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Research Article

PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODEL FOR PLANT-BASED ANTI-OXIDANT DRUGS

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ABSTRACT

Objective: A pharmacokinetic study is a cumbersome process in clinical research. It is very important in target validation and in shifting a lead compound into a drug. Our major objective was to reveal the most important physiochemical characters of the plant-based anti-oxidants in align with human physiology. The *in silico* studies can preferably be the best solution to identify the physiologically-based pharmacokinetic (PBPK) behavior of the anti-oxidants.

Methods: Anti-oxidants are found in many foods including fruits and vegetables. Few of the important anti-oxidants, i.e. around 10 plant-based anti-oxidant compounds were taken for this research. These compounds were evaluated based on their pharmacokinetic parameters. The properties such as Lipinski's rule of 5, absorption, distribution, metabolism, excretion, and toxicity (ADMET) of the compounds were screened thoroughly with the help of tools such as molinspiration and gastroplus.

Results: The physiological studies of these compounds had shown different compartmental absorptions of the compound in the human gastrointestinal tract. Certain compounds were found to pass the physiological barriers and had the ability to become a drug. The compounds were filtered using the risk and toxicity factors. These risk factors caused the compounds to fail in the process of becoming a drug.

Conclusion: The compounds which passed the PBPK studies were eligible to become a drug. Of the 10 compounds investigated, eugenol, gingerol, zingerone, and geraniol were found to have higher fraction of absorption to become a drug. Out of these compounds, the compounds gingerol and eugenol have shown the best factor of absorption, and hence, have a better probability of becoming a drug.

Keywords: Anti-oxidants, Lipinski, Absorption, Distribution, Metabolism, Excretion and toxicity, Physiological properties, GastroPlus, *In silico.*

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INTRODUCTION

Anti-oxidants are substances that are capable of counteracting the damaging but normal effects of the physiological process of oxidation in animal tissue. Anti-oxidants are nutrients (vitamins and minerals) as well as enzymes (proteins in your body that assist in chemical reactions) [1]. They are believed to play a role in preventing the development of chronic diseases such as cancer, heart disease, stroke, Alzheimer's disease, rheumatoid arthritis, and cataracts [2]. Anti-oxidants block the process of oxidation by neutralizing free radicals. In doing so, the anti-oxidants themselves become oxidized [2] and that is why there is a constant need to replenish our anti-oxidant resources. The objective of this study was to find which of the 10 anti-oxidants obtained from the *in silico* studies can be taken to the next level of lead optimization. Anti-oxidants can become drugs to combat many diseases easily, but the drawbacks occur in the physiological barriers, which do not allow them to be formulated into drug [3]. Hence, the need of physiologically-based pharmacokinetic (PBPK) study is necessary for the validation of drug. PBPK is cumbersome and tedious in the regular lead identification process. Through highly curated data sets, this model can be effectively derived to identify better anti-oxidant compound from the plant origin. Several drug-based PBPK studies have been carried out to make a plant compound into a drug [4]. PBPK modeling and simulation is a tool that can help predict the pharmacokinetics of drugs in humans and evaluate the effects of intrinsic (e.g., organ dysfunction, age, and genetics) and extrinsic (e.g., drug-drug interactions) factors, alone or in combinations on drug exposure. The use of this tool is increasing at all stages of the drug development process. This report reviews the recent instances of the use of PBPK in decision-making during regulatory review [5]. The objectives of PBPK studies were to develop a risk assessment methodology for chemical mixtures that accounts for pharmacokinetic interactions among components and to apply this methodology to assess the health risk [6]. These PBPK studies have helped the scientists and researchers to identify different toxicity and risk factors when a drug is administered into the human body. Based on the clinical trials conducted on rats, these studies have been found to be of great use [7]. They also help the researchers using the *in silico* tools for different properties. PBPK studies make the compound enter into *in vitro* research, which then gets formulated into drug. This aids in knowing the adverse effects of the drugs beforehand [8].

Mostly, the animal models suggest the drug candidate which is often considered for clinical trials. Distinctive oral delivery models have been used in the pharmacological research for increasing the patient's compliance, safety and convenience. So far the interest is often provided to identify the PBPK model for oral delivery, which follows the advanced compartmental and transit (ACAT) model. ACAT model was parameterized to capture distinct properties such as fluid volume, absorptive surface area, and bile concentrations [9-11].

MATERIALS AND METHODS

Materials

10 anti-oxidant compounds were obtained from the *in silico* literature studies. The compounds' SMILE structures were obtained using PUBCHEM [12]. These SMILE structures were used in the tool called molinspiration [13] (cheminformatics software tool) to find their absorption, distribution, metabolism, excretion and toxicity (ADMET) properties based on the Lipinski's rule. The literature studies based on GastroPlus were obtained to show the solubility, dosage, and fraction of absorption of the compound [14].

Methods

Lead compound screening

A set of 10 phytochemical compounds that possess anti-oxidant properties were taken for the study. The compounds taken for this study were aloesin, eugenol, cyanidin, gingerol, zingeron, paradol, Geraniol, corilagin, and Asiaticoside. These compounds were obtained from the literature studies, which were already conducted. Using PubChem, the SMILES formats were obtained for these 10 compounds for knowing the ADMET parameters and measuring the GastroPlus model [15].

Lipinski's rule of study

Identification of drug molecules is one of the major challenges in the field of drug discovery. Existing approach like Lipinski's rule of 5 plays an important role in the screening of compounds. Thus, there is a need to develop a computational method that can predict the drug-likeness of a molecule with precision [16]. The compounds with their SMILE formats were evaluated based on the Lipinski's rule. The rule consists of 4 parameters as follows:

- No more than 5 hydrogen bond donors
- No more than 10 hydrogen bond acceptors
- Molecular mass less than 500 Da
- Log P not greater than 5.

Apart from these rules, the rotational bonds and TPSA values were also calculated. Under these conditions, only the compounds were proved to have good pharmacokinetic properties.

ADMET risk factors

ADMET prediction is an extremely challenging area because many of the properties that we tried to predict were as a result of multiple physiological processes. The compounds based on the properties such as hydrogen donor and acceptors, the charges, log p octanol, permeability, and solubility were validated [17,18]. While such models are not sufficiently accurate to act as a replacement for *in vivo* or *in vitro* methods; *in silico* methods nevertheless can help us to understand the underlying physiochemical dependencies of different ADME properties, and thus, can give us inspiration on how to optimize them [19]. The compounds which violate these properties are considered to be in risk zone. The threshold values of the risk and toxicity factors are given in Tables 1-3 [20].

PBPK studies

PBPK studies are frequently used for pharmacokinetic (PK) analysis when only blood or plasma data is available. The body and model are represented as actual blood and tissue (usually total body weight) volumes, fractions (f (d)) of cardiac output and systemic or intrinsic clearance [21,22]. Analyzing only blood or plasma concentrations versus time, the minimal-PBPK models parsimoniously generate physiologically-relevant PK parameters [22]. The intake and dosage of these compounds have been formulated by using the tool GastroPlus. The fraction of absorption was found in each compartment of gastrointestinal tract. The compounds violating these factors have a huge risk of toxicity [23].

RESULTS

The various risk factors and their contributions have been shown in Tables 4-9 along with Figures 1-2

DISCUSSION

This study was pursued on a list of anti-oxidant phytocompounds from various plant sources and identifies the PBPK model which could

Table 1: PK absorption risk factors threshold

Rules	Values
Sz: Size (too big)	MWt>550 OR N_Atoms>35 OR MolVol>500 OR N_ Bonds>40
RB: Too flexible	N_FrRotB>10
HD: Too many good H-bond donors	HBDH>4 AND HBDch>1.8
HA: Too many good H-bond acceptors	HBA>9 AND HBAch <−5.8
ch: Excessive charge	NPA_ABSQ>21 OR T_PSA>140
ow: High logPoctanol-water	S+logP>5 OR S+logD>4.1 OR MlogP>4.1
Pf: Low permeability	S+Peff<0.1 OR MDCK<25
Sw: Low solubility	S+Sw<0.005
Fu: Low fraction unbound in plasma	S+PrUnbnd<3.5%
Vd: High steady-state volume of distribution	S+Vd>5.5
Sz	Size
Mwt	Molecular weight
N_atoms	Number of atoms
MolVol	Molal volume
RB	Rotational bonds
N_FrRotB	Number of fractional rotational bonds
HD	Hydrogen donors
HBDH	The number of hydrogen bond donor hydrogens
HBDch	Sum of estimated NPA partial atomic charges on HB donor hydrogen
HA	Hydrogen acceptors
HBA	Number of hydrogen bond acceptors
HBAch	Sum of estimated NPA partial atomic charges on HB acceptors
ch	Charge
NPA_ABSQ	Sum of absolute values of estimated NPA partial atomic charges
T_PSA	Topological polar surface area
ow	Octanol
S+logP	Octanol-water partition coefficient, log P; Simulations Plus model
S+logD	Octanol-water distribution coefficient, log D; calculated from pKa and S+logP
S+Peff	[cm/s×104] (human jejunal effective permeability; Simulations Plus)
MDCK	Madin-Darby Canine Kidney (MDCK) apparent permeability data ([cm/ s×107] (apparent MDCK COS permeability; Simulations Plus))
Sw	Water solubility
S+Sw	[mg/ml] (Native water solubility; Simulations Plus)
Fu	low fraction unbound in plasma
S+PrUnbnd	(Percent unbound to blood plasma proteins; Simulations Plus)
Vd	Volume of distribution
S+Vd	[L/kg] (pharmacokinetic volume of distribution in human; Simulations Plus)

help us in understanding the distinctive properties for defining an effective drug candidate. PBPK model is often derived to study about the influencing parameters for oral absorption which mostly has drawbacks with respect to the risks during and after absorption in the gastrointestinal tract.

Based on the above *in silico* studies, certain drugs had drawbacks with respect to the risk involved in the absorption, distribution, toxicity, and metabolism. On the evaluation of compounds SMILES format, data are studied based on the physiological parameters obtained from GastroPlus simulation tool which had guided the data to evaluate for the drug candidate. Various parameters tell us about the dosage levels, different compartmental models, and C_{max} and T_{max} values of each compound in detail [24]. This type of software minimizes the time taken for validation of so many compounds. Gastroplus data mainly highlight the risk involved and it is illustrated by the risk codes (Tables 1-3) based on which they explain the physiological parameters under which they are affected. However, compounds such as gingerol, eugenol, zingerone, and paradol had no risks involved with the Lipinski's rule (Table 4). The Lipinski's risk codes such as log p value and molecular weights are the major drawbacks for the other compounds due to which they have lower absorption rate as well as low hydrophilic nature (Table 4) [25]. Although many of the 10 compounds were approved in this rule, they may cause a dilemma in the bio-availability by having less clearance value and may cause toxicity in future [26,27]. Hence, the toxicity studies were performed to predict which compound had the least toxicity. Research results had shown effectively that there are toxic factors for previously screened compound such as under absorption risk factors (Tables 5 and 6). The toxicity factors did not affect any of the four compounds such as gingerol, geraniol, eugenol, and zingerone. However, the other compounds such as gallotannin, aloesin, and asiaticoside had toxicity and mutation factors such as mutation in strains, elevated enzyme levels and high concentration of alkaline phosphates, and caused mutation in rats. Once the compounds were out of toxicity zone, studies were conducted for metabolic risk factors (Table 7). This illustrates better binding ability with the enzymes and hence proven as the better substrate to the few of the important metabolizing enzymes by which they may be metabolized. As seen above, the compounds gingerol and geraniol had good binding with the enzyme sites although there was one risk factor for eugenol

Table 2: PK toxicity risk factors threshold

Rules	Values
hE: hERG liability	TOX_hERG>6
ra: Acute toxicity in rats	TOX_RAT<300
Xr: Carcinogenicity in chronic rat studies	TOX_BRM_Rat<4
Xm: Carcinogenicity in chronic mouse studies	TOX_BRM_Mouse<25
Hp: Hepatotoxicity	(TOX_AlkPhos=Toxic OR TOX_GGT=Toxic OR TOX_LDH=Toxic) AND (TOX_SGOT=Toxic OR TOX_SGPT=Toxic)
SG: SGOT and SGPT elevation	TOX_SGOT=Toxic AND TOX_SGPT=Toxic
Mu:	TOX_MUT_Risk>2
TOX_hERG	Qualitative estimation of the likelihood of hERG potassium channel inhibition in human; Simulations Plus
TOX_RAT	[mg/kg] (LD50 for lethal rat acute toxicity, all mechanisms; Simulations Plus)
TOX_BRM_Rat	[mg/kg/day] (TD50, which is defined as the oral dose of a compound required to induce tumors in 50 percent of rat population after exposure over a standard lifetime; Simulations Plus)
TOX_BRM_Mouse	[mg/kg/day] (TD50, as above, but for mouse; Simulations Plus)
TOX_AlkPhos	Human liver adverse effect as the likelihood of causing elevation in the levels of alkaline phosphatase enzyme; Simulations Plus
TOX_GGT	Human liver adverse effect as the likelihood of causing elevation in the levels of GGT enzyme; Simulations Plus
TOX_SGOT	Human liver adverse effect as the likelihood of causing elevation in the levels of SGOT enzyme; Simulations Plus
TOX_SGPT	Human liver adverse effect as the likelihood of causing elevation in the levels of SGPT enzyme; Simulations Plus
TOX_MUT_Risk	ADMET Risk and ADMET Code for mutagenicity in *S. typhimurium* – a computational filter developed by Simulations Plus summarizing the output of TOX_MUT_* models)
TOX_MUT_97+1537	Qualitative assessment of mutagenicity of the pure compound in TA97 and/or TA1537 strains of *S. typhimurium*; Simulations Plus
TOX_MUT_m97+1537	Qualitative assessment of mutagenicity of the compound and its microsomal rat liver metabolites in TA97 and/or TA1537 strains of *S. typhimurium*; Simulations Plus
TOX_MUT_98	Qualitative assessment of mutagenicity of the pure compound in TA98 strain of *S. typhimurium*; Simulations Plus
TOX_MUT_m98	Qualitative assessment of mutagenicity of the compound and its microsomal rat liver metabolites in TA98 strain of *S. typhimurium*; Simulations Plus
TOX_MUT_100	Qualitative assessment of mutagenicity of the pure compound in TA100 strain of *S. typhimurium*; Simulations Plus
TOX_MUT_m100	Assessment of mutagenicity of the compound and its microsomal rat liver metabolites in TA100 strain of *S. typhimurium*; Simulations Plus
TOX_MUT_102+wp2	Qualitative assessment of mutagenicity of the pure compound in TA102 strain of *S. typhimurium* and/or WP2 uvrA strain of *E. coli*; Simulations Plus
TOX_MUT_m102+wp2	Qualitative assessment of mutagenicity of the compound and its microsomal rat liver metabolites in TA102 strain of *S. typhimurium* and/or WP2 uvrA strain of *E. coli*; Simulations Plus
TOX_MUT_1535	Qualitative assessment of mutagenicity of the pure compound in TA1535 strain of *S. typhimurium*; Simulations Plus
TOX_MUT_m1535	Qualitative assessment of mutagenicity of the compound and its microsomal rat liver metabolites in TA1535 strain of *S. typhimurium*; Simulations Plus

S. typhimurium: Salmonella typhimurium, E. coli: Escherichia coli. ADMET: Absorption, distribution, metabolism, excretion, and toxicity

Table 3: PK metabolism risk factors threshold

Rules	Values
1A:	CYP_1A2_Substr=Yes AND MET_1A2_CLint>30
19:	CYP_2C19_Substr=Yes AND MET_2C19_CLint>30
C9:	CYP_2C9_Substr=Yes AND MET_2C9_CLint>30
D6:	CYP_2D6_Substr=Yes AND MET_2D6_CLint>30
3A:	CYP_3A4_Substr=Yes AND MET_3A4_CLint>30
mi:	MET_3A4_Ki_Mid<1.5 AND (MET_3A4_I_mid=Yes OR MET_3A4_Inh=Yes)
ti:	MET_3A4_Ki_tes<1.0 AND (MET_3A4_I_tes=Yes OR MET_3A4_Inh=Yes)
CYP_1A2_Substr	Qualitative assessment of a molecule being the substrate of CYP 1A2 in human; Simulations Plus
MET_1A2_CLint	µl/min/mg microsomal protein (intrinsic clearance constant for CYP 1A2 mediated metabolism; Enslein Research and Simulations Plus)
CYP_2C19_Substr	Qualitative assessment of a molecule being the substrate of CYP 2C19 in human; Simulations Plus
MET_2C19_CLint	µl/min/mg microsomal protein (intrinsic clearance constant for CYP 2C19 mediated metabolism; Enslein Research and Simulations Plus)
CYP_2C9_Substr	Qualitative assessment of a molecule being the substrate of CYP 2C9 in human; Simulations Plus
MET_2C9_CLint	µl/min/mg microsomal protein (intrinsic clearance constant for CYP 2C9 mediated metabolism; Enslein Research and Simulations Plus)
CYP_2D6_Substr	Qualitative assessment of a molecule being the substrate of CYP 2D6 in human; Simulations Plus
MET_2D6_CLint	µl/min/mg microsomal protein (intrinsic clearance constant for CYP 2D6 mediated metabolism; Enslein Research and Simulations Plus)
CYP_3A4_Substr	Qualitative assessment of a molecule being the substrate of CYP 3A4 in human; Simulations Plus
MET_3A4_CLint	µl/min/mg microsomal protein (intrinsic clearance constant for CYP 3A4 mediated metabolism; Enslein Research and Simulations Plus)
MET_3A4_Ki_Mid	[µM] (specific inhibition constant for the CYP 3A4-mediated metabolism of midazolam; Enslein Research and Simulations Plus)
MET_3A4_I_mid	Qualitative model of a specific inhibition of the CYP 3A4-mediated metabolism of midazolam; Enslein Research and Simulations Plus
MET_3A4_Inh	Qualitative estimation of general inhibitory action against CYP 3A4; Simulations Plus
MET_3A4_Ki_tes	[µM] (specific inhibition constant for the CYP 3A4-mediated metabolism of testosterone; Enslein Research and Simulations Plus)
MET_3A4_I_tes	Qualitative model of a specific inhibition of the CYP 3A4-mediated metabolism of testosterone; Enslein Research and Simulations Plus
MET_3A4_Inh	Qualitative estimation of general inhibitory action against CYP 3A4; Simulations Plus

and zingerone (Fig. 1); this is because they did not have good binding with the enzyme C9 (Fig. 1); whereas, the other compounds had shown drawbacks by being non-substrate to many of the enzymes (Table 7). In this study, we even evaluated the physiological factors. The compounds were taken as immediate release tablets with a dosage of 100 mg and were observed with a time period of 24 hrs. The compounds were absorbed into different compartments of the human intestinal tract. Based on the total fraction of absorption and the C_{max}-T_{max} values, it was found that the previously screened compounds such as eugenol, gingerol, zingerone, and geraniol had a better rate of absorption rather than the other compounds (Table 7). Here, the other compounds faced a disadvantage of having very less absorption fraction along with the high T_{max} values with Asiaticoside being the least satisfying compound. The four compounds have been shown with the maximum fraction of absorption in the human intestinal compartment, out of which gingerol and eugenol have comparatively highest with risk factors as well (Fig. 2). The entire compartment model that occurred in the PBPK was literally suggested to have better fraction of absorption for the very few compounds that were screened earlier. The pharmacokinetic studies paved the way for the lead compound to be taken to the next level of validating the candidate for the drug in the clinical research [28].

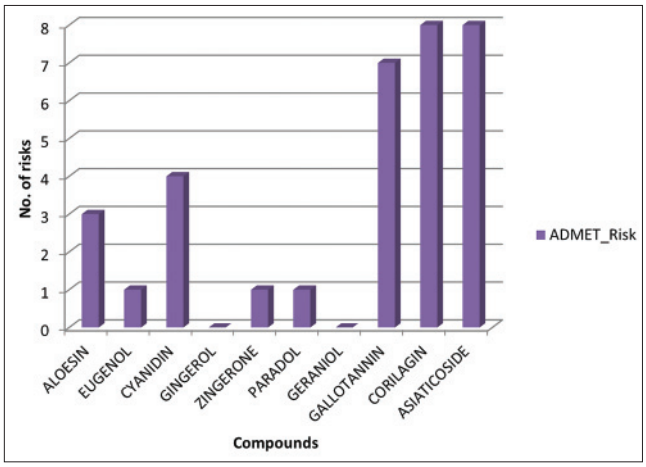

Fig. 1: Total risks of compounds based on absorption, distribution, metabolism, excretion, and toxicity. A total number of absorption, distribution, metabolism, excretion, and toxicity risk factors were analyzed for each compound Aloesin (3), Eugenol (1), Cyanidin (4), Gingerol (0), Zingerone (1), Paradol (1), Geraniol (0), Gallotannin (7), Corilgin (8), Asiaticoside (8)

CONCLUSION

Pharmacokinetics plays an important role in the validation of a lead compound. Many a time the compounds obtained from plant sources are left without being taken to the next level of becoming an appropriate drug. These studies have taken our compound of interest to the various PBPK models which helped our research to identify the best anti-oxidant compound. The PBPK models were illustrated with four better anti-oxidants that had shown greater fraction of absorption and with less toxicity (Table 8 and Fig. 1) and it was proven under ADMET risk factors (Fig. 2). Hence, the graph shows that those 4 compounds, namely eugenol, gingerol, zingerone, and geraniol had better ADME and physiological properties (Figs. 1 and 2) although one or two risk factors might cause certain negligible side effects. Since eugenol and gingerol have higher factor of absorption, there is more probability of these two compounds of becoming a drug in future.

Our study has given us the scope to carry on further *in vitro* and *ex vivo* [29] studies to test these compounds for a better drug molecule or therapeutic compound. The future prospective of this study will be to

Table 4: Absorption risk factors

Name	Molwt	N_Aatoms	Mol vol	N_Bonds	N_Frrotb	Hbdh	Hdbch	Hba	Hbach	Npa_Absq	T_Psa	S+Logp	S+Logd	Mlogp	S+Peff	Mdck	S+Sw	S+Prunbnd	S+Vd	Risk Codes	Number of risk codes
Aloesin	394.38	29	329	30	4	5*	2.39	9	−5.88	17.37	158	−0.79	−0.79	−1.14	0.01	108.87	7.73E+00	24.64	1.07	HD, CH, Pf	3
Eugenol	164.21	12	168	12	3	1	0.46	2	−1.2	6.6	2.95E+01	2.53	2.52	2.62	7.8	632.81	9.15E-01	44.1	1.26		0
Cyanidin	287.25	21	203	23	0	5	2.49	6	−3.47	11.39	1.14E+02	0.93	0.11	0.6	0.46	20.58	2.06E-01	8.48	0.14	hd, pf	2
Gingerol	294.39	21	322	21	10	2	0.91	4	−2.44	14.25	66.8	2.99	2.99	2.83	3.19	357.3	1.24E-01	26.96	1.23		0
Zingerone	194.23	14	189	14	4	1	0.46	3	−1.67	8.72	46.54	1.6	1.6	2.08	6.22	507.11	1.64E+00	47.45	0.98		0
Paradol	278.39	20	315	20	10	1	0.45	3	−1.68	13.52	46.54	4.54	4.53	3.64	7.62	457.3	1.49E-02	24.21	1.46	ow	1
Geraniol	154.25	11	217	10	4	1	0.45	1	−0.76	8.17	2.02E+01	2.59	2.59	2.64	4.89	408.23	1.46E+00	43.21	1.45		0
Gallotannin	636.48	45	455	48	7	11	5.32	18	−11.5	27.23	3.11E+02	−0.04	−1.51	−4.01	0.27	14.24	3.28E+00	1.57	0.18	SZ, HD, HA, ch, pf, fu	6
Corilagin	634.46	45	441	49	2	11	5.36	18	−11.46	26.47	3.11E+02	0.1	−1.41	−4.01	0.12	14.21	7.89E-01	1.54	0.2	SZ, HD, HA, ch, pf, fu	6
Asiaticoside	959.14	67	966	74	10	12	5.6	19	−13.68	44.72	3.15E+02	0.59	0.59	−1.76	0.01	16.36	4.88E-01	26.95	1.69	SZ, HD, HA, ch, pf	5

*The highlighted values represent the various absorption risk factors of anti-oxidants which exceed the threshold values based on Table 1

Table 5: Mutation risks factor

Name	Tox_Mut_97+ 1537	Tox_Mut_M97+ 1537	Tox_Mut_98	Tox_Mut_M98	Tox_Mut_100	Tox_Mut_M100	Tox_Mut_102+ Wp2	Tox_Mut_M102+ Wp2	Tox_Mut_1535	Tox_Mut_M1535	Tox_Mut_Risk	Tox_Mut_Code
Aloesin	Undecided	Toxic	Nontoxic	Nontoxic	Nontoxic	Nontoxic	Undecided	Nontoxic	Nontoxic	Nontoxic	1.5	m1, SU
Eugenol	Nontoxic	Nontoxic	Nontoxic	Nontoxic	Nontoxic	Nontoxic	Nontoxic	Nontoxic	Nontoxic	Nontoxic	0	
Cyanidin	Nontoxic	Toxic	Nontoxic	Nontoxic	Nontoxic	Nontoxic	Toxic	Nontoxic	Nontoxic	Nontoxic	2	m1, S4
Gingerol	Nontoxic	Nontoxic	Nontoxic	Nontoxic	Nontoxic	Nontoxic	Nontoxic	Nontoxic	Nontoxic	Nontoxic	0	
Zingerone	Nontoxic	Nontoxic	Nontoxic	Nontoxic	Nontoxic	Nontoxic	Nontoxic	Nontoxic	Nontoxic	Nontoxic	0	
Paradol	Nontoxic	Nontoxic	Nontoxic	Nontoxic	Nontoxic	Nontoxic	Nontoxic	Nontoxic	Nontoxic	Nontoxic	0	
Geraniol	Nontoxic	Nontoxic	Nontoxic	Nontoxic	Nontoxic	Nontoxic	Nontoxic	Nontoxic	Nontoxic	Nontoxic	0	
Gallotannin	Nontoxic	Nontoxic	Nontoxic	Nontoxic	Nontoxic	Nontoxic	Nontoxic	Nontoxic	Nontoxic	Nontoxic	0	
Corilagin	Toxic	Nontoxic	Nontoxic	Nontoxic	Nontoxic	Nontoxic	Nontoxic	Nontoxic	Nontoxic	Nontoxic	1	s1
Asiaticoside	Nontoxic	Nontoxic	Nontoxic	Nontoxic	Nontoxic	Nontoxic	Nontoxic	Nontoxic	Nontoxic	Nontoxic	0	

Table 6: Toxicity risks factor

Name	Tox_Herg	Tox_Rat	Tox_Brm_Rat	Tox_Brm_Mouse	Tox_Alkphos	Tox_Sgpt	Tox_Ggt	Tox_Ldh	Tox_Sgot	Tox_Code	Tox_Risk
Aloesin	3.6	901.75	113.04	1516.75	Nontoxic	Toxic	Nontoxic	Nontoxic	Nontoxic		0
Eugenol	3.96	1299.83	141.96	679.33	Nontoxic	Nontoxic	Toxic	Nontoxic	Nontoxic		0
Cyanidin	4.43	1699.22	393.09	519.84	Nontoxic	Toxic	Toxic	Nontoxic	Toxic	hp, SG	2
Gingerol	4.97	3516.66	109.78	1212.79	Nontoxic	Nontoxic	Nontoxic	Nontoxic	Nontoxic		0
Zingerone	4.32	2194.53	149.15	999.92	Nontoxic	Nontoxic	Nontoxic	Nontoxic	Nontoxic		0
Paradol	5.29	3440.73	121.01	1151.51	Nontoxic	Nontoxic	Nontoxic	Nontoxic	Nontoxic		0
Geraniol	3.78	2350.73	101.61	267.61	Toxic	Nontoxic	Toxic	Toxic	Undecided		0
Gallotannin	4.52	2614.57	574.97	2167.39	Nontoxic	Undecided	Nontoxic	Nontoxic	Toxic		0
Corilagin	4.24	1740.31	535.02	1174.89	Nontoxic	Nontoxic	Toxic	Nontoxic	Toxic	hp	1
Asiaticoside	3	32.52	11.49	1559.5	Nontoxic	Toxic	Nontoxic	Nontoxic	Toxic	ra, SG	2

Table 7: Metabolic risk factors

Name	CYP_1A2_ Substr	CYP_1A2_ Sites	MET_1A2_ Clint	CYP_2C19_ Substr	CYP_2C19_ Sites	MET_2C19_ Clint	CYP_2C9_ Substr	CYP_2C9_Sites	MET_2C9_ Clint	CYP_2D6_ Substr	CYP_2D6_ Sites	MET_2D6_ Clint	CYP_3A4_ Substr
Aloesin	No	Non Substrate	0.815	No	Non Substrate	26.911	No*	Non Substrate	135.047	No	Non Substrate	0.43	No
Eugenol	Yes	C9 (0.925); C1 (0.846)	0.599	Yes	C1 (0.96); C9 (0.871)	0.104	Yes	C1 (0.938); C9 (0.904)	66.909	Yes	C1 (0.973); C9 (0.779)	0	No
Cyanidin	Yes	C12 (0.688)	7.414	No	Non Substrate	0.449	No	Non Substrate	2.01	No	Non Substrate	0.87	No
Gingerol	No	Non Substrate	1.479	Yes	C19 (0.988)	2.778	No	Non Substrate	187.161	Yes	C19 (0.996); C2 (0.624)	2.1	No
Zingerone	No	Non Substrate	0.271	Yes	C14 (0.959)	0.203	Yes	C14 (0.938)	87.689	Yes	C14 (0.979); C1 (0.706)	0.07	No
Paradol	No	Non Substrate	1.575	Yes	C20 (0.964)	0.486	No	Non Substrate	195.27	Yes	C20 (0.992); C2 (0.714); C3 (0.584); C1 (0.532)	1.59	No
Geraniol	Yes	C8 (0.965); C11 (0.937); C1 (0.937)	0.415	Yes	C11 (0.924); C1 (0.924); C8 (0.734)	0.392	Yes	C11 (0.937); C1 (0.937); C8 (0.9)	5.704	Yes	C11 (0.932); C1 (0.932)	0.08	No
Gallotannin	No	Non Substrate	0.033	No	Non Substrate	0.18	No	Non Substrate	2.077	No	Non Substrate	0.07	No
Corilagin	No	Non Substrate	0.042	No	Non Substrate	0.076	No	Non Substrate	0.661	No	Non Substrate	0.17	No
Asiaticoside	No	Non Substrate	618.2	No	Non Substrate	7598.4	No	Non Substrate	0.389	No	Non Substrate	0.06	No

*The highlighted values represent the various metabolic risk factors of anti-oxidants which exceed the threshold values based on Table 3

CYP_1A2_Substr	Qualitative assessment of a molecule being the substrate of CYP1A2 in human; Simulations Plus
CYP_1A2_Sites	Specific sites of human CYP1A2 mediated oxidation; Simulations Plus
MET_1A2_CLint	[µl/min/mg microsomal protein] intrinsic clearance constant for CYP1A2 mediated metabolism; Enslein Research and Simulations Plus
CYP_2C19_Substr	Qualitative assessment of a molecule being the substrate of CYP2C19 in human; Simulations Plus
CYP_2C19_Sites	Specific sites of human CYP2C19 mediated oxidation; Simulations Plus
MET_2C19_CLint	[µl/min/mg microsomal protein] intrinsic clearance constant for CYP2C19 mediated metabolism; Enslein Research and Simulations Plus
CYP_2C9_Substr	Qualitative assessment of a molecule being the substrate of CYP2C9 in human; Simulations Plus
CYP_2C9_Sites	Specific sites of human CYP 2C9 mediated oxidation; Simulations Plus
MET_2C9_CLint	[µl/min/mg microsomal protein] intrinsic clearance constant for CYP2C9 mediated metabolism; Enslein Research and Simulations Plus
CYP_2D6_Substr	Qualitative assessment of a molecule being the substrate of CYP2D6 in human; Simulations Plus
CYP_2D6_Sites	Specific sites of human CYP2D6 mediated oxidation; Simulations Plus
MET_2D6_CLint	[µL/min/mg microsomal protein] intrinsic clearance constant for CYP2D6 mediated metabolism; Enslein Research and Simulations Plus
CYP_3A4_Substr	Qualitative assessment of a molecule being the substrate of CYP3A4 in human; Simulations Plus

Table 8: PBPK model based on the human compartmental study

Name/Id	Dose	Stom Fa %	Duod Fa %	Jej1 Fa %	Jej2 Fa %	Ile1 Fa %	Ile2 Fa %	Ile3 Fa %	Caec Fa %	Col Fa %	Pred Fa %	Pred C_{max}	Pred T_{max}
Aloesin	100	0	0.196	0.699	0.554	0.435	0.315	0.22	0.063	0.264	2.746	0.029	24
Eugenol	100	0	55.11	33.21	7.216	1.73	0.391	0.083	1.739	0.166	99.64*	1.102	3.88
Cyanidin	100	0	8.373	22.03	13.86	8.979	5.579	3.449	3.091	9.433	74.79	7.167	24
Gingerol	100	0	10.61	34.94	23.21	13.19	6.686	3.368	7.037	0.732	99.77	1.129	6.66
Zingerone	100	0	50.78	34.78	8.341	2.225	0.568	0.145	2.134	0.475	99.44	1.418	4.74
Paradol	100	0	2.203	8.294	7.619	6.273	4.616	3.23	31.87	28.75	92.85	0.886	12.32
Geraniol	100	0	46.29	37.36	9.976	2.965	0.911	0.309	1.687	0.196	99.7	0.956	4.8
Gallotannin	100	0	4.267	12.99	9.097	6.413	4.226	2.704	1.166	3.025	43.89	3.355	24
Corilagin	100	0	1.837	6.236	4.64	3.433	2.349	1.553	0.467	1.296	21.81	1.531	24
Asiaticoside	100	0	0.06	0.332	0.287	0.23	0.167	0.116	0.095	0.331	1.617	0.0088	24

*The highlighted values represent the predicted fraction of absorption of anti-oxidants which are < 90%. PBPK: Physiologically-based pharmacokinetic

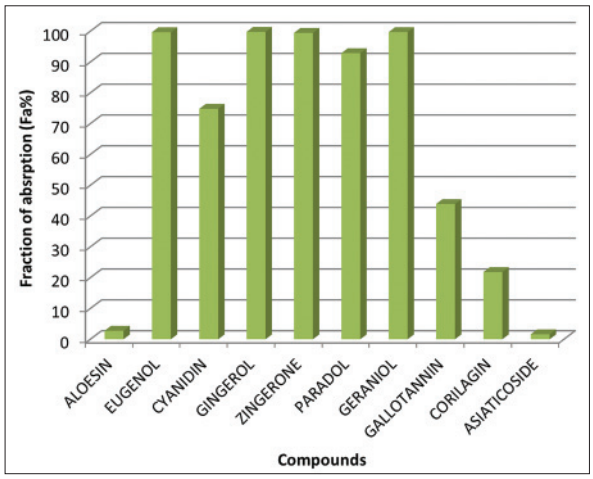

Fig. 2: Total predicted fraction of absorption of each compound in different compartments of human gastrointestinal tract. Percentage of absorption fraction were analyzed for each compound Aloesin (2.76), Eugenol (99.64), Cyanidin (74.79), Gingerol (99.97), Zingerone (99.44), Paradol (92.85), Geraniol (99.7), Gallotannin (43.87), Corilgin (21.81), Asiaticoside (1.617)

facilitate the four compounds to be formulated as a drug with the help of pharmacodynamic studies.

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