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ACTIVITY OF CLOVE AND FEVERFEW CONSTITUENTS TOWARDS TOOTHACHE: *IN SILICO* ANSWER.

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ABSTRACT

Clove and Feverfew are medicinal herbs in various applications screened *in vitro* and *in vivo*. Bio-Clove activity is recognizable in toothache, antispasmodic, capscaicin agonist, and join pain medications. Eugenol (E) as mainly bio-Clove compound; also found in feverfew; is the active player in ganglionar cell through enhancement of Calcium –Chloride channel. Both Clove and Feverfew plants contain many bioactives play remarkable roles as medicinal constituents.

In dental medication, it is important to specify toxicity of any chemical material and its role on human organs through inhibition, enhancement, metabolism, absorption, and other biological processes. In this study, computational online websites:https://tox-new.charite.de/protoxII/, http://biosig.unimelb.edu.au/pkcsm/, and http://virtualtaste.charite.de were applied to predicate many effective properties as a first try in Iraqi studies especially in dental subject.

Toxicity class and LD50 predications of Clove and Feverfew bioactives are important characters in related medications. In this paper, online predications showed that Clove and Feverfew constituents were non- fatal class with an acceptable LD50 as good indication of safety intake individually or in mixture state under controlled quantities. These and other *in Silico* results in this study ensure using these bioactives in oral and skin treatment.

Rhamnetin (R) and Tanetin (T) in Clove and Artecanin (AN) in Feverfew had the lowest toxicological characters. This foundation and other calculations achieved as Quantitative Structural Activity Relationship - Adsorption, Distribution, Metabolism, Excretion, and Toxicity QSAR -ADMET characters confirmed non- inhibition predications of hERG I, CYP2C19, CYP2C9, CYP2D6, and CYP3A4 towards using Clove as medicinal type with limited cautions of side effects.

Primary indications were obtained to use Feverfew bioactives with higher limited cautions compared to Clove. Also, taste predication suggests highly sour presence so oral intake is in low concentration to avoid unlikely savour.

In conclusion, these bioactives under test can be taken as a dental medication or others individually or as an extracted mixture but in limited concentration and time of repeating. Also, further *in vitro* and *in vivo* studies particularly in dental field is necessary to determine its ADMET with covering all studying factors.

KEYWORDS: clove, feverfew, in Silico, toothache, ADMET.

INTRODUCTION

Clove and Feverfew are medicinal herbs in various applications such as antioxidant, antibacterial, antifungal, antiviral, anticancer, and others. Clove contains phenolic compounds mainly Eugenol (E) that also found in Feverfew, Acetyl Eugenol (AE); Humilene (H) and flavonoids: Kaemferol (K) and Quercetin (Q); that also found in Feverfew; and volatiles in its essential oil [1].

Pharmaceutical effects of Clove that screened in vitro and in vivo in is not the only application of this spice that used as nutritional and food preservatives especially dairy products [2] because of its high contain of many polyphenols.

Traditional and lab Clove extraction processes [3] such as Solvent, Supercritical CO2, ultrasonic, and microwave extraction methods and drying are varied in their final presentation such as capsule, solid lipid nanoparticle, complex and other models. The most known of bio- Clove activities are toothache, antispasmodic, capscaicin agonist, and join pain medications where Eugenol (E) is the active player in ganglionar cell through enhancement of Calcium – Chloride channel. Antinociceptive activity of Eugenol in Clove is not the main research target in academic centres, its cytotoxicity or genotoxicity for cancer treatment of various cell strains showed that E is not carcinogenic to rats and suppressed malign

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melanoma, inhibition of human metastasis enzyme [1,3, 4, 5, 6, 7, 8].

World Health Organization (WHO) stated Clove daily intake according to human weight (in Kg) with 2.5 mg and this oral intake of Clove permit E reaching blood in more than 12 hours to be as a pain reliever [8].

Feverfew (fever reducer – feathery leaves) [1,9] represents an interesting aromatic plant that contains flavonoids, volatile oils, thirty sesquiterpene lactone, and others and has important biological activities including anti-inflammatory, anticancer, inhibition of smooth muscle spasm, growth of bacteria, fungi, and yeast, prostaglandin synthesis, and release of histamine and serotonin.

Treatment or prevention of psoriasis, rheumatoid arthritis, and migraine headache under controlled herbal dose of this non- chronic toxic plant depending on disease state and human age and weight to avoid inhibition of blood clotting substance, nerve-muscle – joint reaction (post feverfew syndrome), mouth ulceration, tongue or mucosa inflammation, loss of taste, and dermatitis [1, 9, 10]. Also, ref. [1] stated that "Mouth ulcers, sore mouth, abdominal pain and indigestion, diarrhoea, flatulence, nausea, dizziness and skin rash are some of Feverfew side effects".

According to ref. [1], "Clove oil is a dermal and mucous membrane irritant, cheilitis and stomatitis attributed to the E so its use orally (not on skin) must be with caution. Repeated application of it as a toothache remedy may result damage of gingival tissue and it is not suitable for internal use larger than those recommended".

Above biological and medicinal information particularly of tooth and gingival tissue sensations encourage us to investigate from in Silico point of view the activity of several bioactive constituents in Clove and Feverfew toward important biological characters. This computational predication was done through online websites https://tox-new.charite.de/protox_II/ to determine toxicity class, LD50, Hepatotoxicity, Carcinogenicity, Immunotoxicity, Mutagenicity, Cytotoxicity, and other toxicological characters, http://biosig.unimelb.edu.au/pkcsm/ to determine Quantitative Structural Activity Relationship - Adsorption, Distribution, Metabolism, Excretion, and Toxicity QSAR -ADMET characters. Finally, taste characterization of each bioactives in both plants were tested by http://virtualtaste.charite.de. Prediction results will expand our knowledge of their toxicity as a major concern of using Clove and Feverfew as herbal medicinal source especially in toothache and related diseases.

Experimental details

- Bioactive constituents of Clove and Feverfew [1].

In Clove: Eugenol (E) and acetyl Eugenol (AE) were tested in this paper beside Ylangene (Y), terpenoids: Humulene (H) and Oleanolic acid (O); phytosterols: Campesterol (C), Sitosterol (S), and Stigmastanol (ST); and flanonoids: Kaempferol (K) and Rhamnetin (R). (Figure (1)).

In Feverfew: sesquiterene lactones: Parthenolide (P), Costunolide (CT), Artemorin (A), Artecanin (AN), Magnoliadlide (M), Santamarine (SA), and Reynosin (RE) and flavonoids: Apigenin (AP), Luteolin (L), Querectin (Q), Centaureidin (CE), Jaceidin (J), Santin (SN), Chrysoeriol (CO), and Tanetin (T). (Figure (2)).

- Online websites predication.

Predication characters in this paper were toxicity: (Class: Predicated toxicity class; LD50, Predicated LD50, mg/Kg; Hepat, Hepatotoxicity; Carcino, Carcinogenicity; Immuno., Immunotoxicity; Mutag., Mutagenicity; Cyto., Cytotoxicity; Aryl hydrocarbon Receptor (AhR); Androgen Receptor (AR); Estrogen Receptor Alpha (ER); Heat shock factor response element (HSE); Mitochondrial Membrane Potential (MMP); Phosphoprotein (Tumor Supressor) p53) by https://tox-new.charite.de/protox_II/ website (Table (1) and Figure (3) for Clove constituents and Table (4) and Figure (4) for Feverfew constituents respectively). (Image (1))

ADMET characters (Tables (2) for Clove and Table (5) for Feverfew) were http://biosig.unimelb.edu.au/pkcsm/ website where **Adsorption**: water solubility (H2O Sol., log mol/L), Intestinal absorption in human (Intest. Abs., %), Skin Permeability (Skin Perm., log Kp), P-glycoprotein I and II inhibitor (GlycoproInh. I, GlycoproInh. II), Distribution: Blood Brain Barrier Permeability (BBB, log BB), Central Nerve System Permeability (CNS, log PS), **Metabolism**: Cytochromes P450 inhibitor (CYP1A2, CYP2C19, CYP2C9, CYP2D6, CYP3A4), **Excretion**: Renal Organic Cation Transporter 2 substrate (OCT2 Subs.), **Toxicity**: Ames Toxicity (Ames), Maximum

tolerated dose for human (Max. Dose, log mg/Kg/day), Human Ether –à-go-go- related gene I and II inhibitor (hERG I and hERG II), Oral Rat Acute Toxicity (LD50 –rat, mol./Kg), Hepatotoxicity (Hepat.), and Skin Sensitisation (Skin Sens.). (Image (2))

Also, taste probability was predicated by http://virtualtaste.charite.de online website to determine the bitter, sweet, or sour of individual constituents of Clove (Table (3)) and Feverfew (Table (6)). (Image (3))

Table (1):- Various toxicity characters of bioactives in Clove.

Property	Phenyl propanoid		Sesquiterpenoid	Phytosterol					Flavonol	
	E	AE	H	Y	C	S	ST	O	K	R
Class	4	4	5	5	4	4	4	4	5	5
LD50	1930	1670	3650	3700	890	890	500	2000	3919	5000
Hepat.	0.67	0.57	0.82	0.85	0.85	0.87	0.79	0.52	0.68	0.73
Carcino.	0.73	0.55	0.76	0.77	0.59	0.60	0.76	0.57	0.72	0.59
Immuno.	0.83	0.89	0.99	0.68	0.99	0.99	0.97	0.79	0.96	0.55
Mutag.	0.97	0.66	0.87	0.88	0.96	0.98	0.89	0.85	0.52	0.69
Cyto.	0.90	0.88	0.79	0.69	0.95	0.94	0.81	0.99	0.98	0.91
AhR	0.98	0.98	0.98	0.95	0.99	0.99	0.99	0.99	1.0	0.86
AR	0.99	0.95	0.99	0.99	0.92	0.83	0.88	0.81	0.99	0.99
Aromatase	0.99	0.99	0.88	0.60	0.98	0.96	0.99	0.94	0.96	0.86
ER	0.97	0.88	0.82	0.96	0.87	0.80	0.80	0.71	1.0	0.66
HSE	0.99	0.87	0.90	0.98	0.54	0.61	0.94	0.70	0.99	0.91

Property	Phenyl propanoid		Sesquiterpenoid	Phytosterol					Flavonol	
	E	AE	H	Y	C	S	ST	O	K	R
MMP	0.98	0.98	0.90	0.90	0.68	0.63	0.81	0.72	1.0	0.84
P53	0.99	0.98	0.99	0.99	0.99	0.99	0.97	0.98	0.92	0.88

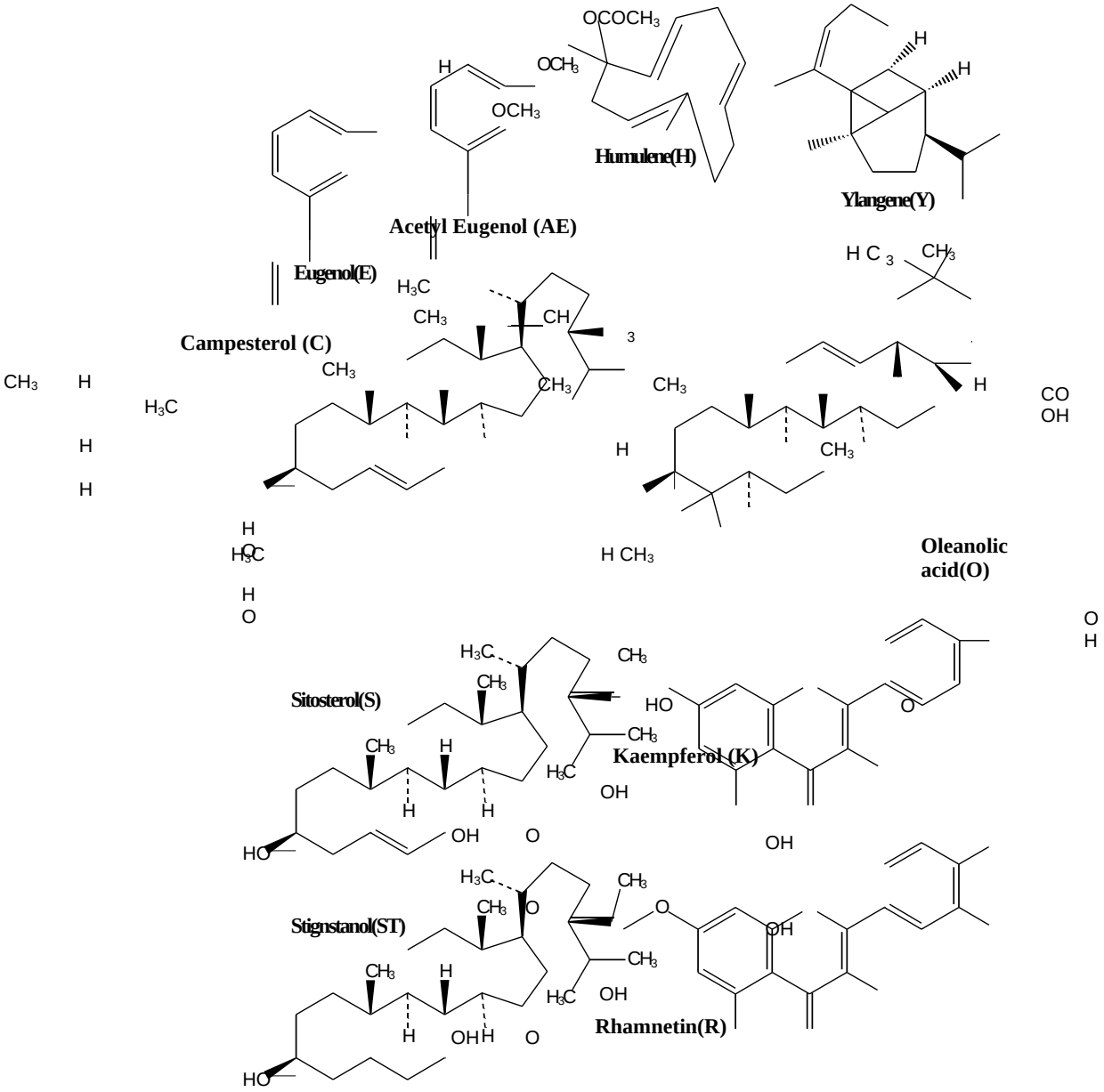

Fig. (1): Chemical structures of bioactive Clove constituents under test.

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Table (2):-ADMET data of active Clove constituents.

Property	Phenyl propanoid		Sesquiterpenoid			Phytosterol		Flavonoid		
	E	AE	H	Y	C	S	ST	O	K	R
H2O sol.	-2.25	-2.846	-5.191	-5.705	-6.818	-6.773	-6.682	-3.261	-3.04	-3.212
Intest .abs.	92.041	94.755	94.682	96.221	94.757	94.464	94.97	99.558	74.29	80.214
Skin Perm.	-2.207	-2.257	-1.739	-2.225	-2.81	-2.783	-2.783	-2.735	-2.735	-2.735
GlycoproInh. I	No	No	No	No	No	Yes	Yes	No	No	No
GlycoproInh. II	No	No	No	No	Yes	Yes	Yes	No	No	No
BBB	0.374	0.401	0.663	0.887	0.771	0.781	0.771	-0.143	-0.939	-1.345
CNS	-2.007	-2.077	-2.555	-1.659	-1.805	-1.705	-1.652	-1.176	-2.228	-3.235
CYP1A2	Yes	Yes	No	Yes	No	No	No	No	Yes	Yes
CYP2C19	No	No	No	No	No	No	No	No	No	No
CYP2C9	No	No	No	No	No	No	No	No	No	No
CYP2D6	No	No	No	No	No	No	No	No	No	No

Property	Phenyl propanoid		Sesquiterpenoid			Phytosterol		Flavonoid		
	E	AE	H	Y	C	S	ST	O	K	R
CYP3A4	No	No	No	No	No	No	No	No	No	No
OCT2 subs.	No	No	No	No	No	No	No	No	No	No
AMES	Yes	No	No	No	No	No	No	No	No	No
Max. Dose	1.024	1.102	0.551	-0.302	-0.641	-0.621	-0.664	0.094	0.531	0.56
hERG I	No	No	No	No	No	No	No	No	No	No
hERG II	No	No	No	No	Yes	Yes	Yes	No	No	No
LD50 - rat	2.118	2.198	1.766	1.644	2.28	2.552	2.54	2.196	2.449	2.453
Hepat.	No	No	No	No	No	No	No	Yes	No	No
Skin Sens.	Yes	Yes	Yes	No	No	No	No	No	No	No

Table (3):- Predication of taste probability of bioactive Clove constituents.

Taste	Phenyl propanoid		Sesquiterpenoid			Phytosterol		Flavonoid		
	E	**AE**	**H**	**Y**	**C**	**O**	**S**	**ST**	**K**	**R**
Bitter	0.7	0.521	0.842	0.847	0.511	0.51	0.531	0.669	1.0	0.718
Sweet	0.885	0.843	0.854	0.894	0.819	0.722	0.86	0.758	0.911	0.717
Sour	0.92	0.769	0.911	0.962	0.994	0.994	0.99	0.997	1.0	1.0

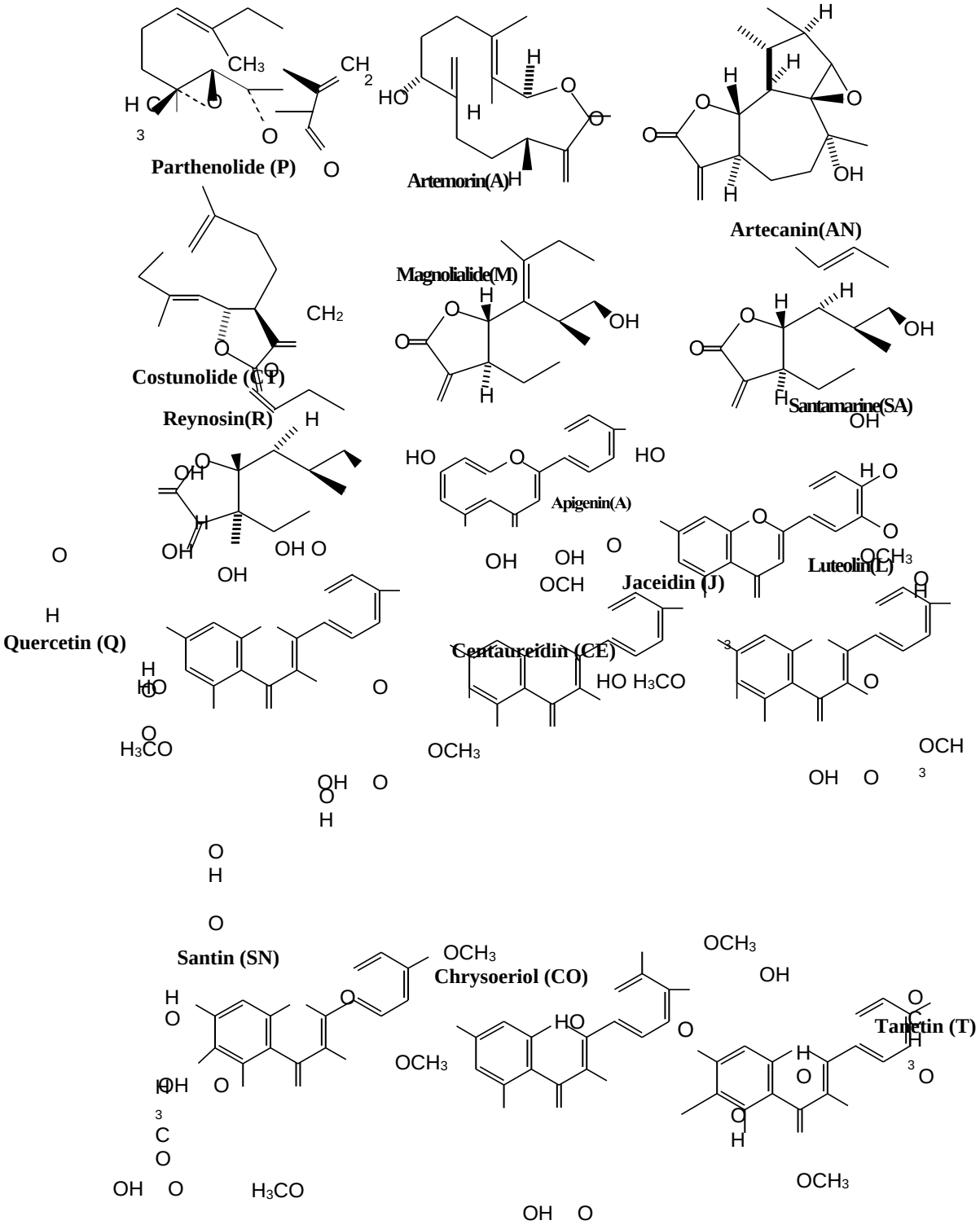

Fig. (2): Chemical structures of bioactive Feverfew constituents under test.

Table (4):-Various toxicity characters of bioactives in Feverfew.

Property	Sesquiterpene lactone									Flavonoid					
	P	CT	A	AN	M	SA	RE	AP	L	Q	CE	J	SN	CO	T
Class	4	5	4	6	4	5	4	5	5	3	5	5	5	5	5
LD50	1330	3140	841	39800	841	5000	1330	2500	3919	159	5000	5000	5000	4000	5000
Hepat.	0.77	0.62	0.60	0.66	0.50	0.53	0.50	0.68	0.69	0.69	0.70	0.70	0.70	0.72	0.71
Carcino.	0.52	0.57	0.63	0.57	0.59	0.64	0.68	0.62	0.68	0.68	0.69	0.69	0.69	0.68	0.51
Immuno.	0.98	0.99	0.99	0.91	0.85	0.99	0.99	0.99	0.97	0.87	0.97	0.94	0.85	0.77	0.93
Mutag.	0.74	0.88	0.81	0.53	0.83	0.79	0.82	0.57	0.51	0.51	0.82	0.82	0.82	0.94	0.54
Cyto.	0.81	0.83	0.89	0.66	0.92	0.98	0.96	0.87	0.99	0.99	0.75	0.75	0.75	0.95	0.72
AhR	0.98	0.96	0.98	0.96	0.99	0.99	0.99	1.0	0.91	0.91	0.84	0.84	0.84	0.97	0.73
AR	0.93	0.92	0.96	0.92	0.76	0.61	0.59	0.99	0.99	0.99	0.99	0.99	0.99	1.0	0.99
Aromatase	0.81	0.85	0.83	0.81	0.51	0.53	0.64	0.61	0.91	0.91	0.54	0.54	0.54	0.58	0.86
ER	0.77	0.83	0.68	0.70	0.52	0.58	0.57	1.0	0.87	0.87	0.69	0.69	0.69	0.88	0.54
HSE	0.83	0.88	0.89	0.86	0.91	0.84	0.86	0.99	0.99	0.99	0.93	0.93	0.93	0.96	0.85
MMP	0.92	0.93	0.89	0.66	0.84	0.78	0.80	1.0	1.0	1.0	0.86	0.86	0.86	0.92	0.79
P53	1.0	0.81	0.85	0.63	0.92	0.84	0.88	1.0	0.97	0.97	0.7	0.73	0.73	0.86	0.73

Table (5):- ADMET data of active Feverfew constituents.

Property	Sesquiterpene lactone									Flavonoid					
	P	CT	A	AN	M	SA	RE	AP	L	Q	CE	J	SN	CO	T
H2O sol.	-3.161	-3.764	-2.822	-3.112	-3.032	-2.954	-2.864	-3.329	-3.094	-2.925	-3.221	-2.892	-3.326	-3.237	-3.366
Intest .abs.	97.599	97.18	96.204	75.915	96.886	96.824	97.003	93.25	81.13	77.207	77.207	82.633	78.996	82.844	89.136
Skin Perm.	-3.278	-2.423	-3.165	-3.297	-3.246	-3.469	-3.296	-2.735	-2.735	-2.735	-2.735	-2.735	-2.747	-2.735	-2.77
GlycoproInh. I	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
GlycoproInh. II	No	No	No	No	No	No	No	No	No	No	Yes	No	Yes	No	Yes
BBB	0.444	0.512	0.089	-0.165	0.087	0.083	0.062	-0.734	-0.907	-1.098	-1.466	-1.027	-0.809	-0.943	-0.821
CNS	-3.007	-2.672	-2.88	-3.034	-2.82	-2.879	-2.828	-2.061	-2.251	-3.065	-3.256	-1.604	-3.083	-2.32	-3.077
CYP1A2	No	No	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
CYP2C19	No	No	No	No	No	No	No	Yes	No	No	No	No	Yes	Yes	Yes
CYP2C9	No	No	No	No	No	No	No	No	Yes	No	No	No	Yes	Yes	No
CYP2D6	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
CYP3A4	No	No	No	No	No	No	No	No	No	No	No	No	No	No	Yes

Property	Sesquiterpene lactone								Flavonoid						
	P	CT	A	AN	M	SA	RE	AP	L	Q	CE	J	SN	CO	T
OCT2 subs.	Yes	No	No	No	No	No	No	No	No	No	No	No	No	No	No
AMES	Yes	No	No	Yes	No	No	No	No	No	No	No	Yes	No	No	No
Max. Dose	0.306	0.449	0.577	0.161	0.409	0.283	0.331	0.328	0.499	0.499	0.594	0.438	0.328	0.436	0.254
hERG I	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
hERG II	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
LD50 - rat	2.096	1.889	2.093	2.908	2.103	1.956	2.136	2.45	2.455	2.471	2.286	2.482	2.113	2.337	2.179
Hepat.	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
Skin Sens.	Yes	Yes	Yes	No	No	No	No	No	No	No	No	No	No	No	No

Table (6):- Predication of taste probability of active Feverfew constituents.

Taste	Sesquiterpene lactone								Flavonoid						
	P	CT	A	AN	M	SA	RE	AP	L	Q	CE	J	SN	CO	T
Bitter	0.953	0.835	0.946	0.897	0.85	0.874	0.877	0.998	1.0	1.0	0.635	0.635	0.635	0.656	0.746
Sweet	0.678	0.738	0.809	0.635	0.611	0.562	0.593	0.628	0.989	0.989	0.708	0.708	0.708	0.817	0.648
Sour	0.998	0.924	0.929	0.994	0.993	0.993	0.98	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0

RESULTS AND DISCUSSION

Pharmacokinetic characters is *in Silico* predicated characters in many online websites like pkCSM and protox-II that applied in this study. pkCSM easily screens these multiple properties by introducing Simplified Molecular – Input-Line- Entry- System (SMILES) string of the target molecule (Image (1)) while protox-II website by introducing Pubchem- name or canonical SMILES beside the more interesting method to chemist (drawing) (Image (2)). pkCSM gives ADMET characters in numeric and (Yes/No) categories whilst presents toxicity characters in numeric.

Drug, food, or herb usage is strongly related to taste that can be predicted easily with http://virtualtaste.charite.de/VirtualTaste/ online website. This website gives numerical bitter, sweet, and sour probabilities companied with colour as a calculation confidence together with its high, medium, or low strength (Image (3)). The main target of taste predication as this website stated is to "The taste of a chemical compound present in food stimulates us to take in nutrients and avoid poisons. Many active ingredients present in drugs taste bitter and thus are aversive to children as well as many adults. Bitterness of medicines presents compliance problems and early flagging of potential bitterness of a drug candidate may help its further development. Taste prediction of a compound is of large interest for the food industry". Glucose is very known compound was selected to give a close look about the obtained results.

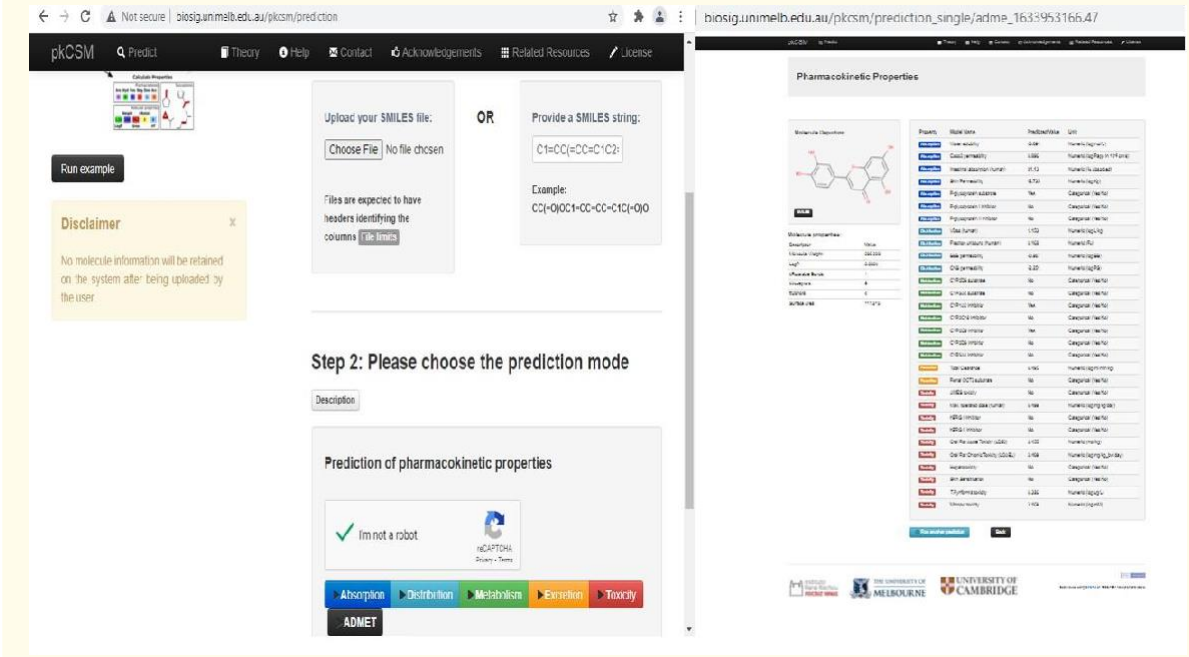

Image (1): pkCSM predication steps and results.

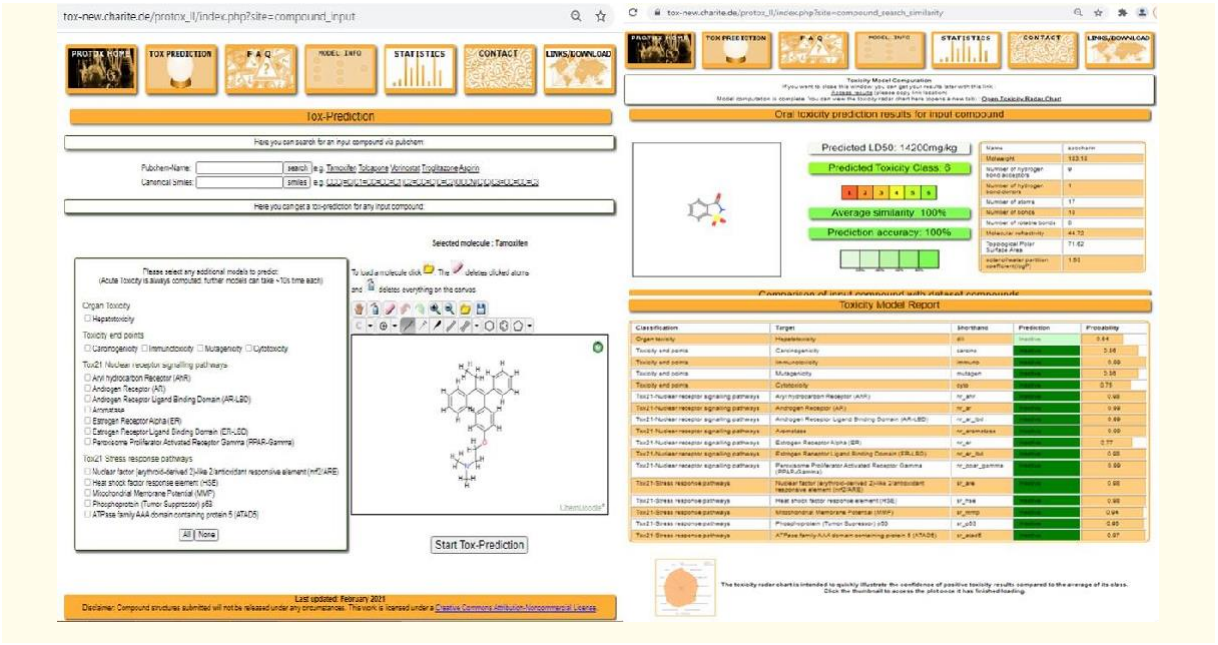

Image (2):-wqsaProtox-II predication steps and results.

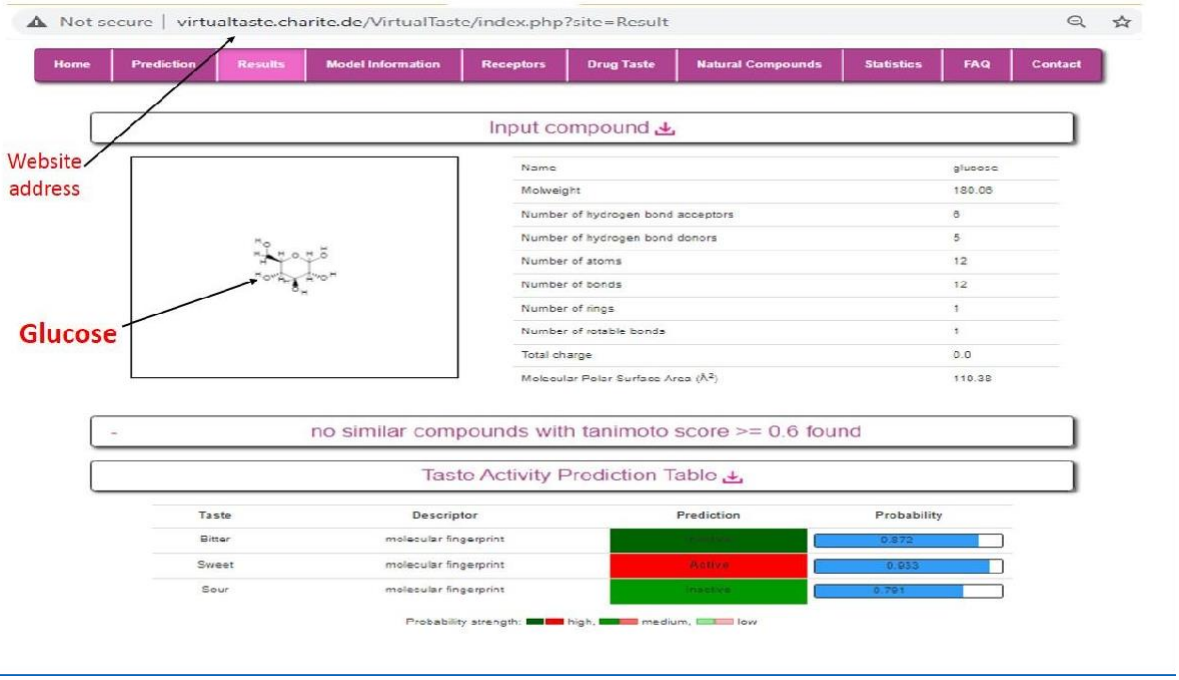

Image (3):- Result of applying http://virtualtaste.charite.de/VirtualTaste/ website to Predicate taste of glucose.

Predication calculations by https://tox-new.charite.de/protox_II/ website (Table (1) and Figure (3)) of individual Clove constituents included probability of Predicated toxicity class; LD50, Predicated LD50, mg/Kg; Hepat, Hepatotoxicity; Carcino, Carcinogenicity; Immuno., Immunotoxicity; Mutag., Mutagenicity; Cyto., Cytotoxicity; Aryl hydrocarbon Receptor (AhR); Androgen Receptor (AR); Estrogen Receptor Alpha (ER); Heat shock factor response element (HSE); Mitochondrial Membrane Potential (MMP); and Phosphoprotein (Tumor Supressor) p53) that their ranges as below:

Toxicity Class: 4 and 5	LD50 (mg/Kg):500-5000	Hepatotoxicity: 0.52-0.87	Carcinogenicity: 0.55-0.77	Androgen Receptor (AR): 0.81-0.99	Estrogen Receptor Alpha (ER): 0.66-1.00	Mitochondrial Membrane Potential (MMP): 0.63-1.00
Immunotoxicity: 0.55-0.99	Mutagenicity: 0.52-0.98	Cytotoxicity: 0.69-0.99	Aryl hydrocarbon Receptor (AhR): 0.86-1.00	Aromatase: 0.60-0.99	Heat shock factor response element (HSE): 0.54-0.99	Phosphoprotein (Tumor Supressor) p53: 0.88-0.99

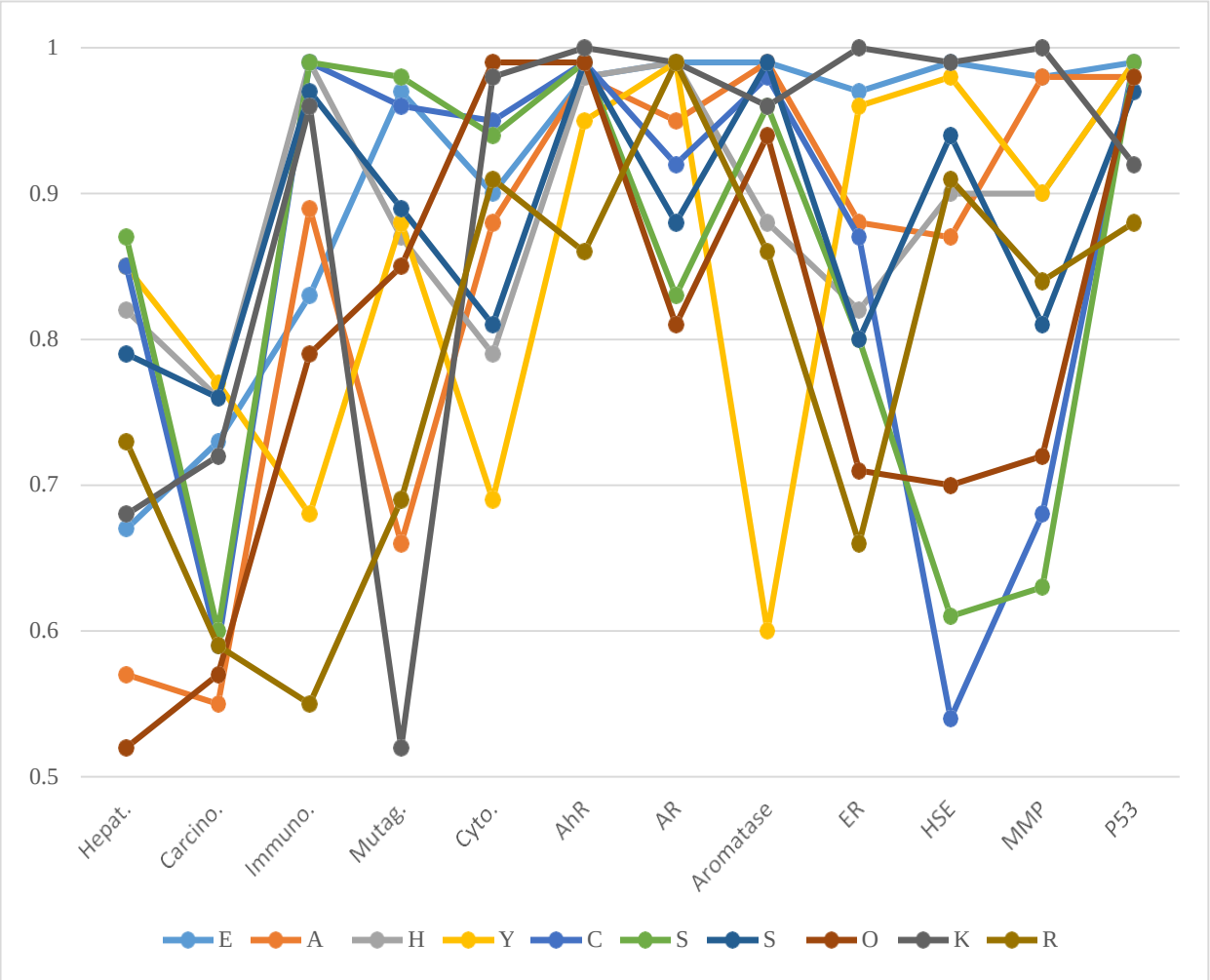

Fig. (3): Hepat. (Hepatotoxicity), Carcino. (Carcinogenicity), Immuno. (Immunotoxicity), Mutag. (Mutagenicity), Cyto. (Cytotoxicity), Aryl hydrocarbon Receptor (AhR); Androgen Receptor (AR); Estrogen Receptor Alpha (ER); Heat shock factor response element (HSE); Mitochondrial Membrane Potential (MMP); Phosphoprotein (Tumor Supressor) p53) of several active **Clove** constituents.

The same website was used to predicate toxicity probabilities of active Feverfew constituents (Table (4), Figure (4)) that were ranged as below:

Toxicity Class: 3-6	LD50 (mg/Kg): 159-39800	Hepatotoxicity: 0.50-0.55	Carcinogenicity: 0.51-0.69	Androgen Receptor (AR): 0.59-1.00	Estrogen Receptor Alpha (ER): 0.52-1.00	Mitochondrial Membrane Potential (MMP): 0.66-1.00
Immunotoxicity: 0.77-0.99	Mutagenicity: 0.51-0.94	Cytotoxicity: 0.66-0.99	Aryl hydrocarbon Receptor (AhR): 0.86-1.00	Aromatase: 0.51-0.91	Heat shock factor response element (HSE): 0.83-0.99	Phosphoprotein (Tumor Supressor) p53: 0.63-1.00

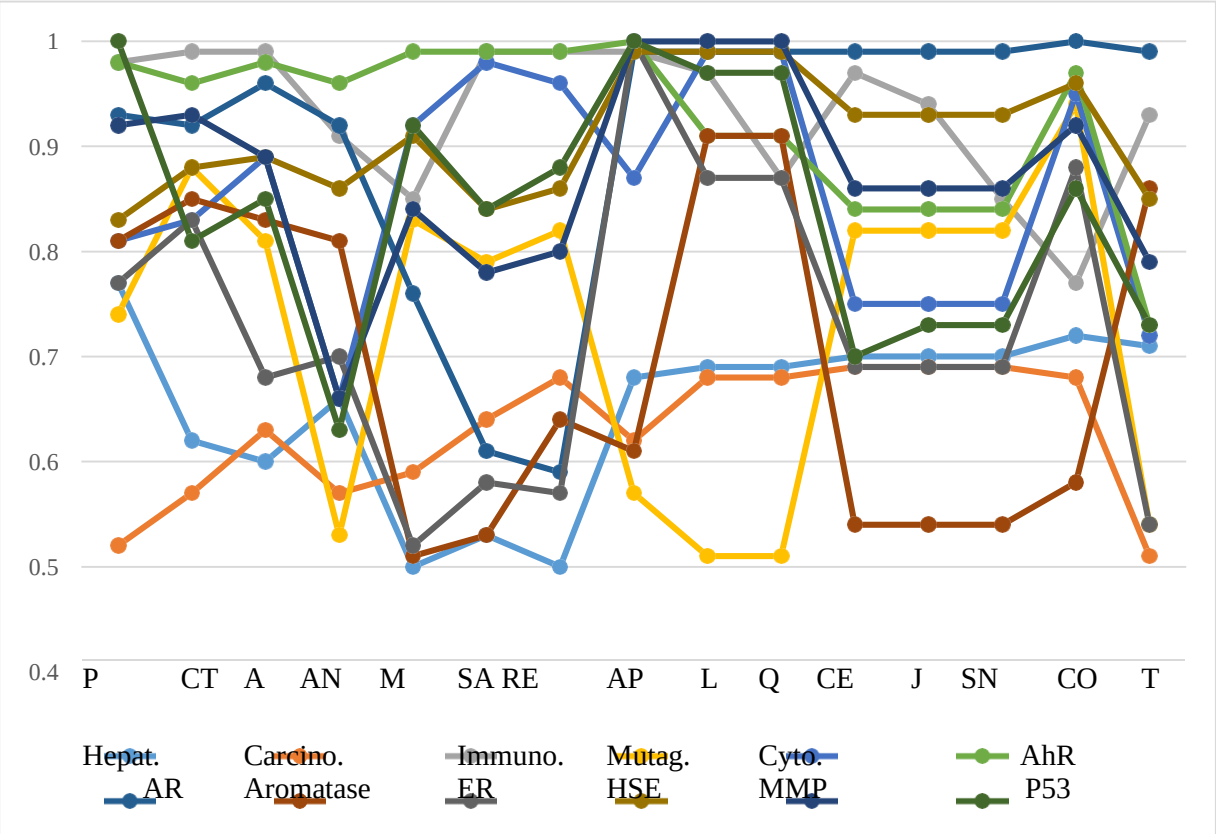

Fig. (4): Hepat. (Hepatotoxicity), Carcino. (Carcinogenicity), Immuno. (Immunotoxicity), Mutag. (Mutagenicity), Cyto. (Cytotoxicity), Aryl hydrocarbon Receptor (AhR); Androgen Receptor (AR); Estrogen Receptor Alpha (ER); Heat shock factor response element (HSE); Mitochondrial Membrane Potential (MMP); Phosphoprotein (Tumor Supressor) p53) of several active **Feverfew** constituents.

Numeric and categorical ADMET values of Clove and Feverfew constituents (Table (2) and Table (5) respectively) were calculated and their numerical ranges as below:

Plant	Adsorption	Distribution	Toxicity
Clove	water Sol.: -6.818 to -2.250 Intest. Abs.: 74.29-99.558 Skin Perm.: -2.810 to -1.739	BBB: -1.345 – 0.887 CNS: -3.235 – 0.887	Max. Dose: -0.664 – 1.102 LD50 –rat: 1.644-2.552
Feverfew	water Sol.: -3.764 to -2.822 Intest. Abs.: 75.915-97.599 Skin Perm.: -3.469 to -2.423	BBB: -1.466 to 0.512 CNS: -3.256 to -1.604	Max. Dose: 0.161 to 0.594 LD50 –rat: 1.889-2.908

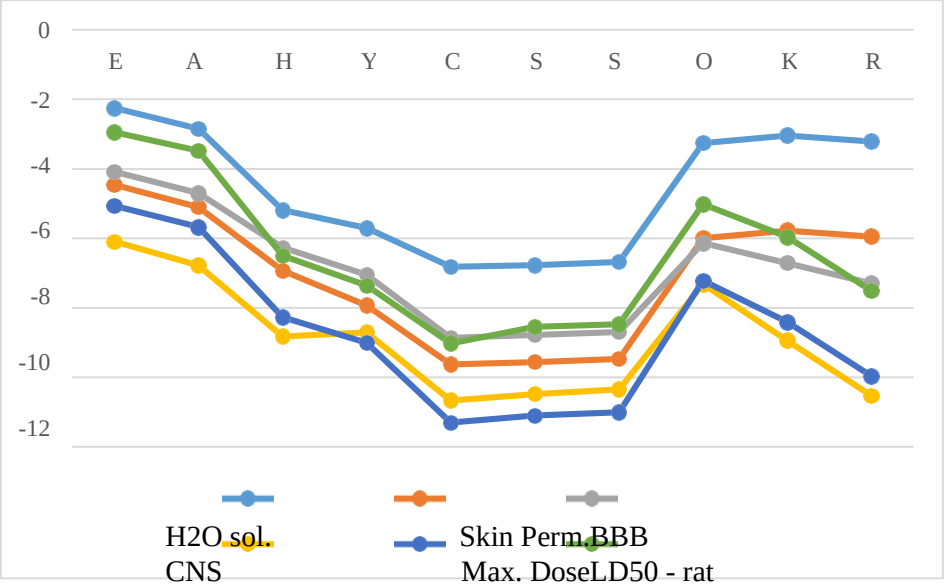

Fig. (5): Some ADT characters of Clove constituents.

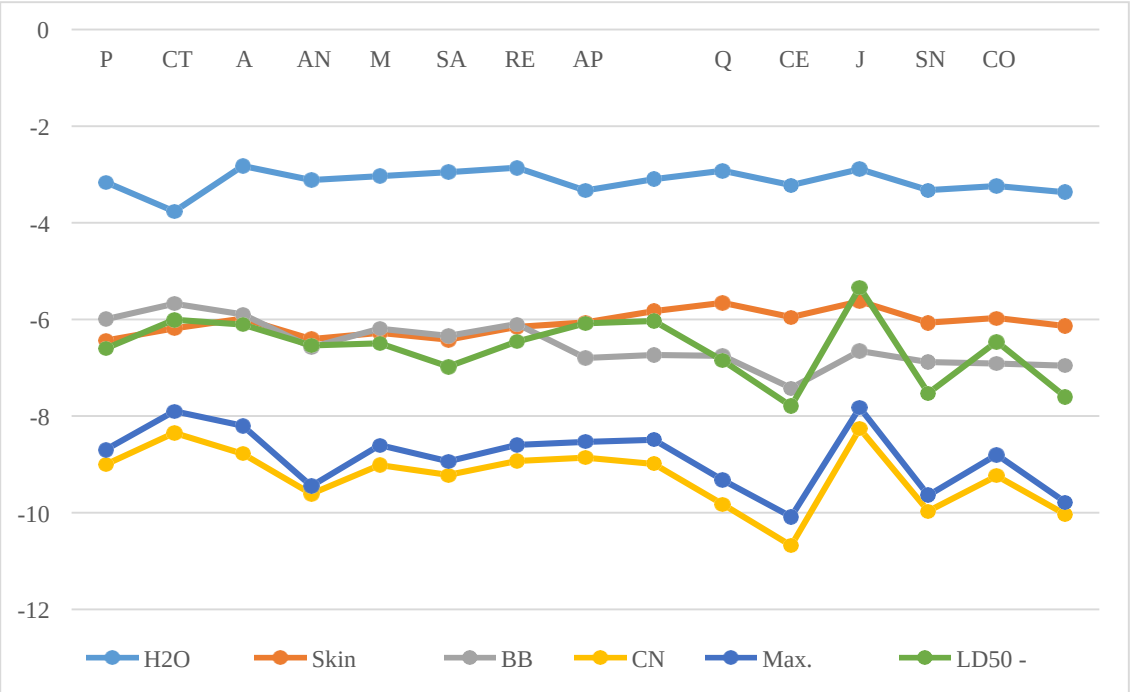

Fig. (6): Some ADT characters of Feverfew constituents.

Human health can be in danger state with exposure to chemical causing acute liver damage (Hepatotoxicity) or inducing tumor (Carcinogenicity), affecting immune system (Immunotoxicity), changing DNA (Mutagenicity), reflecting primary cell function (Cytotoxicity), and other toxicological characters that calculated in all tested bioactives. What affects human cell or organ is the sum of many important properties like chemical composition and structure that impact formation of hydrogen bonding, water solubility, interaction with cell components, and effecting enzyme or protein structure and role [11-20].

Global Harmonized System (GHS) specify toxicity to six classes [21] according to swallowing state where Class I and II, fatal; Class III, toxic; Class IV, harmful; Class V, may be harmful while Class VI, non-toxic. These six classes range their LD50 from 5 mg/Kg or less to more than 5000 mg/Kg. Clove constituents under predication (Table (1)) by https://tox-new.charite.de/protox_II/ website showed phenyl propaneoids (E and AE) and phytosterols (C, S, ST, O) were Class IV while H, Y, and flavonols (K and R) were Class V. Additionally, feverfew constituents (Table (4)) showed class range from III to VI where Class III contains Q; Class IV has P, A, M, and RE, while (CT, SA, AP, L, CE,J, SN, CO, and T) and (AV) predicated to be in Class V and Class VI respectively.

In the same manner, LD50 values of both Clove (Table (1)) and Feverfew (Table (4)) were calculated as toxicity class predication. In general, toxicity class and LD50 predications showed that Clove and Feverfew contain major constituents ensuring safety intake individually or in mixture state under controlled quantities.

From Tables (1 and 4) and Figures (3 and 4), it can be noticed that Hepatotoxicity, Carcinogenicity, Immunotoxicity, Mutagenicity, Cytotoxicity, Aryl hydrocarbon Receptor, Androgen Receptor, Estrogen Receptor Alpha, Heat shock factor response element, Mitochondrial Membrane Potential, Phosphoprotein (Tumor Supressor) p53) characters showed the same pattern of increasing and decreasing of these bioactives in Clove and Feverfew.

Structural flavone presence in R (Clove – Toxicity Class V) and T (Feverfew –Toxicity Class V) and structural lactone AN (Feverfew – Toxicity Class VI) were as the most repeated constituent(s) with the lowest toxicological characters Tables (1 and 4) and Figures (1, 2, 3 and 4).

Quantitative Structural Activity Relationship - Adsorption, Distribution, Metabolism,

Excretion, and Toxicity QSAR -ADMET characters as showed in Tables (2) for Clove and Table (5) for Feverfew were predicated by http://biosig.unimelb.edu.au/pkcsm/ website in numeric and (Yes/No) categories.

Adsorption: water solubility, Skin Permeability; Distribution: Blood Brain Barrier Permeability, Central Nerve System Permeability; Toxicity: Maximum tolerated dose for human as ADT relationship were predicated in (log) term with negative values in most of them.

In general, water solubility was very low for both plants where the lowest range was for clove (log mol/L: -6.818 to -2.250; mol./L: 1.5E-07 to 5.6E-03) than for Feverfew (log mol/L: -3.764 to -2.822; mol./L: 1.7 E-04 to 1.5 E-03).

From ADT Tables (2 and 5), Clove and Feverfew showed remarkable actions of their constituents towards P-glycoprotein I and II inhibitor, Cytochromes P450 inhibitor, Renal Organic Cation Transporter 2 substrate Human Ether related gene I and II inhibitor Hepatotoxicity, and Skin Sensitisation.

Clove constituents did not inhibit CYP2C19, CYP2C9, CYP2D6, CYP3A4, hERG I, and OCT2 subs. while other characters showed (No) response in most of these bioactives. Table

showed that (Yes) response was available as below and these results are good indications to use this plant as medicinal type with limited cautions of side effects.

GlycoproInh. I: S, and ST; GlycoproInh. II: C, S, and ST; CYP1A2: E, AE, Y, K, and R; Ames: E; hERG II: C, S, and ST; Hepat.: O; Skin Sens.: E, AE, and H.

In Feverfew (Table (5)), there was some changing in response of its bioactives towards categorical (Yes/No) predication.

GlycoproInh. I, CYP2D6, hERG I and hERG II, and Hepat. were with (No) response to all Feverfew constituents while others have (Yes) response as below where (Yes) response gave primary indication of these bioactives capabilities to interact with a specific unit(s) or bond(s) in these targets (gene, enzyme, or others) so limited cautions in this plant is increased:

GlycoproInh. II: CE SN, and T; CYP1A2: all (Yes) except P, CT, A, and AN were with (No); CYP2C19: AP, SN, CO, and T; CYP2C9: L, SN, and CO; CYP3A4: T; OCT2 Subs.: P; Ames:

P, AN, and J; Skin Sens.: P, CT, and A.

Presence of these cautions can be controlled with taste predication (Tables (3 and 6)) where highest percentage of probability of these bioactives in both plants showed sour taste so their oral intake is in low concentration.

CONCLUSION

Both Clove and Feverfew plants contain bioactives play important roles as medicinal constituents particularly dental field. In this study, computational online websites were applied to predicate many toxicological properties.

As it known in dental medication, it is important to specify toxicity of any chemical material and its role on human organs through inhibition, enhancement, metabolism, absorption, and other biological processes. In this study as a first attempt in Iraqi studies principally in dental science, computational online websites: https://tox-new.charite.de/protox_II/, http://biosig.unimelb.edu.au/pkcsm/, and http://virtualtaste. charite.de were applied to predicate many effective properties.

Toxicity class and LD50 predications of Clove and Feverfew bioactives are important characters in related medications. These online predications showed that Clove and Feverfew constituents were non- fatal class with an acceptable LD50 as good indication of safety intake individually or in mixture state under controlled quantities. These and other *in Silico* results in this study ensure using these bioactives in oral and skin treatment.

Rhamnetin and Tanetin in Clove and Artecanin in Feverfew had the lowest toxicological characters. This foundation and other calculations achieved as Quantitative Structural Activity Relationship - Adsorption, Distribution, Metabolism, Excretion, and Toxicity QSAR - ADMET characters confirmed non- inhibition predications of hERG I, CYP2C19, CYP2C9, CYP2D6, and CYP3A4 towards using Clove as medicinal type with limited cautions of side effects.

In Feverfew, primary indications were obtained to use these bioactives with higher limited cautions in this plant compared to Clove. Also, taste predication of both herbs suggests highly sour presence so oral intake is in low concentration to avoid unlikely savour.

In conclusion, these bioactives under test can be taken as a dental medication or others individually or as an extracted mixture but in limited concentration and time of repeating. Also, further *in vitro* and *in vivo* studies particularly in dental field is necessary to determine its ADMET with covering all studying factors.

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