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RESEARCH ARTICLE

FOODINFORMATICS OF VANILLIN-RICH DRINK AGAINST CANONICAL TARGETS OF BREAST CANCER

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Abstract

Aim and objective: Vanillin-rich drink (Vimto®) is a popular drink among women. The aim of this study was to evaluate anti-breast cancer potential of Vimto®.
Methods: The chemical composition of Vimto® has been determined by gas chromatography and foodinformatics used to identify its putative binders to canonical targets of breast cancer. The binding affinity (kcal/mol) of the target proteins of breast cancer and the phyto compounds was computationally obtained.
Results: Major aliphatic hydrocarbons of Vimto® were hydroxymethyl furfurole, tetradecane, hexadecane, dodecane, octadecane and 9-octadecenamide while aromatic hydrocarbons were vanillin, benzoic acid, diisooctyl phthalate, butanoic acid, piperonal, bis (2-ethylhexyl) phthalate, and germacrene D. 9-octadecenamide bound firmly to HER2 (human epidermal growth factor receptor 2) and warrant experimental studies. All aromatic hydrocarbons expect benzoic acid and vanillin showed reliable BA with HER2. Germacrene D showed acceptable binding affinity with estrogen receptor alpha (ERα) and estrone sulfatase. Benzoic acid and diisooctyl phthalate showed strong BA with phosphoinositide 3-kinases (PI3Ks) and AKT (protein kinase B), respectively. Bis (2-ethylhexyl) phthalate and diisooctyl phthalate have been docked with HER2. Butanoic acid and bis (2-ethylhexyl) phthalate showed binding affinity with aromatase. Benzoic acid and diisooctyl phthalate, and bis (2-ethylhexyl) are not safe compounds while bounded to target proteins of breast cancer.
Conclusions: To sum up, Vimto® is an amalgam of safe compounds possess possible anti-breast cancer, however it needs further experimental and biosafety evaluation.
Keywords: Breast cancer, foodinformatics, molecular docking, vanillin Vimto®drink.

INTRODUCTION

Vimto (Vimto®) dubbed as a vanillin-rich non-carbonated drink produced mainly in the United Kingdom and the Kingdom of Saudi Arabia, however distributed globally. The major formula of Vimto® is still ambiguous while it is an admixture of the juice of grapes, raspberries, and blackcurrants flavored with unknown herbs and spices. The principal recipe of Vimto®, developed in 1908 by (John) Noel Nichols in the Lancashire, Blackburn, is available in cans and bottles and served as a draught soft drink[1]. The major component of Vimto®, vanillin, is a plant secondary metabolite of *Vanilla* which has a phenolic phenyl propane C6-C1 derivatives. Vanillin has been used as ubiquitous flavor and aroma and found principally *V. planifolia, V. tahitensis,* and *V. pompona*[2] and often employed in beverages, processed foods, pharmaceutics, and perfumery[3,4]. Vanillin has anti-oxidative and antitumor activities and frequently used in cosmetic products[5-7]. In addition, antimicrobial, anti-inflammatory, anti-mutagenic, and hypotriglyceridemic activities of vanillin have been reported elsewhere[8-10]. More specifically, vanillin solely possesses anti-cancer effects by interfering various pathways of tumorigenesis in an array of cancers including breast cancer (BC[11-19]). However, studies focused on cytotoxicity of vanillin and vanillin-rich formulations are scarce[20].

Breast cancer is one of the leading causes of mortality among women worldwide[21]. The majority of BCs are carcinoma-forming cells that make up milk constituents in the mammary gland. The presence or absence of hormone receptors (estrogen and

progesterone subtypes) and human epidermal growth factor receptor-2 (HER2) are the basis of categorization of BCs. In this scenario, hormone receptor positive and HER2 negative (luminal A subtype), hormone receptor positive and HER2 positive (luminal B subtype), hormone receptor negative and HER2 positive (HER2 positive), and hormone receptor negative and HER2 negative (basal-like or triple-negative breast cancers (TNBCs) are major categories of BCs. Hormone receptor positive BCs are largely governed by the estrogen signaling pathway[22]. In HER2 positive BCs, HER2 activates the PI3K/AKT and the RAS/RAF/MAPK pathways and stimulate cell growth, survival and differentiation[23]. In this line, searching of novel and botanical anti-BC compounds still is an active research avenue. To speed up the process of drug discovery and bio-designing new functional anti-cancer drinks, foods, and formulations, employing *in silico* tools are a necessity to treat or to prevent various cancer phenotypes.

Many molecular modeling methods have been employed in pharmaceutical researches to discover new drugs and their biological and chemical systems. The conglomerate of computational and experimental strategies has a great value in the identification and development of new promising compounds. Molecular docking method has been vastly used in novel drug designing, exploring the binding and ligand conformations accepted within the binding sites of macromolecular targets[24]. This study has been aimed to investigate anti-BC activity of Vimto® using foodinformatics tools.

MATERIALS AND METHODS

Chemical composition

A commercial brand of soft drink Vimto® was a sweet glass bottle version made in Saudi Arabia (Fimto in Arabic) imported and distributed in Iranian and global markets. To prepare extracted oil of Vimto®, liquid-liquid extraction procedure has been employed. Briefly, solvent dichloromethane mixed with NaCl saturated Vimto® extracted in an ultrasonic batch. After that, the extracted oil was desiccated over anhydrous magnesium sulfate and refrigerated at 4°C before analysis (*vide infra*).The gas chromatography (GC) analysis was accomplished using a Hewlett-Packard chromatograph 5890 series equipped with flame ionization detector (FID) and a HP-1 fused silica column (30 m×0.25 mm and film thickness 0.25 µm). Gas chromatography/mass spectrometry (GC/MS) was accomplished on a Hewlett-Packard 5973 coupled with a mass detector HP 6890 using aforementioned HP-1 column. Oven temperature was set on 40-250°C with an ascending temperature rate of 3°C/min for both GC/FID and GC/MS while injector and detector were set on 320 and 310°C, respectively. Helium was carrier gas which flew at a rate of 1 mL/min. The mass spectrometer was operated at 70 eV with the mass range of 40-350 amu and scan time 1 s. Exploration was pursued based on model retention data and evaluation with reliable standards like NIST MS library. The exploration was also qualified by comparison of the retention indices with data in the literature[25,26]. The percentages of compounds were calculated by the area normalization method and the retention indices were calculated for all volatile components using a homologous series of *n*-alkanes[27].

In silico anti-breast cancer assay

Molecular docking of target protein of BC (Table 1) and selected bioactive compounds of Vimto® was performed by VINA WIZARD docking program mounted on PyRx software version 0.8 (Table 2;28). First, crystal structures of BC target proteins were taken from the Protein Data Bank (PDB; http://www.RCSB.org). The PDB format of target protein has been primed for docking using Molegro Virtual Docker (29) and Chimera 1.12.1 software before loading to PyRx software. The structures of the major phytocompounds of Vimto® were repossessed from ZINC database version 12.0 and/or PubChem.

The docking outcomes were achieved as binding affinity (BA; kcal/mol) values. More negative the binding affinity of ligand declares superior docking to target proteins. The best pose of ligand in target protein in Molegro Virtual Docker or Chimera and its atomic interactions have been visualized by Lig+Plot software[30].

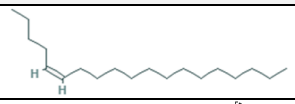
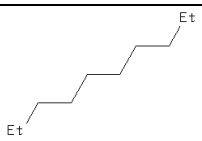
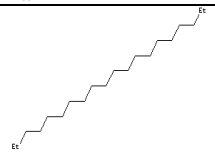
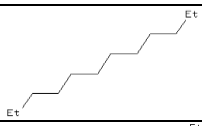
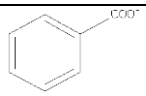
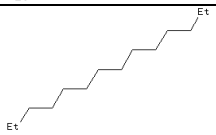
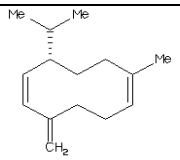
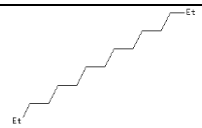
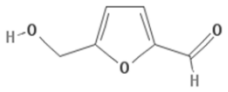
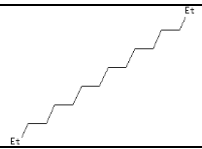
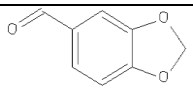
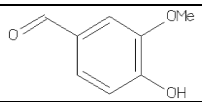
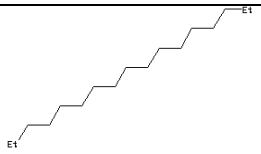
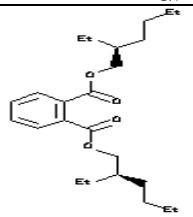
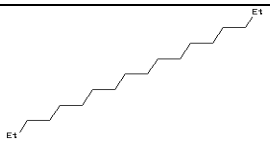
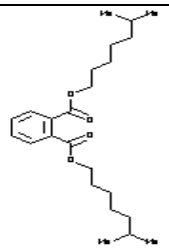
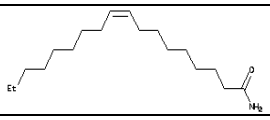
RESULTS AND DISCUSSION

Figure 1 showed GC of the Vimto® drink and their chemical constituents of Vimto® were demonstrated in Table 2. The main component that isolated from Vimto® in the presence of CH2Cl2 solvent was vanillin (Table 3, Figure 1). According to the results of chromatography (Table 3, Figure 1), the vanillin had the highest amount in this beverage (26.604%) as compared to benzoic acid (10.994%), 5-hydroxy-methylfurfural (5.197%), and diisooctyl phthalate (2.353%).

Table 1: The canonical proteins involved in breast cancer, their PDB codes and inhibitors.

Target protein	PDB code	Inhibitor	Reference
AKT serine/threonine-protein kinase	3E88	MK2206	31
Receptor tyrosine-protein kinaseerbB-2 (HER2)	3PP0	Lapatinib	32
Mitogen-activated protein kinase 1(ERK)	5NGU	GSK2141795	33
Aromatase	3EQM	Exemestane	34
Estrogen receptor (ER)-alpha	1X7E	Tamoxifen	35
Estrone sulfatase	1P49	STX-64	36
Phosphatidylinositol 3-kinase (PI3K)	5SW8	Perifosine	37

Table 2: The chemical identity of compounds isolated from Vimto® beverage.

Compound	Zinc Database (Z)/PubChem (P) code	Chemical structure	Compound	Zinc Database (Z)/PubChem (P) code	Chemical structure
Butanoic acid	Z:895132		z-5-Nanodecane	P:5364560	
Decane	Z:1648227		Eicosane	Z:15638542	
Dodecane	Z:1531085		Benzoic acid	Z:1011	
Tetradecane	Z:1698519		Germacrene D	Z:30730221	
Pentadecane	Z:1531089		5-Hydroxy-Methyl-furfural	P:237332	
Hexadecane	Z:38141452		Piperonal	Z:1953	
n-Hexadecanoic acid	P:985		Vanillin	Z:2567933	
Heptadecane	Z:8217397		Bis (2-ethylhexyl)-phthalate	Z:3860432	
Octadecane	Z:59592152		Diisooctyl phthalate	Z:4655188	
9-Octadecenamide	Z:8036015				

There were also minor compounds with a low percentage (<1%) in the Vimto® drink such as bis (2-ethylhexyl) phthalate (0.497%), butanoic acid (0.630 %), germacrene D (0.142%) and 9-octadecena-mide (0.408%). The best BA (lesser than -7.0) of compounds of Vimto® as presented in the Table 4, as well as, the interactions between the ligand and the proteins were depicted in the following figures. Based on docking results, all of aliphatic hydrocarbons except 9-octadecenamide which found in the Vimto® drink were not trustfully bound to selected target of BC. In addition, among aromatic hydrocarbons, piperonal and vanillin have been docked with selected target of BC with BA greater than -7 kcal/mol. While diisooctyl phthalate showed reliable BA with AKT protein at -7.1 kcal/mol (Table 4 and Figure 2). It has been docked to AKT protein with hydrogen bond (Lys214) and hydrophobic interactions (Val199, Asp326, Leu216,

Phe196 and Leu329) which are common in the interaction of MK-2206 with AKT. Butanoic acid showed reliable BA -7.4 kcal/mol with HER2 protein (Table 4) and it has been docked to HER2 protein with hydrogen bond (Asp444) and hydrophobic interaction (Val315) which are common in lapatinib interaction with HER2 (Figure 3A and Figure 3F). Butanoic acid has been also docked with hydrophobic interactions (Phe177 and Asp265) that are common in exemestane interaction with aromatase (Figure 5B and Figure 5C) and they showed reliable BA with aromatase at identical BA of -7.0 kcal/mol (Table 4).

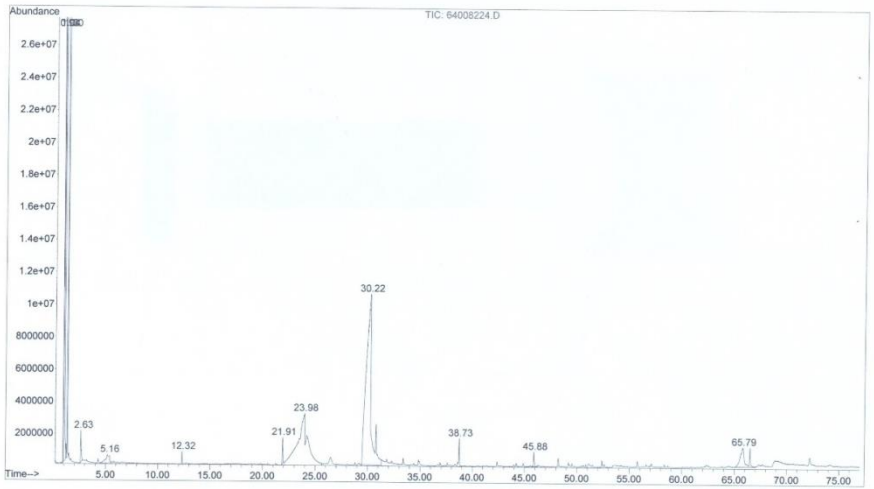


Figure 1: Gas chromatogram of the Vimto® through using dichloromethane as solvent. The main compounds represented from left to right on the chromatogram are benzoic acid,5-hydroxymethylfurfural, vanillin and diisooctyl phthalate.

Butanoic acid has been docked with a hydrogen bond with ER-alpha protein as common in tamoxifen (His213) binding to ER-alpha protein. Butanoic acid has been docked with hydrophobic interactions (Arg129, Pro138, Glu94, Phe97, etc.) and hydrogen bonds (Glu95 and Arg107) with PI3K (Figure 8A) comparable to perifosine. In sum, butanoic acid showed trustful BA with BC classic targets and this short fatty acid can be considered as endogenous or exogenous compound for oncotherapy. One study showed that butanoic acid prodrugs that release formaldehyde, boost doxorubicin (Dox) chemo-therapeutic activity and also possess cardioprotective against Dox induced cardiotoxicity[38].

Hence, clinical co-applications of these prodrugs with Dox chemotherapy need further investigations[38]. 9-Octadecenamid showed reliable BA of -7.3 kcal/mol with HER2 protein (Table 4) and it has been docked to HER2 protein with hydrogen bond (Met96) and hydrophobic interactions (Leu147, Lys48, Leu91, Thr157, Asp158, etc.; Figure 3B). This compound has also antimicrobial and anti-inflammatory effects[39]. Germacrene D showed reliable BA of -7.0 kcal/mol with HER2 protein (Table 4) and it has been docked to HER2 protein with hydrophobic interactions (Val315, Asp444, Leu307, Phe577, and Leu433) that are common in lapatinib with HER2 (Figure 3C and 3F).

Table 3: Chemical composition (%) of the Vimto® in the presence of dichloromethane solvent.

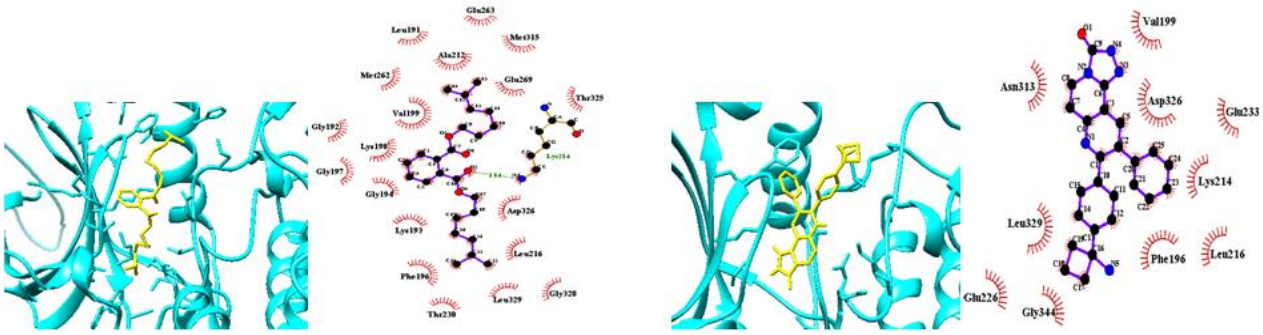
Compound	Percentage	Retention index
Butanoic acid	0.630	
Decane	0.234	920
Dodecane	0.490	1110
Benzoic acid	10.994	1151
5-Hydroxymethylfurfural	5.197	1155
Piperonal	0.533	120
Vanillin	26.604	1306
Tetradecane	0.553	1316
Germacrene D	0.142	1358
Pentadecane	0.112	1382
Hexadecane	0.546	1483
Heptadecane	0.091	1560
Octadecane	0.403	1656
Z-5-Nonadecene	0.199	1728
n-Hexadecanoic acid	0.178	1796
Eicosane	0.144	1825
Diisooctyl phthalate	2.353	2136
Bis(2-ethylhexyl)	0.497	2153
phthalate	0.408	2286
9-Octadecenamide		

Table 4: *In silico* molecular docking of major compounds of Vimto® drink against selected canonical targets in breast cancer expressed as binding affinity (kcal/mol).

Compound	AKT 3E88	HER2 3PP0	ERK 5NGU	Aromatase 3EQM	ER-Alpha 1X7E	Estrone Sulfatase 1P49	PI3K 5SW8
Butanoic acid		-7.4		-7.0	-7.6		
9-octadecenamide		-7.3					
Benzoic acid							-7.5
GermacreneD		-7.0	-7.0		-8.4	-7.0	
Vanillin							
Bis(2-ethylhexyl)phthalate		-8.0		-7.0			
Diisooctyl phthalate	-7.1	-7.7				-7.5	
MK-2206	-9.0						
Lapatinib		-10.3					
GSK-2141795			-7.7				
Exemestane				-9.1			
Tamoxifen					-6.7		
STX-64						-8.0	
Perifosine							-5.9

Note: Blank cell shows that binding affinity of ligand to target proteins was bigger than -7.0 kcal/mol and intentionally not reported.

Bis (2-ethylhexyl) phthalate has been docked with acceptably BA of -8.0 kcal/mol (Table 4) and employed both hydrogen bonds (Thr157 and Asp158) and hydrophobic interactions (Leu147, Lys48, Val29, Thr93, Glu65, etc.) with HER2 protein (Figure 3D) while diisooctyl phthalate has been docked with reliable BA of -7.7 kcal/mol (Table 4) and docked with hydrogen bond (Asp444) and hydrophobic interactions (Val315, Leu307, Leu433, Phe577, and Cys386) to HER2 protein which lapatinib also interacted with these amino acid residues with HER2 (Figure 3E and 3F). Hydrogen bonds are presented using dashed lines, while hydrophobic interactions showed with semicircles with spokes directed to ligand atoms they interact.

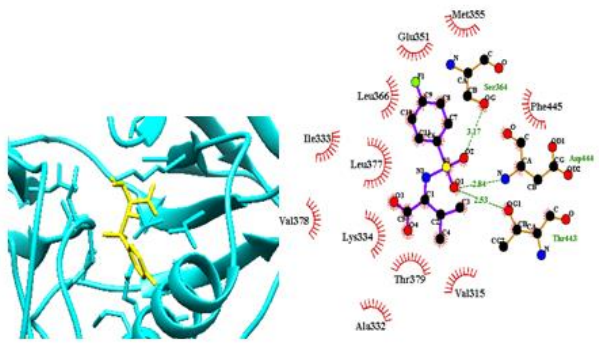


2A: AKT-3E88-diisooctyl-phthalate-4655188 2B: AKT-3E88-MK-2206-24964624

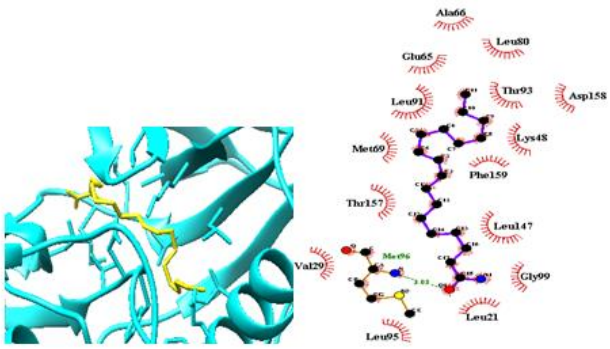
Figure 2: Molecular docking of phytocompounds (in yellow color) of the Vimto® with AKT serine/threonine-protein kinase (PDB code 3E88; in cyan color).

Germacrene D has been docked with hydrophobic interactions (Ala42, Leu146, Asp101, Ser143 and Met98), which GSK-2141795 also interacted via these amino acid residues with ERK2 (Figure 4A and 4B) and showed reliable BA with ERK2 protein at -7.0 kcal/mol (Table 4). Germacrene D has been docked with hydrophobic interactions (Met322, Leu318, Phe338, Thr281, etc.) with ER-alpha (Figure 6B) and showed reliable and acceptable BA of -7.6 and -8.4 kcal/mol with ER-alpha protein, respectively (Table 4). Germacrene D has been interacted hydrophobically using amino acid residues (Trp528, Phe210, Leu206, Gly209, etc.) with estrone sulfatase (Figure 7A) and diisooctyl phthalate has been employed hydrophobic interactions that are common in STX-64 (Gly158 and Phe155) interaction with estrone sulfatase (Figure 7B and 7C) and showed reliable BA with estrone sulfatase protein at -7.0 and -7.5 kcal/mol, respectively (Table 4). Germacrene D has been docked with hydrophobic interactions (Arg93, Ile126, Arg129, Glu92, etc.) with PI3K (Figure 8B) and bis (2-ethylhexyl) phthalate has been docked with hydrophobic interactions (Lys1064, Ile1075, Tyr1052, Val1047, Gln528, etc.) with PI3K (Figure 8C) and showed reliable BA of -7.0, -7.1 and -7.1 kcal/mol with PI3K protein, respectively (Table 4).

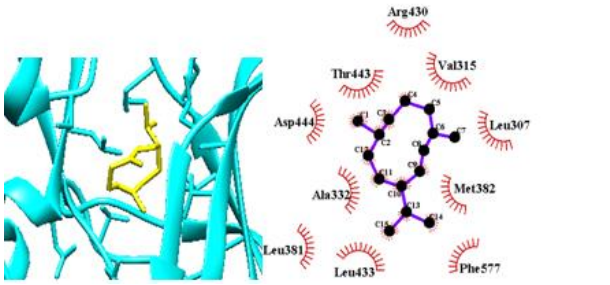
Germacrene, as a sesquiterpene, is a volatile phytocompound has antimicrobial and insecticidal properties, though it also plays a role as insect pheromone. In one study, the antioxidative and cytotoxic capacity of *Kundmannia secula* plant, rich source of sesquiterpene hydrocarbon germacrene D, has been investigated and results supported the applications of this plant as an eco-friendly natural source of germacrene D which could to be used in industrial level (40). Bis (2-ethylhexyl) phthalate has been docked with aromatase using hydrogen bond (Arg71) and hydrophobic interactions (Met330, Trp180, Leu328, Phe177, Thr266, Leu433, Val326 and Ile89) that are common in the interaction of exemestane with aromatase (Figure 5A). Phthalates are commonly fortified to plastics to boost their flexibility, transparency, durability and longevity.



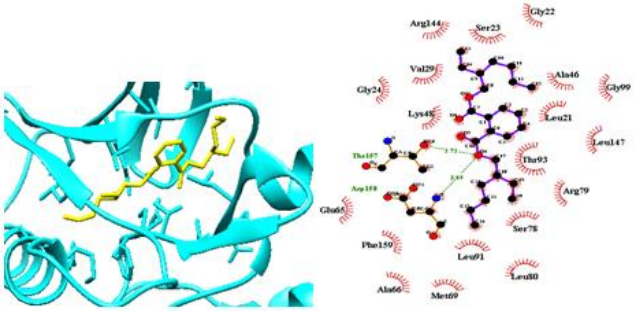
3A: HER2-3PP0-Butanoic-acid-166179



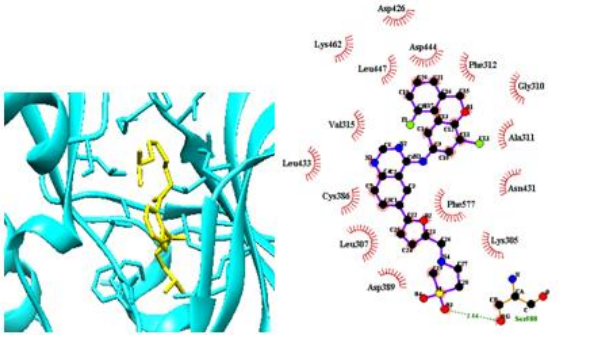
3B: HER2-3PP0-9-Octadecenamide-8036015



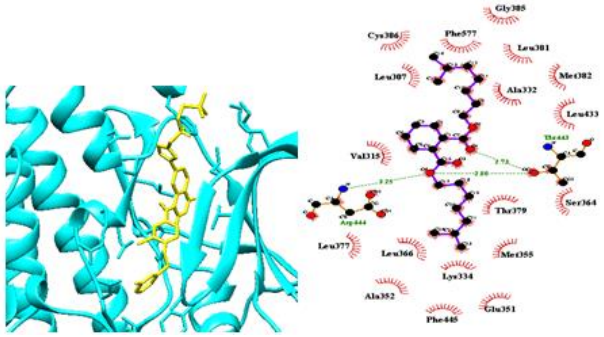
3C: HER2-3PP0-Germacrene-D-3073



3D: HER2-3PP0-Bis (2-ethylhexyl) phthalate-3860432



3E: HER2-3PP0-Diisooctyl-phthalate-4655188

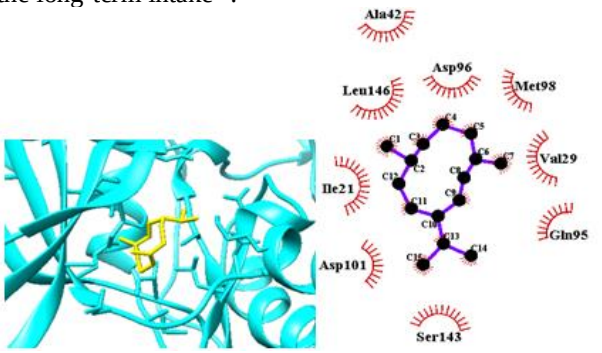


3F: HER2-3PP0-Lapatinib-208908

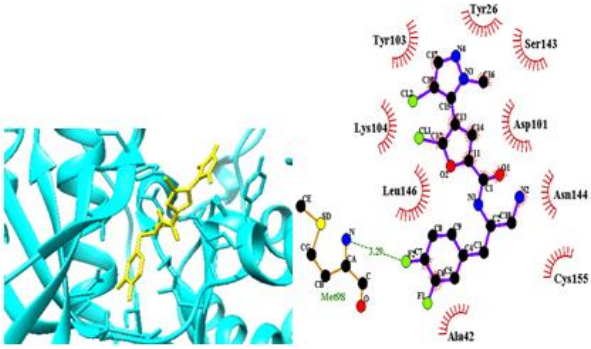
Figure 3: Molecular docking of phytocompounds (in yellow color) of the Vimto® with HER2 (PDB code 3PP0; in cyan color).

In addition, phthalates are used in cosmetic and food products which they are released into the environment. In this context, diet is believed to be the main source of phthalates because fatty foods such as milk, butter and meats are commonly packaged or stored in plastics containing this dangerous pollutant[41]. These compounds are toxic substances and have adverse effects on liver, kidney, endocrine system, and re-production and it may also have carcinogenic effects in the long-term intake[42].

From these data, it can be concluded that phthalates present in the Vimto® may pollute manufacturing process during production and packaging, although its level is tolerable. Since the *in silico* methods function based on probabilities, compounds that have not been properly docked with proteins may also affect the physiology. The most important of these compounds are benzoic acid and vanillin which have high percentages in Vimto®.

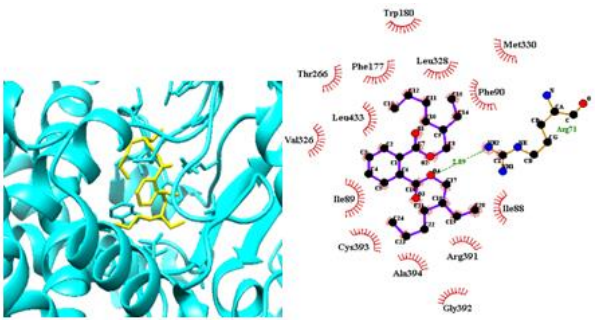


4A: ERK-5NGU-Germacrene-D-30730221

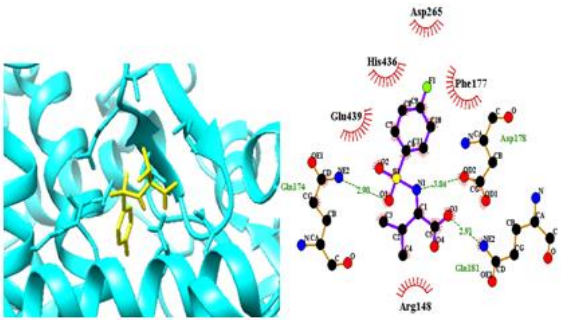


4B: ERK-5NGU-GSK-2141795-51042438

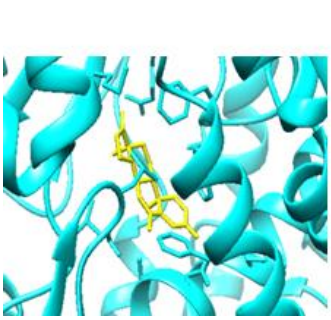
Figure 4: Molecular docking of phytocompounds (in yellow color) of the Vimto® with ERK (PDB code 5NGU; in cyan color).



5a: Aromatase-3EQM-Bis (2-Ethylhexyl) Phthalate-3860432

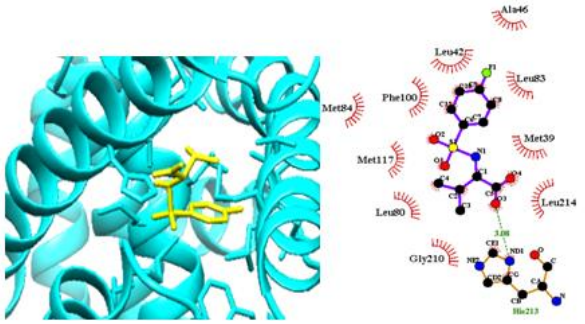


5B: Aromatase-3EQM-Butanoic-acid-166179

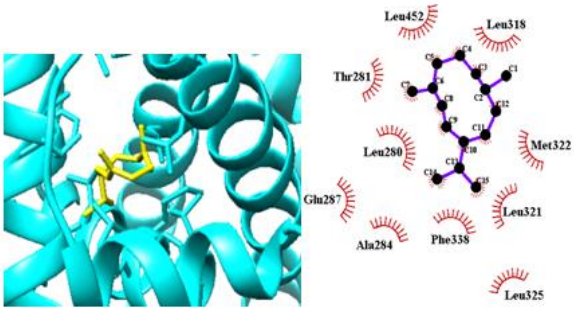


5C: Aromatase-3EQM-Exemestane-60198

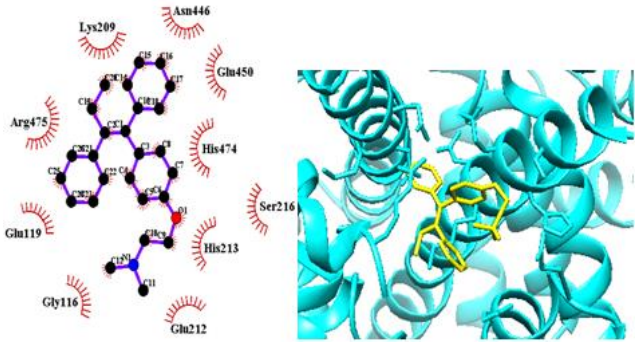
Figure 5: Molecular docking of phytocompounds (in yellow color) of the Vimto® with aromatase (PDB code 3EQM; in cyan color).



6A: ER-Alpha-1X7E-Butanoic-acid-166179



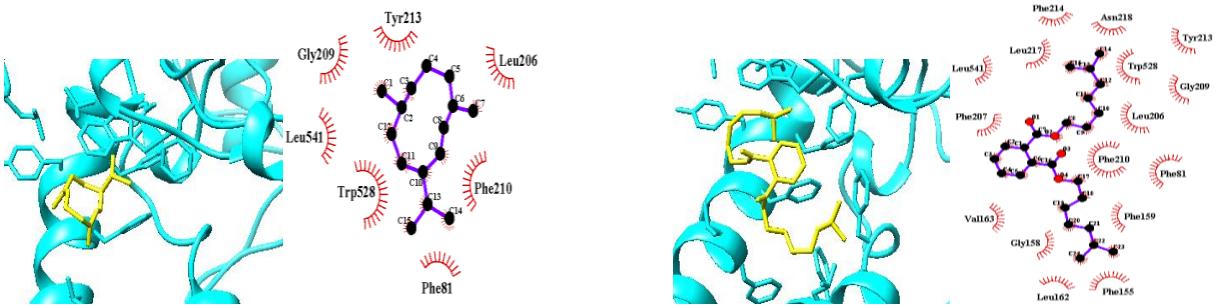
6B: ER-Alpha-1X7E-Germacrene-D-30730221



6C: ER-Alpha-1X7E-Tamoxifen-2733526

Figure 6: Molecular docking of phytocompounds (in yellow color) of the Vimto® with ER-Alpha (PDB code 1X7E; in cyan color).

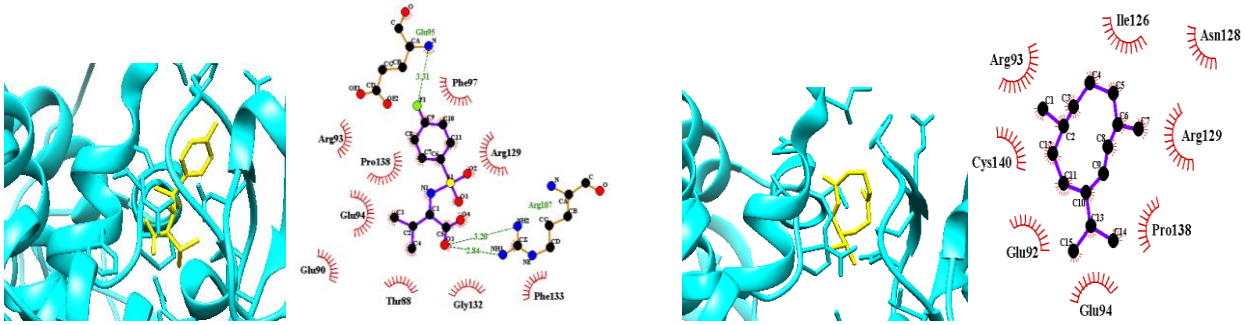
Benzoic acid is a white and crystalline phytocompound and is an important parent compound employed for organic synthesis of colognes, colorants, topical medications, and insect repellents[43]. It is one of these preservatives in processed foods like cheese, sauce, and meat. Miserably, it is also used in cosmetic products and various pharmaceutical products, therefore it can be hard to avoid[44]. Vanillin (4-hydroxy-3-methoxy-benzaldehyde) is a phytocompound found in the pods of *Vanilla* species was found to significantly alter functional gene network of human hepatocellular carcinoma cell line, HepG2[18] and to suppress the metastasis of mammary adenocarcinoma cancer cells[12]. Vanillin also has anti-mutagenic properties against spontaneous mutations in the mammalian cells[9]. In one hand, Vimto® has anti-cancer effects due to its antitumor components and on the other hand, Vimto® may be putative cancer inducer due to the presence of phthalate. Therefore, experiments are needed to know the whole composition of Vimto® and its pharma-cological and toxicological effects.



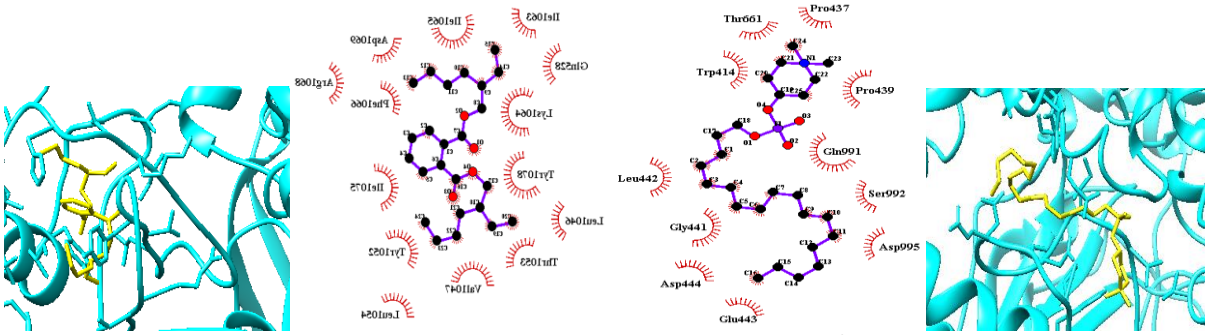
7A: Estrone-Sulfatase-1P49-Germacrene-D-30730221 7B: Estrone-Sulfatase-1P49-Diisooctyl-Phthalate-4655188

7C: Estrone-Sulfatase-1P49-STX-64-5287541

Figure 7: Molecular docking of phytocompounds (in yellow color) of the Vimto® with estrone sulfatase (PDB code 1P49; in cyan color).



8A: PI3K-5SW8-Butanoic-acid-166179 8B: PI3K-5SW8-Germacrene-D-30730221



8C: PI3K-5SW8-Bis (2-Ethylhexyl) Phthalate-3860432 8D: PI3K-5SW8-Prifosine-8214653

Figure 8: Molecular docking of phytocompounds (in yellow color) of the Vimto® with PI3K (PDB code 5SW8; in cyan color).

CONCLUSIONS

The cardinal target proteins of BC including AKT, ERK2, HER2 and aromatase showed better docking with compounds of Vimto®, because its components have been docked with the proteins at their binding sites with their classic inhibitors. Therefore, Vimto®drink showed the both anticancer and carcinogenic potentials due to the presence of diisooctyl phthalate and bis (2-ethylhaxyl) phthalate, a sterilizing agent. Here, we were only focused on binding affinities less than -7.0 kcal/mol to consider a ligand as anti-cancer and/or inducer of cancer. None of compounds found in Vimto® showed BA to PIK3 except benzoic acid as compared to classic PIK3 inhibitor, perifosine. Among all reported compounds, germacrene D showed trustful BA with ER-alpha, ERK2, and estrone sulfatase which shows the promiscuity of this compound in fighting breast cancer. In essence, the compounds of Vimto® that dock to proteins of BC are not necessarily anticancer agents and may activate carcinogenic pathways since many of them are poisonous and harmful. Finally, according to the presence of anticancer and antibacterial compounds in Vimto® (9-octadecenamide, germacrene D, and vanillin), and also due to the many enthusiasms to

drink Vimto®, it is necessary to exclude its harmful and carcinogenic compounds (phthalates and benzoic acid), and a healthier drink offered to its customers.

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AUTHOR'S CONTRIBUTION

Karimi I: writing original draft, collecting data and analysis. **Yousefvand N:** statistical analysis. **Shamspur T:** conceptualization. **Moloodi B:** literature searches, research design. All the authors reviewed the results and approved the final version of the manuscript.

DATA AVAILABILITY

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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