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Mutagenicity and Carcinogenicity Prediction of Compounds from Cardamom (*Elettaria cardamom* Maton)

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Abstract

In silico approaches are currently not employed in any of the spices to study the toxicity. The aim of this study is to find the most efficacious molecule which does not have any adverse effects. In the present study one hundred and eight compounds from cardamom were used to predict mutagenicity and carcinogenecity. The results of these studies indicate that only four compounds are non-mutagenic and non-carcinogenic. The rest of the compounds do not have the characteristics necessary to become therapeutic agents have been identified early and prevented (i.e., the fail early, fail fast approach) from entering the drug development process.

Introduction

Herbs and spices have been an essential factor in health care through the ages in all cultures. Many crude drugs are used medicinally because of their volatile oil content or other chemical constituents that posses biological activities. Cardamom is very popular as a spice and food additive because of its delicious flavour. The constituents of its volatile oil are responsible for the flavour and fragrance. It also possesses carminative, stomachic and antimicrobial actions. These biological activities bring about many advantages to the seasoned and prepared foods. Apart from this, cardamom finds application in the indigenous systems of medicine (Ravindran and Madhusoodanan, 2002).

Cardamom seed oil is obtained naturally from dried ripe seeds of *Elettaria cardamomum*. The essential oil (2-8%) contains eucalyptol (cineol), sabinene, d,a-terpineol and acetate, borneol, etc. The fixed oil (1-2%) consisits of glycerides of oleic, stearic, linolenic, palmitic, caprylic and caproic acids. It is used in the flavouring of liqueurs. Essential oil and their constituents (the resultant of secondary metabolism in plants) have been shown to be a potent source of botanical pesticide. The toxicity of a large number of essential oils and their constituents has been evaluated against a number of bruchid pests (Keita, *et al.*, 2000, 2001, Tripathi *et al.*, 2002). Plant essential oils and their constituents in relation to contact and fumigant insecticidal actions have been well demonstrated against stored product pests. Especially their main compounds monoterpenoids, offer promising alternatives to classical fumigants (Papachristos & Stamopoulos, 2003) and also have some effects on biological parameters such as growth rate, life span and reproduction (Pascual-Villalobos, 1996). Cardamom oil is shown to have antibacterial and antifungal action. Badei *et al.* (1991a,b) studied the chemical composition, physicochemical properties and anti-microbial activity of dried fruits of cardamom to assess the potential usefulness of cardamom oil as a food preservative. The antimicrobial effect of the oil was tested against 9 bacterial strains, 1 fungus and 1 yeast, the oil was 28.9 % as effective as phenol.

To a small extent it is used in flavouring cigarette and tobacco (Ravindran and Madhusoodanan, 2002). Cardamom is used as an adjuvant to carminative drugs. It is officially recognized in British and US pharmacopoeias and used as an aromatic stimulant, carminative and flavouring agent. Cardamom seed oil is a common cosmetic ingredient. This material appears on the list of “Permitted Additives to Tobacco Products in the United Kingdom" (Department of Health, 2003) at a

maximum level permitted for inclusion in cigarettes of 0.15 % w/w tobacco. However cardamom seed oil (CAS No. 8000-66-6) is currently used worldwide at levels below 5 ppm in selected cigarette brands manufactured by Philip Morris International Inc., New York. Toxic effects on humans are not currently available, because as a food flavouring additive, the material has been assessed under the provisions of the Federal Food, Drug and Cosmetic Act, section 201 (s), by the Expert Committee of the USA Flavour and Extract manufacturer's Association (FEMA), to be generally recognized as safe (GRAS) under current conditions of use. In contrast, it was found in literature that it can also be used to ease cigarette addiction. Eating a few seeds of cardamom can safely be recommended to initially minimize the number of cigarettes being smoked and slowly the smoker may give up the chronic addiction to chain smoking (Peter, 2001).

Ironically, in a maximization test, a concentration of 4% of cardamom seed oil in petrolatum produced no sensitization reactions in 25 male volunteers (Kligman, 1973 cited in Opdyke, 1979). In a study of 25 workers in a spice factory, one worker was positive to cardamom on patch-test (Meding, 1993). In control test on 22 dermatitis patients without occupational exposure, one patient reacted to cardamom. No phototoxic effects were reported for cardamom seed oil (Urbach and Forbes, 1972 cited in Opdyke, 1979). But it has been identified the lethal dose in rat and rabbit as well as the genetic effects and mutations in bacteria. The present study was carried out to verify the contradictory statements pertaining to cardamom. It is well known that *in silico* approaches are comparatively cheaper than *in vivo* and *in vitro* screenings. Hence in the present study *in silico* methods were used to test one hundred and eight chemical structures from cardamom essential oil.

Methodology

Data collection

The aim of this study is to screen the diverse array of chemical compounds for removing non-drug-like compounds from the drug discovery lifecycle in the early stages. "Fail early and fail fast" is the current paradigm that the pharmaceutical industry has adopted widely. To achieve this, 108 chemical compounds from cardamom were collected from literature of reported compounds and from the NCBI PubChem database (http://pubchem.ncbi.nlm.nih.gov/).

Ames mutagenicity assay

Mortelmans and Zeiger (2000) described a short-term bacterial mutation assay caused by chemical substances. According to the data set of National Toxicology Program (NTP), the built biological model for toxicity prediction includes 3 strains: TA98, TA100, and TA1535.

Rodent carcinogenicity

The Predictive Toxicology Challenge (PTC) was initiated for the development of advanced technology for predictive toxicology models. We have used computational models for carcinogenicity prediction created by Helma and Kramer (2003) with the data set of both National Toxicology Program (NTP) (Benigni, 1997) and Food and Drug Administration (FDA).

Results & Discussion

The following compounds were found to be non-mutagenic in the biological model for 3 strains: TA98, TA100, and TA1535 [**Table 1**].

1-decanol, 1-heptanol, 1-hexanol, 1-octanol, a-ylangene, ß-guriunene, cedrol, citronellal, decanal, decyl acetate, dodecyl acetate, eicosanoic acid, farnesol, farnesyl acetone, geranyl acetone, humulene, octyl acetate, trans-2-cis-6-dodecadienal, trans-farnesol and undecan-2-one.

The following compounds are found to be non-carcinogenic in the computational model for Rodent carcinogenicity prediction [**Table 1**].

1-heptanol, 1-hexanol, 2methyl-3-buten-2-ol, 2-methylbutanal, 3-methylbutanal, a,p-dimethylstyrene, a-terpinyl acetate, carvone oxide, decanal, delta-terpineol, ethyl 2-hydroxyhexanoate, hexanal, nonanal, oct-1-en-3-ol, octanal, p-dimethylstyrene, phenol, p-menth-8-en-2-ol, terpinyl acetate, tetrahydrolinalool, thymol, trans-2-butenal, trans-dec-2-enal, trans-nerolidol and trans-oct-2-enal.

The mutagenecity and carcinogenicity prediction of the analyzed data set of 108 compounds yielded 1-heptanol, 1-hexanol and decanal, they were found to be non-mutagenic as well as non-carcinogenic. Hence, we conclude that these compounds may be a lead for drug discovery. All other compounds were predicted as mutagenic and carcinogenic.

Toxicity of essential oil from cardamom was investigated against the cowpea weevil, *Callosobruchus maculatus* (Fab.) adults (an important pest of several pulses), through contact and fumigation bioassay (Mahfuz and Khalequzzaman, 2007). In the contact bioassay the toxicity of cardamom oil was higher than neem. In the fumigation bioassay, the efficacy in respect of the toxicity, cardamom oil was higher than neem and eucalyptus oils. This confirms that cardamom is a good fumigant.

Currently, none of the human studies was conducted on the health effects of ingredients used in cigarette manufacture, studies have been conducted using scientifically accepted *in vitro* and *in vivo* toxicity assays with various ingredient mixtures. These studies show there is no meaningful difference in the composition or toxicity of smoke when the smoke from cigarettes with added ingredients is compared to the smoke from cigarettes without added ingredients. These findings are supported by similar studies from the published literature on burnt material (Gaworski *et al*., 1998, 1999; Carmines, 2002; Rustemeier *et al*., 2002 ; Roemer *et al.*, 2002 ; Vanscheeuwijck *et al*., 2002). But in contrast, the *in silico* studies showed that most of the chemicals from the cardamom were mutagenic and carcinogenic [**Table 1**]. This is in concordance with the study of Meding (1993) and moreover several research works illustrated that essential oils and their constituents may have potential as alternative compounds to currently used fumigants (Huang *et al*., 2000; Tunc *et al*., 2000; Lee *et al*., 2001a, b).

Conclusion

The results of these studies indicate that only four compounds are non-mutagenic and non-carcinogenic. The rest of the compounds do not have the characteristics necessary to become therapeutic agents have been identified early and prevented (i.e., the fail early, fail fast approach) from entering the drug development process. Because removing non-drug-like compounds from the drug discovery lifecycle in the early stages can lead to tremendous savings of resources (Cheng and Merz, 2003).

Table 1: Toxicity prediction profile of compounds from *Cardamom.*

Sl No	Chemical Compounds	Ames Mutagenicity test							Rodent Carcinogenicity		
		-S9			+S9			Total			Total
		TA98	TA100	TA1535	TA98	TA100	TA1535	Result	Mouse	Rat	Result
1	(E)-limonene oxide	+	-	+	-	+	+	+	+	+	+
2	(Z)-b-ocimene	+	-	-	+	-	-	+	+	+	+
3	1,3,8-menthatriene	+	-	-	+	+	-	+	+	+	+
4	1,4-cineole	-	-	-	-	-	+	+	+	+	+
5	1-decanol	-	-	-	-	-	-	-	+	-	+
6	1-heptanol	-	-	-	-	-	-	-	-	-	-
7	1-hexanol	-	-	-	-	-	-	-	-	-	-
8	1-nonanol	-	-	-	-	-	-	-	+	-	+
9	1-octanol	-	-	-	-	-	-	-	+	-	+
10	2,3-dehydro-1,8-cineole	-	-	-	-	-	+	+	+	+	+
11	2methyl-3-buten-2-ol	-	-	+	-	+	+	+	-	-	-
12	2-methylbutan-1-ol	-	+	+	-	-	+	+	+	-	+
13	2-methylbutanal	-	+	+	-	-	+	+	-	-	-
14	2-methylpropan-1-ol	-	+	+	-	-	+	+	+	-	+
15	3-methylbutanal	-	+	+	-	-	+	+	-	-	-
16	3-methylpentan-2-ol	-	-	+	-	-	+	+	+	+	+
17	4-thujanol	-	-	-	-	-	+	+	-	+	+
18	6-methyl-5-hepten-2-one	-	+	+	+	-	-	+	-	+	+
19	a-copaene	-	-	-	-	-	-	-	-	+	+
20	a,p-dimethylstyrene	+	-	-	+	+	-	+	-	-	-

21	a-ionone	-	-	-	-	-	+	+	+	-	+
22	a_phellandrene	+	-	-	+	+	-	+	+	+	+
23	a_pinene	-	-	-	-	+	-	+	-	+	+
24	a_terpinene	+	-	-	+	-	-	+	+	+	+
25	a_terpineol	-	-	-	-	-	+	+	-	-	-
26	a-terpinyl acetate	-	-	-	-	-	+	+	-	-	-
27	a-terpinyl propionate	-	-	-	-	-	+	+	+	-	+
28	a-thujene	-	-	+	-	+	-	+	-	+	+
		TA98	**TA100**	**TA1535**	**TA98**	**TA100**	**TA1535**	**Result**	**Mouse**	**Rat**	**Result**
29	a-ylangene	-	-	-	-	-	-	-	-	+	+
30	b_caryophyllene	+	-	-	-	+	-	+	-	+	+
31	b-elemene	+	-	-	-	-	-	+	-	+	+
32	b-gurjunene	-	-	-	-	-	-	-	+	+	+
33	b_pinene	+	-	-	-	+	-	+	-	+	+
34	bornyl acetate	-	-	+	-	-	+	+	-	+	+
35	Camphene	+	-	-	-	+	-	+	-	+	+
36	Camphor	-	-	-	-	-	+	+	-	+	+
37	Carvacrol	+	-	+	+	+	+	+	-	-	-
38	Citronellol	-	-	-	-	-	-	-	+	-	+
39	carvone oxide	+	-	+	-	+	+	+	-	-	-
40	Cedrol	-	-	-	-	-	-	-	-	+	+
41	cis-carveol	-	-	-	+	-	+	+	+	-	+
42	cis-linalol oxide	-	-	+	-	+	+	+	+	+	+
43	cis-ocimene	+	-	-	+	-	-	+	+	+	+
44	Citronellal	-	-	-	-	-	-	-	+	-	+
45	Cubenol	-	-	-	-	-	+	+	+	-	+
46	Decanal	-	-	-	-	-	-	-	-	-	-
47	decyl acetate	-	-	-	-	-	-	-	+	-	+
48	Delta-cadinene	+	-	-	-	-	-	+	+	+	+
49	delta-terpineol	-	-	-	-	-	+	+	-	-	-
50	dodecyl acetate	-	-	-	-	-	-	-	+	-	+
51	eicosanoic acid	-	-	-	-	-	-	-	-	+	+
52	ethyl 2-hydroxyhexanoate	-	+	+	-	-	-	+	-	-	-
53	Farnesol	-	-	-	-	-	-	-	+	-	+
54	farnesyl acetone	-	-	-	-	-	-	-	+	+	+
55	gamma-cadinene	+	-	-	-	-	-	+	-	+	+
56	gamma-terpinene	-	-	-	+	-	-	+	+	+	+
57	geranyl acetone	-	-	-	-	-	-	-	-	+	+
58	germacrene-D	+	-	-	-	-	-	+	+	+	+
59	Globulol	-	-	-	-	+	+	+	-	+	+
60	g-murolene	+	-	-	-	-	-	+	-	+	+
		TA98	**TA100**	**TA1535**	**TA98**	**TA100**	**TA1535**	**Result**	**Mouse**	**Rat**	**Result**
61	Guaiene	+	-	-	-	-	-	+	-	+	+
62	hexadecanoic acid	-	-	+	-	-	-	+	-	+	+
63	Hexanal	-	+	+	-	-	-	+	-	-	-
64	Humulene	-	-	-	-	-	-	-	+	+	+
65	isopiperitenol	+	-	-	+	-	+	+	+	-	+
66	Isosafrole	+	+	+	+	+	-	+	+	+	+
67	Limonene	+	-	-	+	-	-	+	-	+	+
68	Linalool	-	-	+	-	+	+	+	-	-	+
69	limonene-1,2-epoxide	+	-	+	-	+	+	+	+	+	+
70	linalyl acetate	-	-	+	-	-	+	+	+	+	+
71	menthatriene	+	-	-	+	+	-	+	+	+	+
72	methyl eugenol	-	-	+	+	+	+	+	+	+	+
73	Myrcene	-	-	+	+	-	-	+	-	+	+
74	Nerol	-	+	-	+	-	-	+	+	-	+
75	Nerolidol	-	-	-	-	-	-	-	-	-	-
76	neryl acetate	-	+	-	-	-	-	+	+	-	+
77	neryl propionate	-	+	-	-	-	-	+	+	-	+
78	Nonanal	-	-	+	-	-	-	+	-	-	-
79	oct-1-en-3-ol	-	+	+	-	-	-	+	-	-	-
80	octadecanoic acid	-	-	+	-	-	-	+	-	+	+
81	Octanal	-	-	+	-	-	-	+	-	-	-
82	octyl acetate	-	-	-	-	-	-	-	+	-	+
83	p-dimethylstyrene	+	-	-	+	+	-	+	-	-	-
84	Perillene	-	+	+	+	-	+	+	+	+	+
85	Phenol	+	+	+	+	+	-	+	-	-	-

86	Piperitenone	+	+	-	+	-	+	+	+	+	+
87	p-menth-8-en-2-ol	-	-	-	-	-	+	+	-	-	-
88	Sabinene	+	-	+	+	+	-	+	+	+	+
89	terpinen-4-ol	+	-	-	-	-	+	+	+	-	+
90	Terpinolene	-	-	-	+	-	-	+	+	+	+
91	terpinyl acetate	-	-	-	-	-	+	+	-	-	-
92	tetrahydrolinalool	-	-	+	-	+	+	+	-	-	-
93	Thymol	+	+	-	+	+	+	+	-	-	-
94	T-muurolol	-	-	-	-	-	+	+	+	+	+
95	Toluene										
96	trans-2-butenal	+	+	+	+	-	+	+	-	-	-
97	trans-2-cis-6-dodecadienal	-	-	-	-	-	-	-	+	+	+
98	trans-carveol	-	-	-	+	-	+	+	+	-	+
99	trans-dec-2-enal	-	+	-	-	-	-	+	-	-	-
100	trans-farnesol	-	-	-	-	-	-	-	+	-	+
101	trans-linalool oxide	+	-	+	-	-	+	+	+	+	+
102	trans-nerolidol	-	-	-	-	-	+	+	-	-	-
103	trans-ocimene	+	-	-	+	-	-	+	+	+	+
104	trans-oct-2-enal	-	+	+	+	-	-	+	-	-	-
105	trans-p-mentha-2,8-dien-1-ol	-	+	-	+	-	-	+	+	+	+
106	Tricyclene	-	-	-	-	+	-	+	-	+	+
107	undecan-2-one	-	-	-	-	-	-	-	+	-	+
108	Valencene	+	-	-	-	-	-	+	-	+	+

Note: '+' indicates the presence, **'-'** indicates the absence of mutagenicity and carcinogenicity.

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