.1.5. Terpineol (Isomeric mixture)

The isomeric mixture of terpineol was tested at concentrations up to 1250 μg/plate in an OECD-compliant reverse mutation assay. In *S. typhimurium* strains TA98, TA100, TA1535 and TA1537 and *E. coli* WP2*uvr*A, terpineol did not increase the frequency of mutant colonies in treatments with or without S9 metabolic activation (ECHA, 2013b). In addition, two independent *in vitro* chromosomal aberrations assays were conducted on terpineol using cultured human lymphocytes or Chinese hamster lung cells (ECHA, 2010, 2013a). In human lymphocytes, concentrations up to 450 or 625 μg/mL were tested for induction of structural aberrations in the short-term 3 h treatments with and without Aroclor 1254-induced S9, respectively. In continuous 24 h treatments without S9 metabolic activation, concentrations up to 225 μg/mL were tested for induction of aberrations. Based on the conditions tested, terpineol was non-clastogenic in the human lymphocytes in the presence and absence of S9 metabolic activation (ECHA, 2010). In Chinese hamster lung cells, concentrations ranging from 100 to 400 μg/mL were tested in the absence of S9 (6 h or continuous 24 h treatment). In the presence of S9 (6 h treatment only), a range of 100–500 μg/mL was tested. Terpineol did not induce chromosomal aberrations in Chinese hamster lung cells when tested at concentrations similar to the first assay using lymphocytes (ECHA, 2013a).

In an OECD-compliant combined repeated dose and reproductive/development toxicity screening study, groups of male and female Sprague-Dawley rats were administered terpineol in corn oil by oral gavage at dose levels of 100, 300 or 1000 mg/kg bw/day (ECHA, 2019b). Males were treated for a total of 44 days, including the two-week pre-mating through Day 30 after mating. Females were treated during the pre-mating period through Day 4 of lactation for a total of 41–51 days. Additionally, a group of non-mating females was treated with 1000 mg/kg bw/day terpineol on the same schedule as the males, and groups of males and non-mating females were maintained for a two-week recovery period. Six females belonging to the high-dose group were found dead or moribund; moribund animals displayed poor health, including significant clinical observations, decreased body weights and lower food consumption. Necropsy and histopathology of these dead or moribund females showed weight reduction of the spleen and thymus and adverse changes in the kidneys, urinary bladder and liver.

For surviving animals, no treatment related clinical signs, body weight changes, changes in food consumption, hematological, clinical biochemistry or behavior findings were reported. Increased water consumption was observed in high-dose males and non-mating females and corresponded to increased urine volume and low urinary osmotic pressure in these animals. Upon necropsy, significantly higher liver and kidney weights and lower testes and epididymis weights were observed in high-dose males and were also reported following the recovery period. Non-mating females of the 1000 mg/kg bw/day group had increased liver, kidney and adrenal gland weights; only the increased liver weights were present after the recovery period. An increase in relative and absolute liver weights was observed in mid-dose mating females, but not in high-dose mating females. No gross abnormalities were observed in mating females; however, females of the high-dose group were infertile (9/12), which was considered a result of the smaller testes in the males of the group.

Histopathology indicated minimal to mild vacuolation of adrenal cortical cells in mating females dosed with 300 mg/kg bw/day or higher and in high-dose non-mating females. Smaller testes and epididymis in high-dose males (including recovery group males) correlated with moderate atrophy of the seminiferous tubules and other findings reported in these organs. Findings in the kidneys of mid- and high-dose males and of high-dose mating and non-mating females were correlated to higher relative and absolute kidney weights in these groups. Eosinophilic droplets in mid- and high-dose males were positive for α_{2u}-globulin immunochemistry and negative for periodic acid-Schiff staining. Renal effects related to α_{2u}-globulin are widely considered non-relevant to human risk assessment, as this effect is considered unique to male rats (Capen et al., 1999; Flamm and Lehman-McKeeman, 1991; Swenberg and Lehman-McKeeman, 1999; US-EPA, 1991). High-dose animals of both sexes also displayed umbrella cell atrophy and hypertrophy or hyperplasia of transitional epithelial cells of the urinary bladder. Minimal to moderate umbrella cell vacuolation was also observed in the urinary bladder of mid-dose males and mating females. A higher incidence of decreased zymogen granules in the pancreas was reported in mid-dose mating females and high-dose non-mating females. Assessment of reproductive functionality reported no changes to sexual cycles in any of the treatment groups and no significant differences in gestation ratio, pregnancy period, number of corpora lutea, implantation number or ratio, delivery ratio or number of stillborn or live pups. Lower insemination index ($p < 0.01$) and fertility index ($p < 0.01$) were reported in high-dose males and mating females, respectively, and were a result of the histopathological changes observed in male reproductive organs. Live pups born to treatment groups did not present any morphological abnormities or gross pathological findings upon necropsy. Based on these findings, the systemic toxicity NOAEL obtained from the repeated dose portion of the study was determined to be 100 mg/kg bw/day. The reproductive NOAEL was considered 300 mg/kg bw/day in males and 100 mg/kg bw/day in females, while the developmental NOAEL was the highest dose tested, 1000 mg/kg bw/day (ECHA, 2019b).

A second OECD-compliant combined repeated dose toxicity and reproductive/developmental toxicity study was conducted on terpineol (isomeric mixture) via gavage administration at dose levels of 60, 250 or 750 mg/kg bw/day (ECHA, 2019c). The dosing for main (non-recovery group) animals lasted a minimum of five weeks for males and non-mating females, which included the two-week pre-mating period. Mating females were dosed for the pre-mating period and throughout mating and gestation, until Day 6 of lactation. One low-dose male and one low-dose mating female were found dead or morbid but this was not considered a result of test substance administration. Slightly reduced, but not statistically significant, body weight gains were observed in high-dose males and mating females of all dose levels. No adverse changes to body weight, food consumption or hematological parameters were observed for any of the treatment groups. There were no dose-related trends and the effects were minimal in degree. At necropsy, increased liver weights for high-dose males and females were reported, in addition to increased kidney weights in high-dose males. These differences in liver and kidneys weights were not observed in the recovery group. Markedly lower testes and epididymis weights were observed in most high-dose males and in two mid-dose males; high-dose males of the recovery group also displayed the lower testes and epididymis weights. Gross pathology presented a range of testicular findings in high-dose males and recovery group males, including small, flaccid testes and the presence of masses in some epididymides that were correlated to histopathological findings of spermatocele granuloma, a benign cyst-like growth that occurs spontaneously in rats. No gross lesions were reported in any females. Histopathological examinations revealed minimal centrilobular hepatocyte hypertrophy in three high-dose females that showed complete recovery after two weeks. In male rats, examination of the kidneys revealed hyaline droplet formation, characteristic of α_{2u}-globin nephropathy, at doses of 250 and 750 mg/kg bw/day, that persisted in the recovery group high-dose males. Reproductive parameters, such as estrous cyclicity, mating performance and fertility, were generally unaffected by test substance administration. Offspring did not display any differences in body weight or gross findings, and there were no clinical signs of toxicity as a result of maternal exposure. Based on

these findings, the repeated dose toxicity and fertility NOAEL was determined to be 250 mg/kg bw/day for males and 750 mg/kg bw/day for females. The developmental toxicity NOAEL was greater than 750 mg/kg bw/day, the highest dose tested (ECHA, 2019c).

7.1.6. Terpinyl acetate (Isomeric mixture)

Human peripheral blood lymphocytes were exposed to terpinyl acetate in an OECD-compliant *in vitro* micronucleus assay at concentrations up to 36.0 μg/mL for a 24 h exposure period without metabolic activation, concentrations up to 103 μg/mL for the 3 h exposure period without metabolic activation and concentrations up to 158 μg/mL with metabolic activation with S9, prepared from the liver of Aroclor 1254-treated rats. The study concluded that terpinyl acetate did not induce significant increases in micronuclei under the conditions tested (Bhalli, 2015).

7.1.7. α-Terpinyl acetate

Two OECD-guideline bacterial reverse mutation assays were conducted on α-terpinyl acetate in *S. typhimurium* strains TA98, TA100, TA1535 and TA1537 and *E. coli* strain WP2 *uvrA*. In both studies that employed the plate incorporation method, treatment with α-terpinyl acetate up to a concentration of 5000 μg/plate in the presence and absence of Aroclor 1254-induced rat liver S9 did not result in any increases in revertant colony frequencies (Bhalli, 2014a; van den Wijngaard, 2012).

An *in vitro* micronucleus induction assay was conducted in accordance with OECD testing guidelines, in which human peripheral blood lymphocytes were treated with α-terpinyl acetate in the presence and absence of S9 metabolic activation derived from Aroclor 1254-treated rats (Bhalli, 2014b). Based on a preliminary cytotoxicity assay, α-terpinyl acetate was tested up to 225 μg/mL for the 3 h exposure periods with and without S9 and up to 80 μg/mL for the 24 h exposure period without S9. At a single scored concentration of 58.3 μg/mL in the 24 h treatment arm, there was a statistically significant increase in micronuclei frequency, but it was within the laboratory's historical values for the vehicle control and therefore was determined to be not biologically relevant. The overall conclusion was that α-terpinyl acetate was not genotoxic under the conditions tested (Bhalli, 2014b).

7.1.8. Patchouli alcohol

In an OECD-compliant reverse mutation study, patchouli alcohol was not mutagenic when tested in *S. typhimurium* strains TA98, TA100, TA1535 and TA1537 and *E. coli* WP2 *uvrA* at concentrations up to 5000 μg/plate, with and without S9 metabolic activation obtained from the liver of Aroclor 1254-treated male Sprague-Dawley rats (Bhalli, 2014c). In an OECD-compliant *in vitro* micronucleus assay, patchouli alcohol was tested in human peripheral blood lymphocytes for 3 h at concentrations up to 89 μg/mL without S9 metabolic activation and 150 μg/mL with S9 metabolic activation. Concentrations up to 83 μg/mL were tested for the 24 h exposure in the absence of S9 metabolic activation, prepared from the liver of Aroclor 1254-treated rats. The study concluded that patchouli alcohol did not induce an increase in micronuclei in binucleated cells under the conditions tested (Bhalli, 2014d).

7.2. Natural flavor complexes

7.2.1. Bois de rose oil

In an OECD-compliant reverse mutation assay, bois de rose oil was not mutagenic when tested at concentrations up to 5000 μg/plate in *S. typhimurium* strains TA98, TA100, TA1535 and TA1537 and *E. coli* WP2, in the absence and presence of S9 metabolic activation prepared from the liver of Aroclor 1254-treated rats (Mee, 2017). In an OECD-compliant *in vitro* micronucleus study, bois de rose oil was tested at concentrations up to 621 μg/mL in the 3 h treatment in the presence of S9 metabolic activation, 570 μg/mL in the 3 h treatment in the absence of S9 metabolic activation and concentrations up to 176 μg/mL in the 24 h treatment in the absence of S9 metabolic activation. The S9 metabolic activation system was prepared from the liver of Aroclor 1254-treated male rats. Bois de rose oil did not induce the formation of micronuclei in human lymphocytes under the conditions tested (Clare, 2017).

7.2.2. Cardamom Seed Oil

In a GLP guideline study, cardamom seed oil was negative for mutagenicity when incubated with *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 both in the presence and absence of an Aroclor 1254-treated rat liver metabolic activation system at concentrations up to 2325 μg/plate[8] (DeGraff, 1983b; Heck et al., 1989). Another reverse mutation assay with the same *S. typhimurium* strains tested cardamom seed oil at a concentration range of 40–2200 μg/mL.[9] Under the conditions of the study, cardamom seed oil was negative for mutagenic potential with and without S9 prepared from the liver of Aroclor 1254-treated rats (Brusick, 1982). When cardamom seed oil was incubated with L5178Y mouse lymphoma cells in a GLP-compliant assay, an increase in the mutant frequency at the TK locus was not observed in the presence or absence of S9 metabolic activation. Concentrations up to 112 μg/mL[10] were tested in the absence of metabolic activation and up to 233 μg/mL in the presence of Aroclor 1254-treated rat liver S9 metabolic activation (Cifone, 1982; Heck et al., 1989). Cardamom seed oil was negative in an UDS assay in rat hepatocytes at a dose of 50 mg/mL (Heck et al., 1989).

In a GLP-compliant reproductive/developmental toxicity study, cardamom seed oil was administered by oral gavage to virgin female rats (10/group) at doses of 0 (corn oil control), 375, 750 or 1500 mg/kg bw/day for seven days prior to cohabitation, gestation, delivery and a four-day lactation/post-parturition period (Hoberman, 1989a). There was one death in the mid-dose group (750 mg/kg bw/day) on Day 22 of gestation due to pronounced weight loss and petechial hemorrhaging in the gastric mucosa and one moribund sacrifice in the high-dose group (1500 mg/kg bw/day) due to clinical signs of toxicity and pronounced weight loss. Statistically significant numbers of rats with clinical observations such as salivation, decreased motor function, emaciated appearance or tremors and twitches were noted at all dose levels. The onset was dose-related and attributed to the administration of the test substance.

Dose-dependent decreases in maternal body weight gain were observed with significant decreases observed in the middle and high dose groups during the pre-cohabitation period and in all groups during the gestation period. However, significant reduction in feed consumption was only observed in the high dose group. Significant enlargement of the liver was observed in the middle and high dose groups and attributed to hepatic enzyme induction.

Except for the one mortality in the middle dose group during gestation, all dams delivered one or more live pups. There was no dose-related effect observed for implantation incidences or live litter sizes. Decreased pup body weights were observed at the middle and high doses and a significant increase in pup mortality was observed at the high dose. Based on clinical observations and decreased body weight gains and feed consumption in the dams, a maternal NOAEL for toxicity could not be determined. Based on the lack of adverse effects of cardamom oil on mating, fertility, duration of gestation or duration of parturition, a NOAEL of 1500 mg/kg bw/day was determined for reproductive effects. The NOAEL for the offspring was determined to be 375 mg/kg bw/day based on the decreased body weights (Hoberman, 1989a).

[8] Based on a density of 0.93 g/mL (Source: Food Chemical Codex 12th Edition, United States Pharmacopeia (USP), Rockville, MD, USA).

[9] The mean achieved dose is calculated for each treatment group based on the food consumption per day and the mean body weight over each measurement period. Differences in calculated mean achieved dose and the nominal concentration between studies is due to a greater mean body weight (per measurement period) in the longer duration (28-day) study.

In a 28-day repeat-dose study, cardamom oil was administered by oral gavage to male and female Sprague-Dawley rats at 0 (control), 240, 600 or 1500 mg/kg bw/day (Serota, 1991). The constituent analysis reported a composition of 36% terpinyl acetate, 38% eucalyptol, 6% linalool, 6% α-terpineol and several minor constituents for the cardamom oil used in the study. The vehicle control was a 1% methyl cellulose solution. Examination for clinical signs and measurement of food consumption failed to reveal any differences between test and control groups. A significant decrease in body weight was reported in males in the high dose group. Females showed no changes in body weight gain at any dose level. Clinical hematology values were normal. Clinical chemistry evaluation showed elevated total protein and albumin in the high-dose males and females and decreased glucose in high-dose males. There were no significant changes in any parameter related to liver or kidney function. Morbidity was observed for a single high-dose male which was considered treatment-related, although the cause of death could not be determined by macroscopic or microscopic examinations. Clinical findings were noted for this animal prior to its sacrifice, including dyspnea, urine stains and lacrimation of both eyes.

Enlarged livers were reported in high-dose males and mid- and high-dose female treatment groups and increased incidence of pale livers was observed in all treatment groups except the low-dose male group. Significant increases in absolute and relative liver weight were recorded for at all dose levels in males and females. Histopathological examination of males revealed periportal cytoplasmic vacuolization in the low (7/10), mid- (5/10) and high-dose (5/10) groups. Females showed a similar non-dose-related trend in cytoplasmic vacuolization [low (7/10), mid (8/10) and high (6/10)]. The vacuolization was graded as slight. The authors noted that the vacuoles seemed to be fat-like deposits and were considered to have little effect on the function or health of the animals. Since changes in bilirubin and liver enzymes were not observed in females, the relevance of the lesion to the health of the animals is unknown.

Increases in absolute and relative kidney weights were noted in the mid- and high-dose females and relative kidney weights in mid- and high-dose males. There was no evidence of histopathologic abnormalities in any female group. However, histopathological examination of treated males revealed renal tubule regeneration (hyaline droplet degeneration) and necrosis, likely related to the lysosomal handling of α_{2u}-globulin, an effect specific to the male rat and not of toxicological relevance to humans (Capen et al., 1999; Flamm and Lehman-McKeeman, 1991; Swenberg and Lehman-McKeeman, 1999; US-EPA, 1991). High-dose males showed an increase in absolute and relative testes weights with a significant increase in epididymis weights in the high dose group of male rats. Histopathology revealed testicular giant cell degeneration with associated hypospermia in the epididymis. There was no evidence of histopathology of the testes or epididymis in the mid- and low-dose males and no evidence of any adverse effects to female reproductive organs (ovaries and uterus) at any dose level. Changes in clinical chemistry in high-dose males and females and increased absolute and relative adrenal weights in mid- and high-dose females were noted but did not correlate with microscopic findings, nor were the observations dose-related. Based on the findings of the study, the lowest-observed-adverse-effect-level (LOAEL) was determined to be 240 mg/kg bw per day for both male and female rats (Serota, 1991).

7.2.3. Clary Oil

In an OECD-compliant study, clary oil was negative for mutagenicity when incubated with *S. typhimurium* strains TA98, TA100, TA1535 and TA1537 and *E. coli* WP2 *uvrA*/pKM101 both in the presence and absence of an Aroclor 1254-treated rat liver metabolic activation system at concentrations up to 5000 μg/plate (Mee, 2016a). In a separate study, clary oil was negative for mutagenicity when incubated with *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538, both in the presence and absence of S9 metabolic activation at concentrations up to 5000 μg/plate (Heck et al., 1989). Clary oil was also negative in an OECD-compliant *in vitro* micronucleus test in human lymphocytes in both the presence and absence of S9 at concentrations up to 888.9 μg/mL, the lowest concentration in which cytotoxicity was observed (Mee, 2016b). Genotoxicity was not observed in an UDS assay in rat hepatocytes up to 101 μg/mL or in a rec assay at 10 and 30 μg/disk (Heck et al., 1989; Zani et al., 1991). In summary, the results of these assays demonstrate a lack of genotoxic potential for clary oil.

7.2.4. Coriander Seed Oil

Coriander seed oil was not mutagenic in a GLP study with *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 in the presence and absence of an S9 activation system derived from Aroclor 1254-treated rat liver, at concentrations ranging from 9 to 4350 μg/plate[10] (DeGraff, 1983a; Heck et al., 1989). Similarly, when coriander seed oil was incorporated into *S. typhimurium* cultures TA98 and TA100 at 2 and 7 mg/plate, it was negative for mutagenic potential with and without rat liver S13 metabolic system (Marcus and Lichtenstein, 1982). Coriander seed oil was also negative in an UDS assay in rat hepatocytes up to 300 μg/mL; a CA study in Chinese hamster ovary cells up to 0.125 mg/mL; and a mouse lymphoma mutation assay in the presence and absence of S9 up to 160 nL/mL and 300 nL/mL, respectively (Cifone, 1983; Heck et al., 1989; Ishidate Jr. et al., 1984). In a rec assay, coriander seed oil tested at a concentration of 8 mg/disk was positive without S9 activation and negative with S9 activation (Ueno et al., 1984). The rec assay does not have an OECD testing guideline; the OECD guideline for genotoxicity testing notes that indicator tests such as the rec assay should be weighted relative to the results of other assays that measure DNA damage or mutagenicity that can be passed on to subsequent generations (OECD, 2015). In conclusion, the weight of evidence indicates a lack of genotoxic potential for coriander seed oil due to negative results in the standard Ames, CA and mouse lymphoma assays.

In a 28-day oral gavage toxicity study, Sprague-Dawley rats were administered coriander seed oil, containing 73% linalool, at 0 (control), 160, 400 or 1000 mg/kg bw/day (10/sex/group) with 1% methyl cellulose as the vehicle control (Serota, 1990). No treatment-related effects were observed based on mortality, clinical observations, body weight or food consumption. A significant increase in absolute and relative liver weights was observed in the mid- and high dose groups for both male and female rats and a significant increase in the absolute liver weight was observed in the female low dose group. This effect was accompanied by periportal hepatocellular cytoplasmic vacuolization in the liver of high-dose females with lower incidences in low- and mid-dose female rats but was not observed in any treatment groups in the male rats. For all treatment groups, no histopathological findings were reported in the liver of male rats. Hepatocyte vacuolation observed in the low- and mid-dose female rats was likely due to fatty degeneration, although this was not confirmed by special staining. Significant increases in absolute and relative kidney weight were observed in the high dose male and female groups and increases in relative kidney weight were seen in the middle dose group of male rats. For the male rats, treatment-related degenerative lesions of the renal cortex of the kidney in high-dose males were related to increased absolute and relative kidney weights. Lesions in the non-glandular stomach of the mid- and high-dose female groups were also found including erosion, inflammation and hyperplasia particularly in high-dose females. Increases in total protein and serum albumin were observed in the mid-dose male rats and high-dose male and female rats. Based on these observations, the NOAEL was determined to be 160 mg/kg bw/day for male rats. A NOAEL could not be determined for female rats (Serota, 1990).

In a GLP-compliant reproductive/development study, coriander seed oil, containing 73% linalool, was administered to Crl:CD(SD)BR virgin

[10] Based on a density of 0.87 g/mL (Source: Food Chemical Codex 12th Edition, United States Pharmacopeia (USP), Rockville, MD, USA).

female rats (10/group) at doses of 0 (control), 250, 500 or 1000 mg/kg bw/day through the 7-day pre-cohabitation period, cohabitation (7 days maximum), gestation, delivery and the 4-day lactation/post-parturition period. Significant decreases in body weight gain and feed consumption were observed in the highest dose group during the pre-mating period. During gestation, statistically significant, treatment-related increases in weight gain and feed consumption occurred in all test groups compared to the control group, that were considered biologically relevant. These changes were also observed during lactation with less severity. There were no dose-related or statistically significant changes in duration of cohabitation, pregnancy incidences or implementation averages in the treatment groups. A statistically significant increase in pup mortality was noted in the 1000 mg/kg bw/day treatment group. No differences in duration of gestation, pup sex ratios or pup body weights were found in any of the treatment groups compared to the control group. There were also no adverse developmental effects noted in the offspring. From these observations, the NOAEL for progeny was determined to be 500 mg/kg bw/day. A maternal NOAEL was not determined, based on clinical observations and altered body weights and food consumption at the lowest dose tested (Hoberman, 1989b).

7.2.5. Guaiac Wood Oil

In an OECD-compliant study, guaiac wood oil was negative for mutagenicity when incubated with *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 and *E.coli* WP2 *uvrA*/pKM101 both in the presence and absence of an Aroclor-1254 treated rat liver metabolic activation system at concentrations up to 5000 μg/plate (Mee, 2016b). In an OECD-compliant *in vitro* micronucleus test in human blood lymphocytes, cells were exposed to up to 395 μg/mL of guaiac wood oil in the presence and absence of an S9 metabolic activation system obtained from Aroclor 1254-treated male Sprague-Dawley rat liver Guaiac wood oil was determined to be negative for the induction of micronuclei in the presence and absence of S9 metabolic activation (Mee, 2016c).

In a 90-day toxicity study, FDRL rats (15/sex/group) were administered guaiac wood oil in the diet at a single nominal dose of 31.8 mg/kg bw/day (equivalent to 30.7 and 36.0 mg/kg bw/day for male and female rats, respectively). The test substance was diluted in cotton seed oil at a concentration yielding a dose of 2% in the diet (Oser et al., 1965). During the study, body weight and food consumption were recorded. At Weeks 6 and 12, hematological and blood chemistry analyses were performed. At the end of the study, the animals were autopsied during which liver and kidney weights were measured and tissues were collected for histopathology. Observations included increased efficiency of food utilization, increased red blood cell count and decreased hemoglobin, lymphocytes and blood urea nitrogen. There were no significant adverse effects detected and no histopathological findings. The NOAEL was determined to be the only dose tested, 31.8 mg/kg bw/day (Oser et al., 1965).

7.2.6. Lavender Oil

In a reverse mutation assay conducted with lavender oil at concentrations of 4.4 and 8.8 ng/plate, it was found to be mutagenic in *S. typhimurium* strains TA1535 and TA1537 (concentration of 4.4 ng/plate) and TA98 (concentration of 8.8 ng/plate) (Sivaswamy et al., 1991). This study did neither report the chemical composition of the lavender oil tested nor indicate a dose-response to support its validity (OECD, 1997). Additionally, the concentrations tested were extraordinarily low. Based on these shortcomings, this study is not considered relevant to the safety evaluation of lavender oil. In an OECD-compliant reverse mutation test in *S. typhimurium* strains TA98, TA100, TA1535 and TA1537 and *Escherichia coli* strain WP2 *uvrA*, treated with or without exogenous rat metabolic activation, lavender oil was not mutagenic up to the maximum tested concentration of 5000 μg/plate (Dakoulas, 2014). Another OECD-compliant reverse mutation test also showed no mutagenic potential, in *S. typhimurium* strains TA98 and TA100 and *E. coli* WP2 *uvrA,* with and without S9 metabolic activation derived from phenobarbital/5,6-benzoflavone treated rats, up to 2780 and 2500 μg/plate for *Salmonella* and *Escherichia* strains, respectively (Evandri et al., 2005). In a separate study, lavender oil did not induce mutagenicity in *S. typhimurium* strains TA98 and TA100 both with and without liver S9 from Aroclor 1254-treated rats at concentrations up to 443 μg/plate (De Martino et al., 2009).

In an *in vitro* micronucleus study, lavender oil was tested in human peripheral blood lymphocytes at concentrations up to 100 μg/mL. While there was a small significant increase in the frequency of micronuclei observed at the highest concentration tested, a dose response was not observed. (Di Sotto et al., 2011). In a separate OECD-compliant study, lavender oil was tested in human peripheral blood lymphocytes, in the presence and absence of an exogenous metabolic system. In this study, no increase in the induction of micronuclei was observed up to the highest concentration tested, 450 μg/mL (Roy, 2015b). Altogether, the weight of evidence provided by the results of the three OECD-compliant Ames and *in vitro* micronucleus studies reported here, in addition to the negative genotoxicity reported for linalool and linalyl acetate, the major constituents of lavender oil, supports the conclusion that lavender oil is not of genotoxic concern.

7.2.7. Patchouly Oil

Patchouly oil was tested in a GLP-compliant Ames reverse mutation assay to evaluate its mutagenic potential in a dose range of 0.5–50 μg/plate (Jones, 1988). When incubated with and without an S9 metabolic activation system from the liver of Aroclor 1254-treated rats, patchouly oil did not increase the numbers of revertant colonies in *S. typhimurium* strains TA98, TA100, TA1535 and TA1537 (Jones, 1988). Two separate *in vitro* CA studies in Chinese hamster ovary cells had negative results in the presence and absence of metabolic activation; the highest concentration tested was 90 μg/mL (Brooker, 1989; Song, 2009). Patchouly oil was not mutagenic in an OECD-compliant forward mutation assay conducted with mouse lymphoma cells at concentrations up to 50 μg/mL in the absence of S9 metabolic activation or up to 275 μg/mL in the presence of S9 metabolic activation for 4 h and the results were confirmed in a separate assay testing up to 36 μg/mL for 24 h in the absence of S9 metabolic activation (Kirby, 2009).

In a 14-day pilot dietary study on patchouly oil, the highest tolerable dose was 12000 ppm (equivalent to a mean achieved dose[10] of 979 mg/kg bw/day) (Marr, 2011). Following the dose range-finding study, patchouly oil was tested in an OECD-compliant 28-day combined dietary and reproductive/developmental toxicity study in Wistar Han rats (10/sex/group) (Liwska, 2013a, b). For the repeated dose dietary application of the combined study, patchouly oil was incorporated into the diet at concentrations of 500, 4000 and 13000 ppm, corresponding to mean doses of 41, 323 and 977 mg/kg bw/day, respectively, for both male and female rats (Liwska, 2013b). There were no treatment-related deaths or abnormal clinical observations during the study. At the highest dose tested, lower food consumption and decreases in body weight gain compared to controls were reported in both male and female rats. A significant increase in relative and absolute liver weights was observed in middle and high dose groups for both male and females which was correlated to the observation of minimal to moderate centrilobular hepatocellular hypertrophy in these groups. This effect is considered a consequence of hepatocellular induction of enhanced hepatic metabolism. A significant increase in relative and absolute kidney weights was observed in middle and high dose male groups. Hyaline droplet nephropathy was observed in all the male treatment groups and demonstrated a dose-related severity consistent with the accumulation of α_{2u}-globulin, an effect specific to the male rat and of no toxicological relevance to humans (Capen et al., 1999; Flamm and Lehman-McKeeman, 1991; Swenberg and Lehman-McKeeman, 1999; US-EPA, 1991).

A significant reduction in absolute and relative thyroid weights was observed in all female dose groups. Histopathological analysis revealed an increased incidence and/or severity of follicular hypertrophy in the

thyroid for both male and female rats in the middle and high dose groups and males in the low dose group. This was related to an increased metabolism of the thyroid hormones (T3/T4) due to hepatocellular hypertrophy and were considered a secondary effect of treatment and not adverse. A significant decrease in relative and absolute brain and spleen weights was evident in the high dose male group and a significant reduction in relative and absolute heart weight was observed in the female high dose group. These findings had no histopathological correlation and were not considered to be of toxicological significance by the study authors (Liwska, 2013b). T The FEMA Expert Panel considered ed the findings and assigned a assigned a NOAEL of 41 mg/kg bw/day, the lowest dose tested.

For the assessment of reproductive/developmental toxicity, male and female rats were administered concentrations of 1300, 4000 or 13000 ppm of patchouly oil incorporated into the diet for up to 8 weeks corresponding to mean dose levels of 91.4, 277 or 810 mg/kg bw/day, respectively (Liwska, 2013a). On Day 15 of the study, animals were paired (1 male:1 female) for a maximum of 14 days. Following mating, males were returned to their original cage and the females were transferred to an individual cage. Pregnant females were allowed to give birth and maintain their offspring to Day 5 *post-partum* and were then euthanized. Male rats were euthanized on Day 43. Lower food consumption and food efficiency was observed at the high dose in both sexes which correlated with decreased body weight gain. Also, at the highest dose, there were differences in group mean corpora lutea counts compared with concurrent and historical control values, but there was no obvious effect upon reproductive performance or apparent impairment of estrous cyclicity in the majority of females. No increase in neonatal mortality was observed but there was an indication of reduced individual offspring body weight gain from Day 1 to Day 4 *post-partum*. Due to treatment-related effects observed in maternal rats and in offspring *pre-* and *post-partum* at the highest dose tested of 810 mg/kg bw/day, the NOAEL was determined to be 277 mg/kg bw/day for systemic toxicity in the adult rats and for reproduction and offspring development (Liwska, 2013a).

A 90-day dietary study on patchouli oil observed no adverse effects for male FDRL rats dosed at 11.9 mg/kg bw/day and female FDRL rats dosed at 14.5 mg/kg bw/day (Oser et al., 1965). The test substance was diluted in cotton seed oil at a concentration of 2% in the diet. During the study, observations of body weight and food consumption were taken. At Weeks 6 and 12, hematological and blood chemistry analyses were performed. At the end of the study, the animals were autopsied during which liver and kidney weights were measured and tissues were collected for histopathology. No adverse effects were observed for patchouli oil in this study (Oser et al., 1965).

7.2.8. Tea Tree Oil

In a reverse mutation assay, *S. typhimurium* strains TA98 and TA100 and *E. coli* WP2 *uvrA,* were incubated with tea tree oil at concentrations up to 2000 μg/plate in both the presence and absence of S9 metabolic activation. The tea tree oil sample was characterized by gas chromatography and was composed of 39.1% 4-carvomenthenol (terpinen-4-ol), 20.4% *p*-mentha-1,4-diene (γ-terpinene), 9.2% *p*-mentha-1,3-diene (α-terpinene), 4.1% eucalyptol (1,8-cineole) and other minor components. This test material was not mutagenic under the conditions studied (Evandri et al., 2005). In a similar reverse mutation study, commercially available tea tree oil was tested in *S. typhimurium* strains TA98, TA100 and TA102 in the presence and absence of an S9 metabolic activation system, and there were no induced increases in revertant mutant colonies up to 5000 μg/mL (Fletcher et al., 2005). In human peripheral blood lymphocytes, tea tree oil neither increased the frequency of micronuclei nor the frequency of chromosomal aberrations at concentrations ranging from 95 to 365 μg/mL (Pereira et al., 2014). In all three *in vitro* genotoxicity studies, tea tree oil was non-genotoxic.

7.2.9. Summary of genotoxicity data

With the exception of the non-OECD compliant studies reported by Di Sotto and colleagues (Di Sotto et al., 2008, 2011), assays on the tertiary alcohols, linalool, 4-carvomenthenol, α-terpineol, terpineol (isomeric mixture) and patchouli alcohol and related esters, α-terpinyl acetate and the isomeric mixture of terpinyl acetate (summarized in Table 6) were negative for genotoxicity. Similarly, genotoxicity assays on bois de rose oil, cardamom seed oil, clary oil, coriander seed oil, guaiac wood oil, lavender oil, patchouly oil and tea tree oil were negative. A positive result reported for lavender oil tested in an *in vitro* micronucleus assay (also conducted by Di Sotto and colleagues) and a non-OECD compliant reverse mutation assay in *S. typhimurium* were not considered relevant to the safety evaluation of lavender oil. Overall, the weight of evidence indicates no concern for genotoxicity for the NFCs under consideration.

8. Recognition of GRAS status

The NFCs discussed here were determined to be generally recognized as safe (GRAS) under conditions of intended use as flavor ingredients by the Flavor and Extract Manufacturers Association (FEMA) Expert Panel in 1965 and in subsequent years. Upon application of the safety procedure, it was concluded that the NFCs listed in Table 7 do not present safety concerns. There are adequate margins of safety using conservative estimates of exposure and NOAEL values from short and long-term toxicity studies. Also, the weight of evidence indicates a lack of genotoxic potential for these flavorings. These data indicate that there is no significant safety concern and support the FEMA Expert Panel's affirmation of GRAS status for these NFCs as flavoring ingredients in food under conditions of intended use.

CRediT authorship contribution statement

Shoji Fukushima: Conceptualization, Methodology, Formal analysis, Writing - original draft, Writing - review & editing, Supervision. **Samuel M. Cohen:** Conceptualization, Methodology, Formal analysis, Writing - original draft, Writing - review & editing, Supervision. **Gerhard Eisenbrand:** Conceptualization, Methodology, Formal analysis, Writing - original draft, Writing - review & editing, Supervision. **Nigel J. Gooderham:** Conceptualization, Methodology, Formal analysis, Writing - original draft, Writing - review & editing, Supervision. **F. Peter Guengerich:** Conceptualization, Methodology, Formal analysis, Writing - original draft, Writing - review & editing, Supervision. **Stephen S. Hecht:** Conceptualization, Methodology, Formal analysis, Writing - original draft, Writing - review & editing, Supervision. **Ivonne M.C.M. Rietjens:** Conceptualization, Methodology, Formal analysis, Writing - original draft, Writing - review & editing, Supervision. **Thomas J. Rosol:** Conceptualization, Methodology, Formal analysis, Writing - original draft, Writing - review & editing, Supervision. **Jeanne M. Davidsen:** Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Visualization, Writing - original draft, Writing - review & editing, Supervision. **Christie L. Harman:** Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Visualization, Writing - original draft, Writing - review & editing, Supervision, Project administration, Funding acquisition. **Vivian Lu:** Formal analysis, Investigation, Data curation, Visualization, Writing - original draft. **Sean V. Taylor:** Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Visualization, Writing - original draft, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

Drs. Fukushima, Cohen, Eisenbrand, Gooderham, Guengerich, Hecht, Rietjens and Rosol are members of the Expert Panel of the Flavor and Extract Manufacturers Association. The FEMA Expert Panel's

Statement on Conflict of Interest Protections and Procedures is available at https://www.femaflavor.org/gras#conflict. Authors Davidsen, Harman, Lu and Taylor are employed by Verto Solutions, which provides scientific and management support services to FEMA.

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Appendix A. Supplementary data

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SAFETY DATA SHEET

according to Regulation (EC) No. 1907/2006

Version 6.4
Revision Date 27.09.2023
Print Date 17.03.2025
GENERIC EU MSDS - NO COUNTRY SPECIFIC DATA - NO OEL DATA

SECTION 1: Identification of the substance/mixture and of the company/undertaking

1.1 Product identifiers

Product name	:	Cardamom oil
Product Number	:	W224123
Brand	:	Aldrich
REACH No.	:	A registration number is not available for this substance as the substance or its uses are exempted from registration, the annual tonnage does not require a registration or the registration is envisaged for a later registration deadline.
CAS-No.	:	8000-66-6

1.2 Relevant identified uses of the substance or mixture and uses advised against

Identified uses	:	Laboratory chemicals, Manufacture of substances

1.3 Details of the supplier of the safety data sheet

Company	:	Sigma-Aldrich Chemie GmbH Industriestrasse 25 CH-9471 BUCHS
Telephone	:	+41 81 755 2511
Fax	:	+41 81 756 5449
E-mail address	:	technischerservice@merckgroup.com

1.4 Emergency telephone

Emergency Phone #	:	+41 43-508-2011 (CHEMTREC) +41 44-251-5151 (Tox-Zentrum) 145(Tox Info Suisse)

SECTION 2: Hazards identification

2.1 Classification of the substance or mixture

Classification according to Regulation (EC) No 1272/2008
Flammable liquids (Category 3), H226

For the full text of the H-Statements mentioned in this Section, see Section 16.

2.2 Label elements

Labelling according Regulation (EC) No 1272/2008

Pictogram	
Signal Word	Warning
Hazard statement(s) H226	Flammable liquid and vapor.
Precautionary statement(s) P210	Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
P233	Keep container tightly closed.
P240	Ground and bond container and receiving equipment.
P241	Use explosion-proof electrical/ ventilating/ lighting/ equipment.
P242	Use non-sparking tools.
P243	Take action to prevent static discharges.
Supplemental Hazard Statements	none

Reduced Labeling (<= 125 ml)

Pictogram	
Signal Word	Warning
Hazard statement(s)	none
Precautionary statement(s)	none
Supplemental Hazard Statements	none

2.3 Other hazards

This substance/mixture contains no components considered to be either persistent, bioaccumulative and toxic (PBT), or very persistent and very bioaccumulative (vPvB) at levels of 0.1% or higher.

Ecological information:
The substance/mixture does not contain components considered to have endocrine disrupting properties according to REACH Article 57(f) or Commission Delegated regulation (EU) 2017/2100 or Commission Regulation (EU) 2018/605 at levels of 0.1% or higher.

Toxicological information:
The substance/mixture does not contain components considered to have endocrine disrupting properties according to REACH Article 57(f) or Commission Delegated regulation (EU) 2017/2100 or Commission Regulation (EU) 2018/605 at levels of 0.1% or higher.

SECTION 3: Composition/information on ingredients

3.1 Substances

Synonyms : Elletaria cardamomum

Formula	:	C99H0000
CAS-No.	:	8000-66-6

No components need to be disclosed according to the applicable regulations.

For the full text of the H-Statements mentioned in this Section, see Section 16.

SECTION 4: First aid measures

4.1 Description of first-aid measures

General advice
Show this material safety data sheet to the doctor in attendance.

If inhaled
After inhalation: fresh air.

In case of skin contact
In case of skin contact: Take off immediately all contaminated clothing. Rinse skin with water/ shower.

In case of eye contact
After eye contact: rinse out with plenty of water. Remove contact lenses.

If swallowed
After swallowing: make victim drink water (two glasses at most). Consult doctor if feeling unwell.

4.2 Most important symptoms and effects, both acute and delayed
The most important known symptoms and effects are described in the labelling (see section 2.2) and/or in section 11

4.3 Indication of any immediate medical attention and special treatment needed
No data available

SECTION 5: Firefighting measures

5.1 Extinguishing media

Suitable extinguishing media
Foam Carbon dioxide (CO2) Dry powder

Unsuitable extinguishing media
For this substance/mixture no limitations of extinguishing agents are given.

5.2 Special hazards arising from the substance or mixture
Carbon oxides
Combustible.
Vapors are heavier than air and may spread along floors.
Forms explosive mixtures with air at elevated temperatures.
Development of hazardous combustion gases or vapours possible in the event of fire.

5.3 Advice for firefighters
In the event of fire, wear self-contained breathing apparatus.

5.4 Further information

Remove container from danger zone and cool with water. Prevent fire extinguishing water from contaminating surface water or the ground water system.

SECTION 6: Accidental release measures

6.1 Personal precautions, protective equipment and emergency procedures

Advice for non-emergency personnel: Do not breathe vapors, aerosols. Ensure adequate ventilation. Keep away from heat and sources of ignition. Evacuate the danger area, observe emergency procedures, consult an expert.
For personal protection see section 8.

6.2 Environmental precautions

Do not let product enter drains. Risk of explosion.

6.3 Methods and materials for containment and cleaning up

Cover drains. Collect, bind, and pump off spills. Observe possible material restrictions (see sections 7 and 10). Take up with liquid-absorbent material (e.g. Chemizorb®). Dispose of properly. Clean up affected area.

6.4 Reference to other sections

For disposal see section 13.

SECTION 7: Handling and storage

7.1 Precautions for safe handling

Advice on protection against fire and explosion
Keep away from open flames, hot surfaces and sources of ignition.Take precautionary measures against static discharge.

Hygiene measures
Change contaminated clothing. Wash hands after working with substance.
For precautions see section 2.2.

7.2 Conditions for safe storage, including any incompatibilities

Storage conditions
Keep container tightly closed in a dry and well-ventilated place. Keep away from heat and sources of ignition.

Storage class
Storage class (TRGS 510): 3: Flammable liquids

7.3 Specific end use(s)

Apart from the uses mentioned in section 1.2 no other specific uses are stipulated

SECTION 8: Exposure controls/personal protection

8.1 Control parameters

Ingredients with workplace control parameters

8.2 Exposure controls

Personal protective equipment

Eye/face protection
Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU). Safety glasses

Skin protection
required

Body Protection
Flame retardant antistatic protective clothing.

Respiratory protection
required when vapours/aerosols are generated.
Recommended Filter type: Filter type ABEK

The entrepeneur has to ensure that maintenance, cleaning and testing of respiratory protective devices are carried out according to the instructions of the producer. These measures have to be properly documented.

Control of environmental exposure
Do not let product enter drains. Risk of explosion.

SECTION 9: Physical and chemical properties

9.1 Information on basic physical and chemical properties

a)	Physical state	liquid
b)	Color	No data available
c)	Odor	No data available
d)	Melting point/freezing point	No data available
e)	Initial boiling point and boiling range	188 °C at 1.013 hPa - lit.
f)	Flammability (solid, gas)	No data available
g)	Upper/lower flammability or explosive limits	No data available
h)	Flash point	56,67 °C - closed cup
i)	Autoignition temperature	No data available

j)	Decomposition temperature	No data available
k)	pH	No data available
l)	Viscosity	Viscosity, kinematic: No data available Viscosity, dynamic: No data available
m)	Water solubility	No data available
n)	Partition coefficient: n-octanol/water	No data available
o)	Vapor pressure	No data available
p)	Density	0,924 g/cm3 at 25 °C - lit.
	Relative density	No data available
q)	Relative vapor density	No data available
r)	Particle characteristics	No data available
s)	Explosive properties	No data available
t)	Oxidizing properties	No data available

9.2 Other safety information
No data available

SECTION 10: Stability and reactivity

10.1 Reactivity
Vapor/air-mixtures are explosive at intense warming.

10.2 Chemical stability
The product is chemically stable under standard ambient conditions (room temperature) .

10.3 Possibility of hazardous reactions
No data available

10.4 Conditions to avoid
Heating.

10.5 Incompatible materials
Strong oxidizing agents

10.6 Hazardous decomposition products
In the event of fire: see section 5

SECTION 11: Toxicological information

11.1 Information on toxicological effects

Acute toxicity
LD50 Oral - Rat - 5.000 mg/kg
Inhalation: No data available
LD50 Dermal - Rabbit - > 5.000 mg/kg

Skin corrosion/irritation
Remarks: No data available

Serious eye damage/eye irritation
Remarks: No data available

Respiratory or skin sensitization
No data available

Germ cell mutagenicity
No data available

Carcinogenicity
No data available

Reproductive toxicity
No data available

Specific target organ toxicity - single exposure
No data available

Specific target organ toxicity - repeated exposure
No data available

Aspiration hazard
No data available

11.2 Additional Information

Endocrine disrupting properties

Product:

Assessment	The substance/mixture does not contain components considered to have endocrine disrupting properties according to REACH Article 57(f) or Commission Delegated regulation (EU) 2017/2100 or Commission Regulation (EU) 2018/605 at levels of 0.1% or higher.

RTECS: FH4550300
To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

SECTION 12: Ecological information

12.1 Toxicity

No data available

12.2 Persistence and degradability
No data available

12.3 Bioaccumulative potential
No data available

12.4 Mobility in soil
No data available

12.5 Results of PBT and vPvB assessment
This substance/mixture contains no components considered to be either persistent, bioaccumulative and toxic (PBT), or very persistent and very bioaccumulative (vPvB) at levels of 0.1% or higher.

12.6 Endocrine disrupting properties
Product:

Assessment	:	The substance/mixture does not contain components considered to have endocrine disrupting properties according to REACH Article 57(f) or Commission Delegated regulation (EU) 2017/2100 or Commission Regulation (EU) 2018/605 at levels of 0.1% or higher.

12.7 Other adverse effects
No data available

SECTION 13: Disposal considerations

13.1 Waste treatment methods
No data available

SECTION 14: Transport information

14.1 UN number
ADR/RID: 1993 IMDG: 1993 IATA: 1993

14.2 UN proper shipping name
ADR/RID: FLAMMABLE LIQUID, N.O.S. (Cardamom oil)
IMDG: FLAMMABLE LIQUID, N.O.S. (Cardamom oil)
IATA: Flammable liquid, n.o.s. (Cardamom oil)

14.3 Transport hazard class(es)
ADR/RID: 3 IMDG: 3 IATA: 3

14.4 Packaging group
ADR/RID: III IMDG: III IATA: III

14.5 Environmental hazards
ADR/RID: no IMDG Marine pollutant: no IATA: no

14.6 Special precautions for user

Tunnel restriction code : (D/E)

Further information : No data available

SECTION 15: Regulatory information

15.1 Safety, health and environmental regulations/legislation specific for the substance or mixture

This material safety data sheet complies with the requirements of Regulation (EC) No. 1907/2006.

National legislation

Seveso III: Directive 2012/18/EU of the European Parliament and of the Council on the control of major-accident hazards involving dangerous substances.	P5c	FLAMMABLE LIQUIDS

Other regulations

Take note of Dir 94/33/EC on the protection of young people at work.

15.2 Chemical Safety Assessment

For this product a chemical safety assessment was not carried out

SECTION 16: Other information

Full text of H-Statements referred to under sections 2 and 3.

H226	Flammable liquid and vapor.

Full text of other abbreviations

ADN - European Agreement concerning the International Carriage of Dangerous Goods by Inland Waterways; ADR - Agreement concerning the International Carriage of Dangerous Goods by Road; AIIC - Australian Inventory of Industrial Chemicals; ASTM - American Society for the Testing of Materials; bw - Body weight; CMR - Carcinogen, Mutagen or Reproductive Toxicant; DIN - Standard of the German Institute for Standardisation; DSL - Domestic Substances List (Canada); ECx - Concentration associated with x% response; ELx - Loading rate associated with x% response; EmS - Emergency Schedule; ENCS - Existing and New Chemical Substances (Japan); ErCx - Concentration associated with x% growth rate response; GHS - Globally Harmonized System; GLP - Good Laboratory Practice; IARC - International Agency for Research on Cancer; IATA - International Air Transport Association; IBC - International Code for the Construction and Equipment of Ships carrying Dangerous Chemicals in Bulk; IC50 - Half maximal inhibitory concentration; ICAO - International Civil Aviation Organization; IECSC - Inventory of Existing Chemical Substances in China; IMDG - International Maritime Dangerous Goods; IMO - International Maritime Organization; ISHL - Industrial Safety and Health Law (Japan); ISO - International Organisation for Standardization; KECI - Korea Existing Chemicals Inventory; LC50 - Lethal Concentration to 50 % of a test population; LD50 - Lethal Dose to 50% of a test population (Median Lethal Dose); MARPOL - International Convention for the Prevention of Pollution from Ships; n.o.s. - Not Otherwise Specified; NO(A)EC - No Observed (Adverse) Effect Concentration; NO(A)EL - No Observed (Adverse) Effect Level; NOELR - No Observable Effect Loading Rate; NZIoC - New Zealand Inventory of Chemicals; OECD - Organization for Economic Co-operation and Development; OPPTS - Office of Chemical Safety and Pollution Prevention; PBT - Persistent, Bioaccumulative and Toxic substance; PICCS - Philippines Inventory of Chemicals and Chemical Substances; (Q)SAR - (Quantitative) Structure Activity Relationship; REACH - Regulation (EC) No 1907/2006 of the European Parliament and of the Council concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals; RID - Regulations concerning the International Carriage of Dangerous Goods by Rail; SADT - Self-Accelerating Decomposition Temperature; SDS - Safety Data Sheet; TCSI - Taiwan Chemical Substance Inventory; TECI - Thailand Existing Chemicals Inventory; TSCA - Toxic Substances Control Act (United States); UN - United Nations; UNRTDG - United Nations Recommendations on the Transport of Dangerous Goods; vPvB - Very Persistent and Very Bioaccumulative

Further information

The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. The information in this document is based on the present state of our knowledge and is applicable to the product with regard to appropriate safety precautions. It does not represent any guarantee of the properties of the product. Sigma-Aldrich Corporation and its Affiliates shall not be held liable for any damage resulting from handling or from contact with the above product. See www.sigma-aldrich.com and/or the reverse side of invoice or packing slip for additional terms and conditions of sale.

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