



In Silico Taste, Cytochrome and Toxicity Prediction Study of Major Chamomile Constituents

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Article information

Article history:

Received: October, 30, 2021

Accepted: November, 12, 2021

Available online: December, 14, 2021

Keywords:

Chamomile,
Cytochrome enzymes,
Toxicity,
In Silico

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DOI:

<https://doi.org/10.53523/ijoirVol8I3ID106>

Abstract

Chamomile is an ancient herb used for various medications. It contains many bioactive constituents such as volatile oils, terpenoids, flavonoids, lactones, acid esters, glycosides and others. By reviewing many references, a confliction appeared of using Chamomile preparations to treat primary teeth eruption symptoms as a therapy administered by pediatric dentist or pediatrician. In this study, thirteen bioactive constituents (α -bisabolol (B), chamazulene (C), umbelliferone (U), apigenin (A), apigetrin (AT), apiin (AI), luteolin (L), quercetin (Q), quercimertrin (QT), rutin (R), α -cadinene (CD), α -farnesene (F), and matricarin (M) were subject to computational predication through various online websites to predicate their taste, activity towards several CYP450 enzymes and their action as Hepatotoxic, carcinogenic, immunotoxic, mutagenic, and cytotoxic compounds. Our calculations revealed several points such as high value of taste predication indicated that Chamomile constituents under study were with sour taste, did not classify as individual fatal compound Class (GHS) 1 or 2, 44.87% of them showed inhibition character toward specific cytochrome P450 enzyme while 43.59% were non- inhibition character, more than (0.5) probability predication of various cytochrome P450 enzymes gave a positive activity that may affect liver functions. Also, hepatotoxicity, carcinogenicity, immunotoxicity, mutagenicity, and cytotoxicity predictions were more than (0.5), unsaturated Chamomile constituent (Farnesene, F) had highest immune – and mutagenic properties whilst the disaccharide flavonoid (Rutin, R) had the highest Carcino-immunogenic response. According to the above notes, our conclusion is to use minimum concentration of Chamomile preparation for less period of time and lowest repeating intake that ensure effective treatment of teething symptoms under supervision of pediatricians with minimum side effect.

1. Introduction

Many herbs around the world are known to be used for medicinal applications, Chamomile is one of them. It classifies as *Asteraceae* or *Compositae* family member being in German and Roman varieties. Most uses of its extracts are for healing promotion or drinking as a tea. It interplays as curative, inhibitive, and preventive preparations because it contains many bioactive constituents such as volatile oils, terpenoids, flavonoids, lactones,

acid esters, glycosides and others. Chamomile toxic side effect is limited because of its slow initiation to reach maximum activity. Extraction conditions determine quality and quantity of these bioactives [1].

According to reference [2] in Contra-indication, warning section of German or Roman Chamomile "In view of the documented allergic reactions and cross sensitivities, Chamomile should be avoided by individual with a known hypersensitivity to any members of the *Asteraceae/ Compositae* family. In addition, Chamomile may precipitate an allergic reaction exacerbate existing symptoms in susceptible individuals (e.g. asthmatics). The use of Chamomile preparations for teething babies is not recommended". This medical notice is very important and it opens a gate to research question and answer. But it is conflicting with one of British Medical Journals (BMJ) founding [3] after studying 62 infants with teething symptoms which its results indicated "a significant effect of Chamomile water extract on relieving teething symptoms, especially pain which caused irritability, compared with placebo ($p < 0.0001$)". Also, steam distillation oil of Chamomile flower is administered to children at teething stage beside other medicinal activities was mentioned in [4]. Another reference [5] stated that "Chamomile products are also used to soothe inflammation of the mouth and gums, and for infant teething". Also, the same meaning was written by Sharafzadeh and Alizadeh [6]

To find an scientific answer to this confliction "Is (or not) recommended at this baby stage?", in Silico study was taken to predicate taste, activity of cytochromes, and toxicity of α -bisabolol, chamazulene, umbelliferone, apigenin, apigetrin, apiin, luteolin, quercetin, quercimertrin, rutin, α -cadinene, α -farnesene, and matricarin in Chamomile. This in Silico study was done by applying various online predication websites.

2. Experimental Procedure

Predication characters according to its online website. In this study, predication characters were taste (bitter, sweet, and sour) by <http://virtualtaste.charite.de> (Table (1)), activity of various cytochrome enzymes by <http://insilico-cyp.charite.de/SuperCYPsPred/> (Table (2)) and <https://preadmet.bmdrc.kr/adme/> (Table (3)) websites, and toxicity by https://tox-new.charite.de/prottox_II/ (Table (4)) website.

Bioactives in online predication methods

They were α -Bisabolol(B), chamazulene (C), umbelliferone (U), apigenin (A), apigetrin (PT), apiin (AI), luteolin (L), quercetin (Q), quercimertrin (QT), rutin (R), α -cadinene (CD), α -farnesene (F), and matricarin (M) that chosen as mainly representative bioactive compounds in Chamomile (Figure (1)).

3. Results and Discussion

Chamomile is characterized as low –growing apple scent expressing its Greek name *Chamos* (ground) and *melos* (apple) [7]. It presents a pleasant taste with various medicinal effects including anti- inflammatory, anticancer (skin, breast, ovarian, or prostate), antioxidant, antiphlogisisit, treatment of wound, acute viral nasopharyngitis (known as common cold), infant colic disorder, haemorrhoids, mucositis, osteoporosis, vaginitis, improving cardiac health, managing diabetes, sleep, sedation, anxiety, seizure, eczema, digestive disorders, diminishing hyperglycaemia – related oxidative stress, and others [1, 8, 9].

Numerical Taste predication (Table (1), Figure (2)) confirmed sour probability of the tested constituents ranged (0.537-1) for bitter, (0.546-0.989) for sweet, and (0.834-1) for sour character. These predication results were of individuals not as all in one mixture.

Table (1). Taste character of some Chamomile constituents according to <http://virtualtaste.charite.de> website.

Taste	B	C	U	A	AT	AI	L	Q	QT	R	CD	F	M
Bitter	0.583	0.738	0.625	0.998	0.544	0.65	1.0	1.0	0.537	0.746	0.852	0.687	0.842
Sweet	0.685	0.831	0.581	0.628	0.546	0.732	0.989	0.989	0.684	0.979	0.873	0.629	0.684
Sour	0.974	0.834	0.998	1.0	0.996	0.999	1.0	1.0	1.0	0.999	0.960	0.903	0.952

Table (2). Activity of Cytochrome enzymes towards bioactive Chamomile constituents according to <http://insilico-cyp.charite.de/SuperCYPsPred/> website.

Enzyme	B	C	U	A	AT	AI	L	Q	QT	R	CD	F	M	
MACCS	CYP1A2	0.955	0.675	0.657	1.0	0.959	0.957	1.0	1.0	1.0	0.983	0.85	0.944	0.914
	CYP2C19	0.81	0.679	0.813	0.998	0.963	0.859	0.779	0.779	0.996	0.993	0.769	0.944	0.907
	CYP2C9	0.553	0.606	0.609	0.812	0.862	0.676	0.993	0.993	0.928	0.901	0.662	0.717	0.693
	CYP2D6	0.876	0.757	0.914	0.891	0.947	0.781	0.85	0.85	0.965	0.928	0.688	0.777	0.806
	CYP3A4	0.897	0.915	0.953	1.0	1.0	0.968	0.794	0.794	1.0	0.999	0.951	1.0	0.888
Morgan	CYP1A2	0.918	0.546	0.519	1.0	0.821	0.824	0.986	0.961	0.833	0.729	0.79	0.897	0.942
	CYP2C19	0.909	0.793	0.898	0.672	0.873	0.85	0.886	0.9	0.933	0.971	0.864	0.909	0.953
	CYP2C9	0.791	0.5	0.733	0.63	0.938	0.883	0.678	1.0	0.869	0.928	0.817	0.622	0.904
	CYP2D6	0.722	0.534	0.717	0.868	0.903	0.842	0.868	0.533	0.846	0.854	0.601	0.757	0.85
	CYP3A4	0.859	0.72	0.847	0.894	0.95	0.828	0.579	0.751	0.895	0.824	0.918	0.902	0.842

Table (3). Cytochrome enzymes towards bioactive Chamomile constituents according to <https://preadmet.bmdrc.kr/adme/> website. (Inh.: inhibitor, Sub.: Substrate).

Enzyme	CYP2C19 inhibition	CYP2C9 inhibition	CYP2D6 inhibition	CYP2D6 substrate	CYP3A4 inhibition	CYP3A4 substrate
B	Inh.	Inh.	Non	Non	Non	Sub.
C	Inh.	Inh.	Non	Non	Inh.	Sub.
U	Inh.	Inh.	Non	Non	Non	Non
A	Inh.	Inh.	Non	Non	Non	Non
AT	Inh.	Inh.	Non	Non	Inh.	Weakly
AI	Inh.	Inh.	Non	Non	Inh.	Weakly
L	Inh.	Inh.	Non	Non	Inh.	Non
Q	Inh.	Inh.	Non	Non	Inh.	Non
QT	Inh.	Inh.	Non	Non	Inh.	Weakly
R	Inh.	Inh.	Non	Non	Inh.	Weakly
CD	Inh.	Inh.	Non	Non	Non	Sub.
F	Inh.	Inh.	Non	Non	Inh.	Sub.
M	Inh.	Inh.	Non	Non	Inh.	Sub.

Table (4). Various toxicity characters of bioactives in Chamomile according to https://tox-new.charite.de/prottox_II/ website. (Class: Predicated toxicity class; LD₅₀, Predicated LD₅₀, mg/Kg; Hepat., Hepatotoxicity; Carcino., Carcinogenicity; Immuno., Immunotoxicity; Mutag., Mutagenicity; Cyto., Cytotoxicity).

Property	B	C	U	A	AT	AI	L	Q	QT	R	CD	F	M
Class	4	4	6	5	5	5	5	3	5	5	5	5	3
LD ₅₀	1016	1220	10000	2500	5000	5000	3919	159	5000	5000	4400	3650	125
Hepat.	0.81	0.83	0.68	0.68	0.82	0.85	0.69	0.69	0.82	0.79	0.83	0.79	0.63
Carcino.	0.70	0.60	0.64	0.62	0.86	0.81	0.68	0.68	0.85	0.88	0.80	0.73	0.53
Immuno.	0.86	0.99	0.97	0.99	0.93	0.90	0.97	0.87	0.58	0.99	0.68	0.99	0.64
Mutag.	0.75	0.71	0.83	0.57	0.59	0.63	0.51	0.51	0.76	0.90	0.60	0.98	0.70
Cyto.	0.89	0.75	0.68	0.87	0.69	0.70	0.99	0.99	0.69	0.68	0.76	0.81	0.80

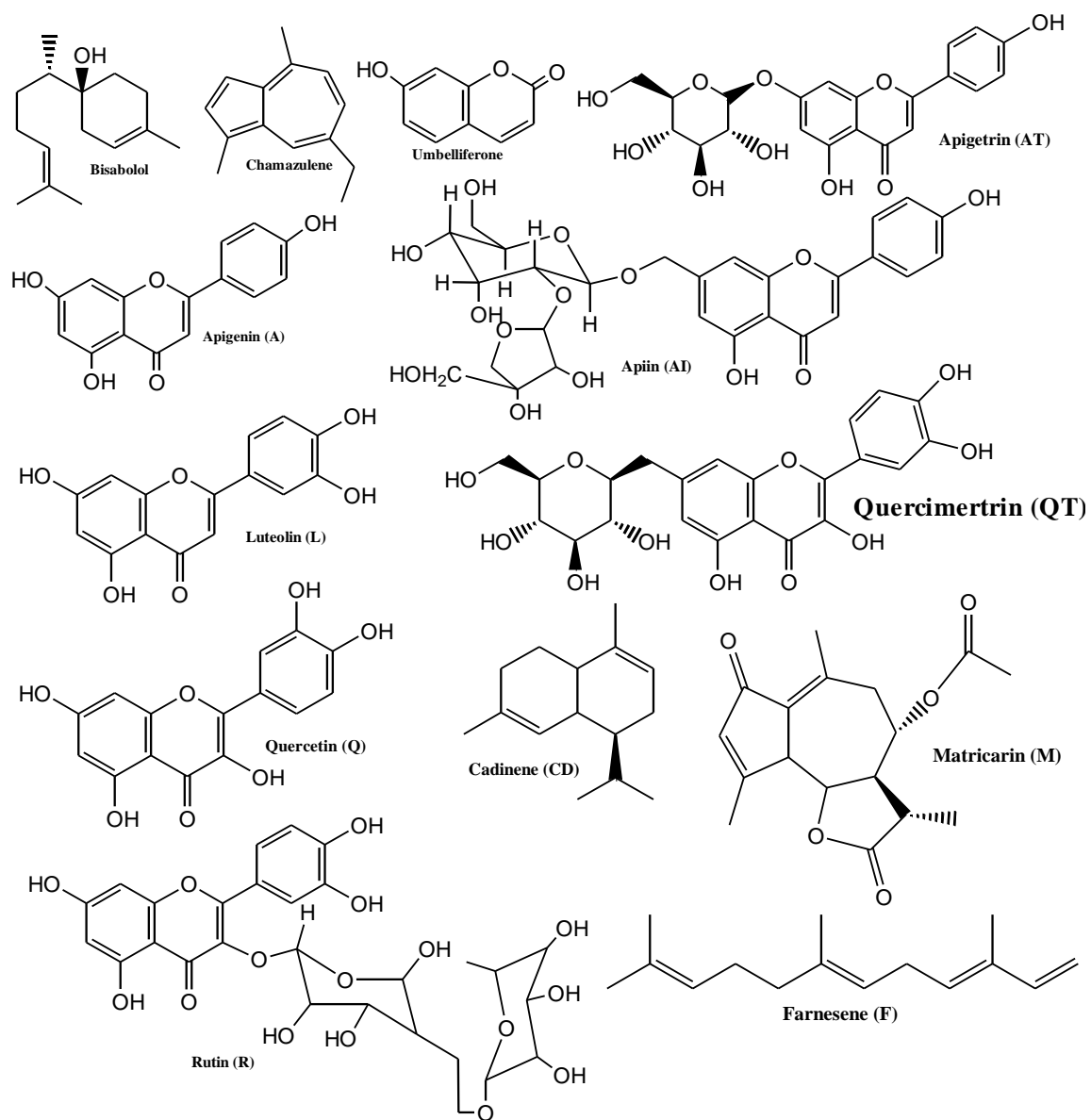


Figure (1). Bioactive constituents in Chamomile under study.

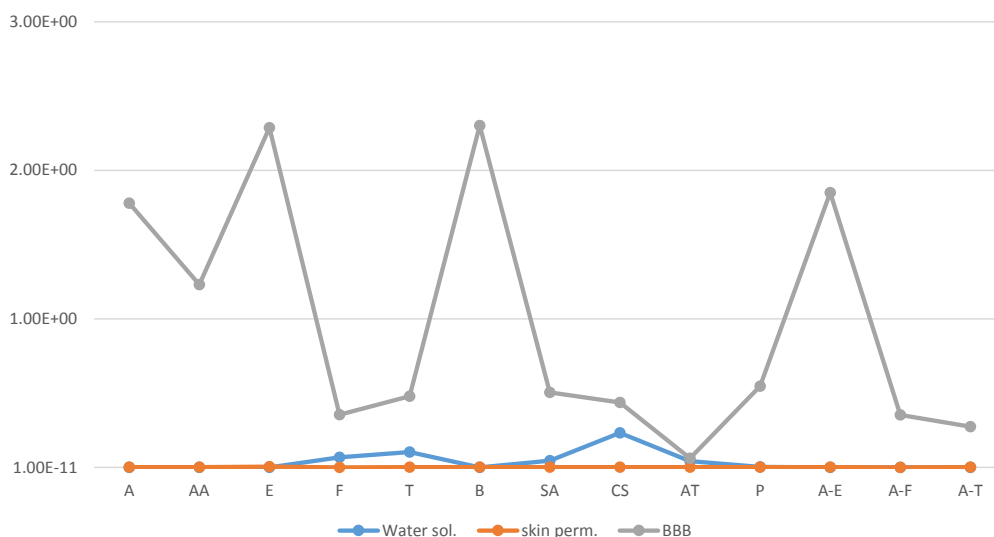


Figure (2). Taste character of Chamomile constituents under test.

Cytochrome P450 family is a superfamily of terminal oxidase enzymes composed of protein with heme group as cofactor found in all living kingdoms. Its coded enzymes based electron transfer (oxidation –reduction) chain like CYP1A2, CYP2C9, CYP2C19, and others that induction, inhibition, and competition properties specify for example drug interaction. So, CYP1A2 (Cytochrome P450 1A2) is member of this oxidase system as monooxygenase to metabolize xenobiotic, drugs, endogenous materials in body and synthesize lipids, cholesterol, steroids. Also, CYP2C19 is another monooxygenase (epoxygenase) works as liver enzyme protein in catalysis of xenobiotic metabolism [10, 11, 12].

Cytochrome activity with tested constituents was studied with two online website <http://insilico-cyp.charite.de/SuperCYPsPred/> (Table (2)) and <https://preadmet.bmdrc.kr/adme/> (Table (3)). According to Table (2), the results were calculated by two methodologies: Molecular Access System MACCS ("166 bit-long structural key descriptor in which each bit is associated with a specific structural pattern") and Molecular fingerprint (Morgan). The maximum difference between both methods was 0.326. Cytochrome response varied between all tested constituents but in general showed highest inhibition value with CYP3A4 (Table 2, Figures (3 & 4)). Also, both MACCS and Morgan fingerprint showed approximately same sequence.

The other website that predicate cytochrome inhibition did not show numerical data and did not compatible in cytochrome types that been calculated (Table (3)). Figure (5) represents Table (3) results by replace inhibitor predication with 2, weakly with 1, non- inhibitor with 0 and substrate with 3.

Both Table (3) and Figure (5) showed that CYP2C19 and CYP2C9 can be inhibited by all constituents under test. Additionally, CYP2D6 (Substrate and Inhibition) were with non- character for all tested compounds while CYP3A4 had been inhibited by all except B, U, A, and CD while substrate predication of the same cytochrome were with active action by B, C, CD, F, and M. Weakly inhibition response of CYP3A4 was predicated in AI, AT, QT, and R whilst U, A, L, Q showed substrate behaviour of the same protein enzyme.

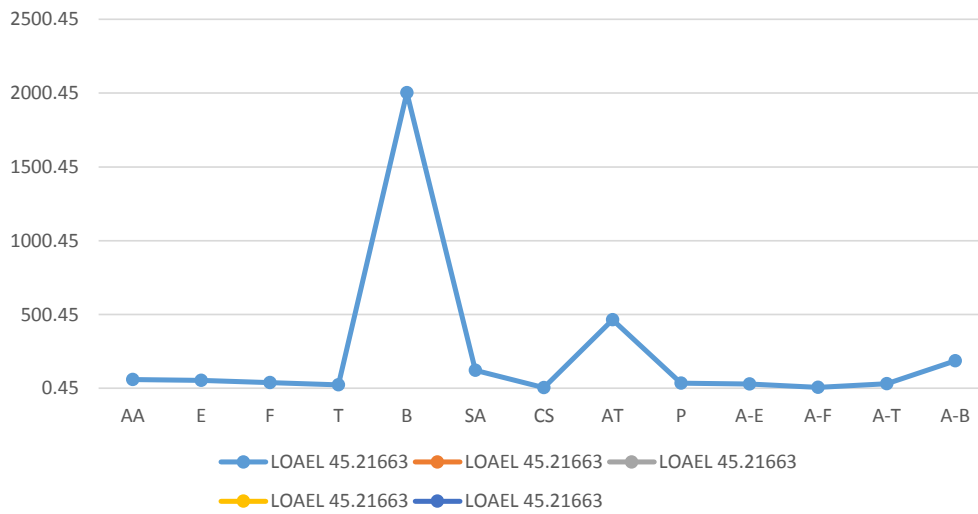


Figure (3). Cytochrome activity prediction according to MACCS method.

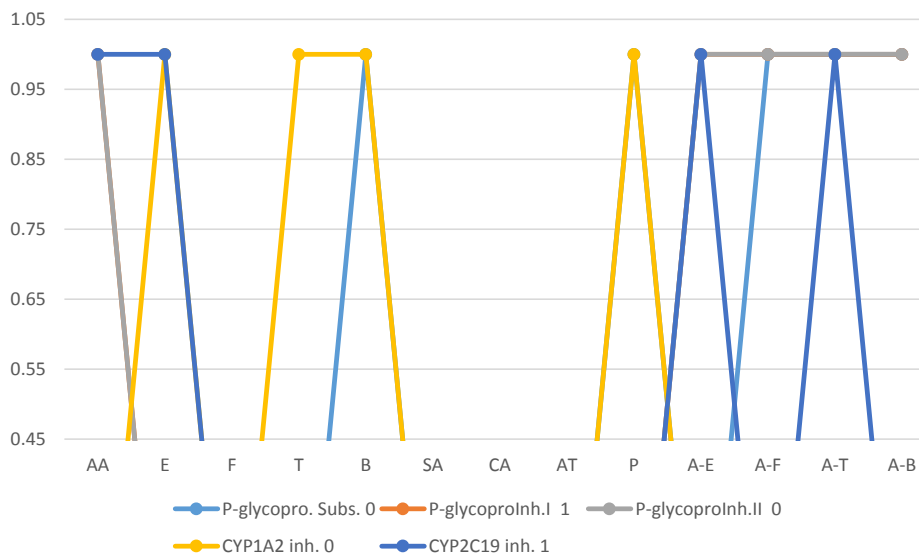


Figure (4). Cytochrome activity prediction according to Morgan method.

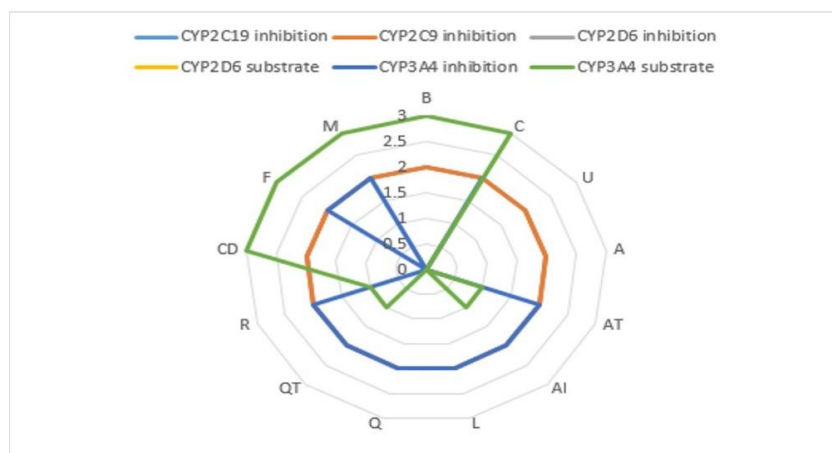


Figure (5). Representative results of Cytochrome inhibition by numbers instead of words (Inh. (2), weakly with 1, non- inhibitor with 0 and substrate with 3).

Toxicity characters of bioactives in Chamomile were predicated by https://tox-new.charite.de/prottox_II/ website and they toxicity class, Predicated LD₅₀, mg/Kg, Hepatotoxicity, Carcinogenicity, Immunotoxicity, Mutagenicity, and Cytotoxicity (Table (4), Figure (6)). Globally Harmonized System (GHS) categorizes toxicity classes to Class: 1, fatal if swallowed with LD₅₀= 5 mg/Kg or less, Class 2-fatal if swallowed (LD₅₀ not more than 50 mg/Kg), Class 3-toxic if swallowed (LD₅₀ not more 300 mg/Kg), Class 4- harmful if swallowed (LD₅₀ not more 2000 mg/Kg), Class 5- may be harmful if swallowed (LD₅₀ less than 5000 mg/Kg), and Class 6- nontoxic (LD₅₀ more than 5000 mg/Kg).

It is a good notice that Chamomile constituents under predication (Table (4)) are not presented in Class 1 or 2 (the fatal category). The other very good sign in this Table (4) is most of these compounds may be harmful if swallowed. Class 4 had be characterized in B and C while Class 3 to Q and M. Also, U has Class 6 the safer class. From Table (4) and Figure (6), other toxicity predictors were ranged as below:

- Hepatotoxicity: 0.63 (M) – 0.85 (AI).
- Carcinogenicity: 0.53 (M) – 0.88 (R).
- Immunotoxicity: 0.58 (QT) – 0.99 (C, A, R, F).
- Mutagenicity: 0.51 (L, Q) – 0.98 (F).
- Cytotoxicity: 0.68 (U, R) – 0.99 (L, Q).

Hepatotoxicity sign caused by chemical is important in human health and drug industry and this acute liver damage sign is resulting by increasing level of specific enzyme [13, 14]. M showed the lowest acute liver damage probability while AI was the highest with 0.85 Table (4) and Figure (6). The reason behind this increase of hepatotoxicity in AI is the presence of furanosyl and glucosyl moieties in the structure of this natural flavonoid (apigenin diglycosidyl flavone) resulting more oxygen atoms that increased interaction with liver active molecules compared to M.

When chemical inducing tumor after long time exposure and an accumulation in the target organ, this chemical identify as carcinogen and studying this sign requires in – life rat, mice, and then human testing of adsorption and metabolism [15, 16, 17]. Carcinogenic probability of γ - butyl lactone M was 0.53 as the least value compared to 0.88 of the citrus disaccharide flavonoid R that as the highest potential hazard (Table (4) and Figure (6)).

Immunotoxicity character of any chemical is endpoint study of *in Vitro*, *in Vivo* and / or computational models and represents the correlation between this chemical and food and cellular actions [18, 19]. Risk probability of immune system with C (bicyclic unsaturated hydrocarbon with aromatic character), A (flavonoid), R (flavonoid), or F (tetraene) was the highest compared with the lowest QT (flavonoid) as shown in Table (4) and Figure (6).

Exposure of environmental species to hazard causing heritable change of DNA is Mutagenicity. This term of securing nucleotide sequence can be screened by *in Vivo* and computational methods to ensure quality of chemical upon living organisms [17, 18, 21, 22, 23]. Cytogenetic character of the semi-identical flavonoids L and Q was 0.51 while α -sesquiterpene isomer (F) showed the highest mutagenic predication response (0.98) (Table (4) and Figure (6)).

In vitro biocompatibility of a primary cell with chemical is a reflection of its function after this biological interaction [24, 25]. Contrary to mutagenicity predication, the toxicity caused by the action of L and Q on living cell was 0.99 as the highest cytotoxicity compared to U and R with 0.68 in spite that Chamomile constituents (L, Q, U, and R) are flavonoids (Table (4) and Figure (6)).

It can be noticed from calculation that the unsaturated Chamomile constituent (F) had highest immune – and mutagenic properties. In addition, the disaccharide flavonoid (R) had the highest Carcino- immunogenic response.

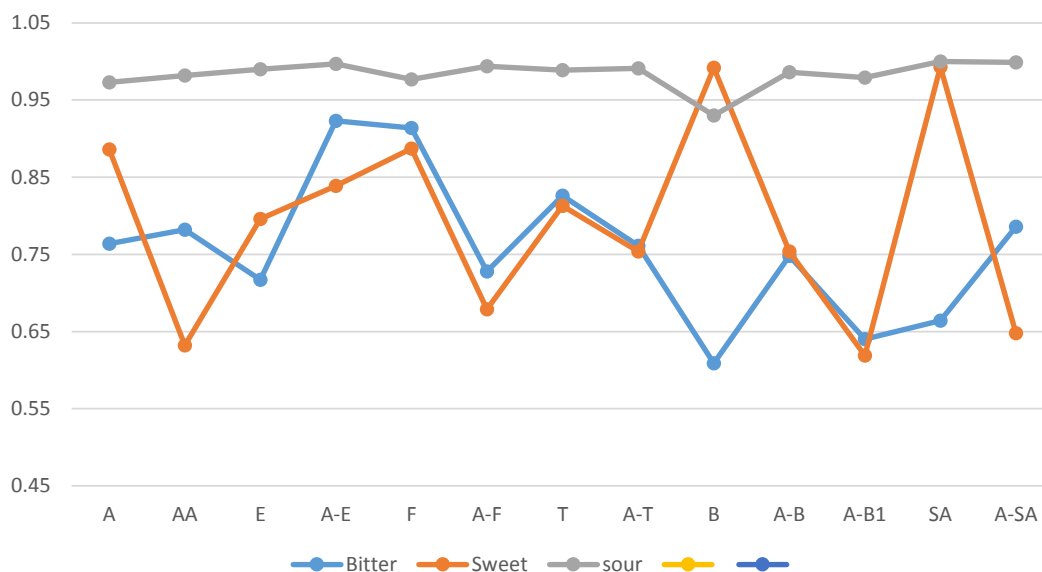


Figure (6). Toxicity predication of Chamomile constituents under test.

By back to the confliction between a notice in a scientific reference concerned of herbal medicine [2] and other published articles [3-6] that in general confirmed using Chamomile preparations to treat primary teeth eruption symptoms as a therapy administered by pediatric dentist or pediatrician followed by antipyretic prescription [26], our calculations revealed several points:

- High value of taste predication indicated that Chamomile constituents under study were with sour taste.
- The studied compounds did not classify as individual fatal compound Class (GHS) 1 or 2.
- 44.87% of them showed inhibition character toward specific cytochrome P450 enzyme while 43.59% were non- inhibition character.
- More than (0.5) probability predication of various cytochrome P450 enzymes gave a positive activity.
- Hepatotoxicity, carcinogenicity, immunotoxicity, mutagenicity, and cytotoxicity prediction probabilities were more than (0.5).
- Unsaturated Chamomile constituent (Farnesene, F) had highest immune – and mutagenic properties.

The disaccharide flavonoid (Rutin, R) had the highest Carcino- immunogenic response.

4. Conclusions

Thirteen bioactive Chamomile constituents were studied with different online website to predicate their taste, activity towards several CYP450 enzymes and their action as Hepatotoxic, carcinogenic, immunotoxic, mutagenic, and cytotoxic compounds. The computational models in these online website showed that these 13 compounds were not fatal as GHS Classification. They had a noticeable inhibition activity against various CYP450 enzymes that may affect liver functions. In general, probable Hepatotoxicity, carcinogenicity, immunotoxicity, mutagenicity, and cytotoxicity of them appeared especially Farnesene as the highest immun –mutagenic properties and Rutin as the highest Carcino- immunogenic response. According to the above notes, our conclusion is to use minimum concentration of Chamomile preparation for less period of time and lowest repeating intake that ensure effective treatment of teething symptoms under supervision of pediatricians with minimum side effect.

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