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ARTICLE

New insight of chemical constituents in *Persea americana* fruit against obesity via integrated pharmacology

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Abstract

Persea americana fruit (PAF) is a favorable nutraceutical resource that comprises diverse unsaturated fatty acids (UFAs). UFAs are significant dietary supplementation, as they relieve metabolic disorders, including obesity (OB). In another aspect, this study was focused on the anti-OB efficacy of the non-fatty acids (NFAs) in PAF through network pharmacology (NP). Natural product activity & species source (NPASS), SwissADME, similarity ensemble approach (SEA), Swiss target prediction (STP), DisGeNET, and online Mendelian inheritance in man (OMIM) were utilized to gather significant molecules and its targets. The crucial targets were adopted to construct certain networks: protein–protein interaction (PPI), PAF-signaling pathways-targets-compounds (PSTC) networks, a bubble chart, molecular docking assay (MDA), and density function theory (DFT). Finally, the toxicities of the key compounds were validated by ADMETlab 2.0 platform. All 41 compounds in PAF conformed to Lipinski's rule, and the key 31 targets were identified between OB and PAF. On the bubble chart, PPAR signaling pathway had the highest rich factor, suggesting that the pathway might be an agonism for anti-OB. Conversely, estrogen signaling pathway had the lowest rich factor, indicating that the mechanism might be antagonism against OB. Likewise, the PSTC network represented that AKT1 had the greatest degree value. The MDA results showed that AKT1-gamma-tocopherol, PPARA-fucosterol, PPARD-stigmasterol, (PPARG)-fucosterol, (NR1H3)-campesterol, and ILK-alpha-tocopherol formed

Abbreviations: AKT1, AKT serine/threonine kinase 1; DFT, density functional theory; FABP4, fatty acid binding protein 4; HOMO, highest occupied molecular orbital; ILK, integrin-linked kinase; LUMO, lowest occupied molecular orbital; MDA, molecular docking assay; MOA, mechanism of action; NFAs, non-fatty acids; NP, network pharmacology; NPASS, natural product activity & species source; NR1H3, nuclear receptor subfamily 1 group H member 3; OB, obesity; OMIM, online Mendelian inheritance in man; PAF, *Persea americana* fruit; PPARA, peroxisome proliferator-activated receptor alpha; PPARD, peroxisome proliferator-activated receptor delta; PPARG, peroxisome proliferator-activated receptor gamma; PPI, protein–protein interaction; PSTC, PFA-signaling pathways-targets-compounds; SEA, similarity ensemble approach; SFAs, saturated fatty acids; SMILES, simplified molecular input line entry system; STP, Swiss target prediction; UFAs, unsaturated fatty acids.

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the most stable conformers. The DFT represented that the five molecules might be promising agents via multicomponent targeting. Overall, this study suggests that the NFAs in PAF might play important roles against OB.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

Currently, *Persea americana* fruit (PAF) studies have been exposed to unsaturated fatty acids (UFAs) for anti-obesity (AOB), but our result shows that non-UFAs might be more valuable against obesity (OB).

WHAT QUESTION DID THIS STUDY ADDRESS?

We postulated that non-UFAs in PAF are more significant agents than known unsaturated fatty acids (UFAs) comprised in other diet. Ultimately, the study manifested that alpha-tocopherol, gamma-tocopherol, fucosterol, stigmasterol, and campesterol in PAF are key chemical constituents against OB.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

The non-UFAs in PAF serve as alleviators for AOB via multiple-compounds and multiple-targets on PPAR signaling pathway.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

The current study represents the integration of bioinformatics, cheminformatics, microbial informatics, and computer screening tools to decode the key mechanism(s), target(s), and compound(s) in complicated relationships. Overall, these results suggest that the merge of rigor biodata, and computational biology is an effective approach to reverse pharmacology concept for translational science.

INTRODUCTION

Obesity (OB) is a prominent global health issue associated with diverse comorbidities, including type 2 diabetes, hypertension, dyslipidemia, and even certain cancers.[1,2] Excessive calory is a main cause to obstruct the balance of body metabolism maintained intake, expenditure, and storage of energy.[3] A fundamental method to assess OB is the body mass index (BMI), an easily calculable value that describes the relationship between height and weight, BMI groups with more than 30 is categorized as OB.[4] Additionally, a report announced that the number of obesity will increase to 1.12 billion by 2030 if the current trend continues consistently.[5] It implies that OB is an urgent health issue facing the globe.

Currently, anti-OB drugs administered over a short period of time are diethylpropion, benzphetamine, phendimetrazine, and phentermine, these medications are effective to suppress appetite.[6] However, the pharmacological effect of these medicines lasts for just a few weeks.[7] Alternatively, the mechanism of action (MOA) of orlistat and liraglutide can be taken over a long period of time to interrupt the absorption of fat by dampening gastrointestinal lipases and appetite, respectively.[8,9] Despite the favorable effects of these drugs, some individuals are under adverse effects, including nausea, diarrhea, vomiting, and constipation.[10] Instead, herbal plants are essential resources as new therapeutic agents due to their few adverse effects.[11] The chemical constituents in herbal plants are significant effectors that can relieve metabolic disorders.[12] Therapeutics against OB are based on treating multiple targets, and plant extracts comprised of diverse compounds might be ideal treatments to exert multitargeted effects.[13]

Commonly, *Persea americana* fruit (PAF) is a plentiful source of monounsaturated fatty acids and a good reservoir of linoleic acid.[14] PAF is a valuable oil-rich fruit with an anti-oxidative effect and enhancement of the immune system through metabolic pathways.[15] PAF oil consists of 84% unsaturated fatty acids (UFAs) and 16% saturated fatty acids (SFAs).[16] However, UFAs are more unstable than SFAs and more susceptible to rancidity.[17] More importantly, an animal experiment demonstrated that both omega-6 and omega-3 fatty acids as representative UFAs in soybean oil cause OB.[18] Additionally, many edible plants contain diverse UFAs in the leaves, roots, and fruits. Therefore, UFAs in PAF might not be key effectors to enhance its therapeutic value. If PAF does not have distinct composites difference from other natural products, its pharmacological values cannot be expected.

Based on that, we reasoned that non-fatty acids (NFAs) of PAF could be alleviative agents against OB. Hereby, we

pioneered bona fide hidden key molecules in PAF for anti-OB with data-driven analysis. Network pharmacology (NP) is an efficient methodology to decipher the therapeutic value of multiple compounds in natural products.[19] This systemic approach is an optimal strategy to elucidate the multiple veiled components (targets, active compounds, and mechanisms) in herbal plants.[20] The relationship between ligand(s) and target(s) can be clarified by NP analysis, which can be a key to promote their therapeutic value.

The study process is represented in Figure 1.

METHODS

Utilization of datasets and the literature for analysis

Biochemically significant datasets were adopted to utilize network pharmacology (NP) as an optimal methodology for new drug discovery or development. There are freely accessible web-based bioinformatics or cheminformatics tools to users who wish to obtain biodata that can be utilized in NP studies. We listed the available software and bioinformatics tools in Table S1. The bioactives in PAF were identified by the Natural Product Activity & Species Source (NPASS) database (accessed on 13 December 2022) and other literature sources.[21–24]

Screening of drug-like compounds from PAF

The sorted chemical constituents were analyzed by SwissADME (http://www.swissadme.ch/) (accessed on 15 December 2022) to confirm their drug-like properties (Lipinski's rule-Molecular Weight: 500 g/mol; Moriguchi octanol–water partition coefficient: 4.15; Number of Nitrogen or Oxygen: 10; Number of NH or OH:5; Topological Polar Surface Area: 140 Å^2).

The confirmed compounds were input into similarity ensemble approach (SEA) database (https://sea.bkslab.org/) (accessed on 15 December 2022) and SwissTargetPrediction (STP) database (http://www.swisstargetprediction.ch/) (accessed on 15 December 2022) to identify their targets with Simplified Molecular Input Line Entry System (SMILE) form.

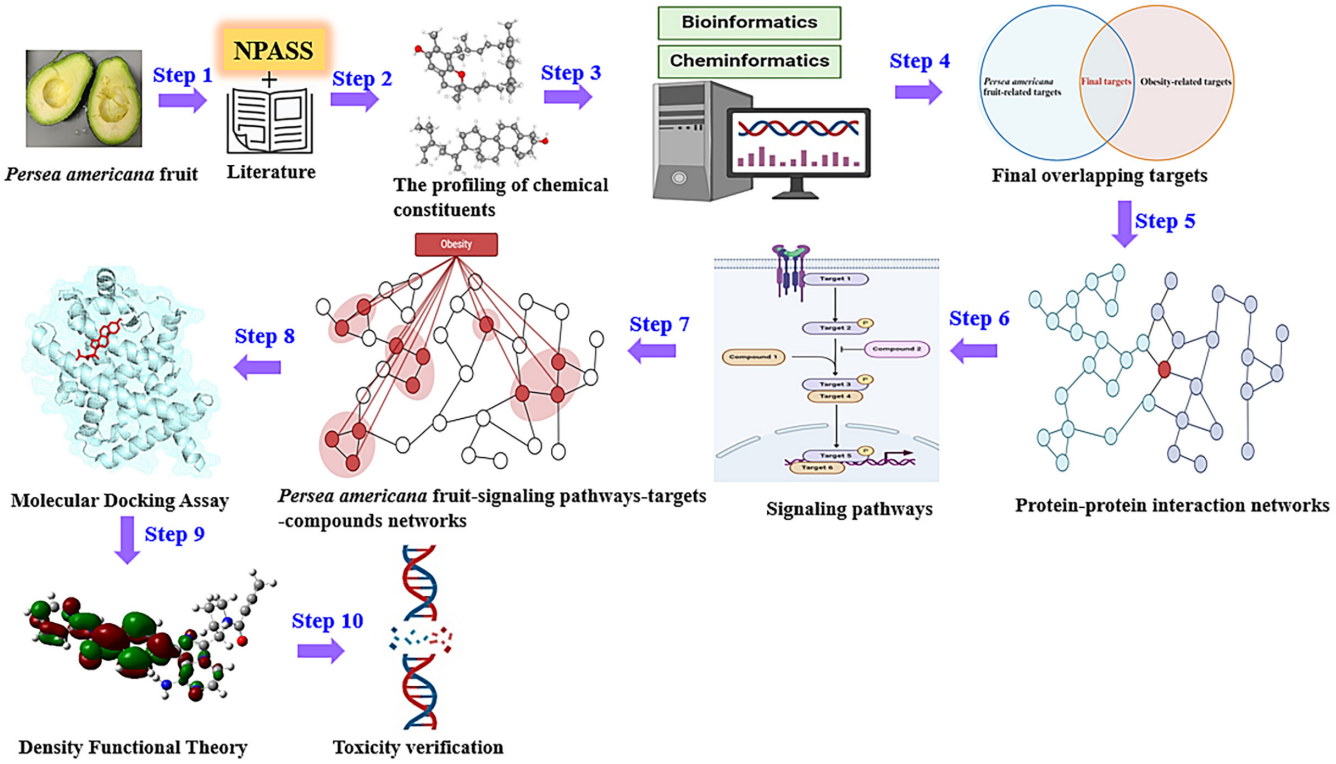

FIGURE 1 The work process of this study. Step 1, Step 2: The browsing of *Persea americana* fruit compounds. Step 3, Step 4: The identification of final overlapping targets. Step 5, Step 6, Step 7: The construction of protein–protein interaction networks, key signaling pathways, and *Persea americana* fruit-signaling pathways -targets-compounds networks. Step 8, Step 9: Molecular Docking Assay and the density functional theory to reveal ligands' reactiveness. Step 10: The verification of toxicity via in silico. The figures were adapted from BioRender.com (2024). Retrieved from https://app.biorender.com/biorender-templates/figures.

Retrieval of OB-related targets

In parallel, OB-related targets in humans were retrieved from the DisGeNET database (https://www.disgenet.org/) (accessed on 16 December 2022) and Online Mendelian Inheritance in Man (OMIM) database (https://www.omim.org/) (accessed on 16 December 2022). The ultimate intersecting PAF-related targets and OB-responsive targets were identified and described with the Venn diagram plotter.

PPI network and bubble chart construction

The critical identified targets were defined as "overlapping targets between PAF-related targets and OB-responsive targets, which were used to construct a protein–protein interaction (PPI) network, which can be determined as the most significant target in the analysis". The target information was identified by the STRING database (https://string-db.org/) (accessed on 17 December 2022), which was built by the R package. Then, we constructed a bubble chart from the expressed gene ratio (known as the rich factor), and the false discovery rate (FDR) of the identifying signaling pathways was setup as a threshold (FDR ≤ 0.05). The threshold (FDR ≤ 0.05) is a significant indicator to measure the statistical validation in dataset.[25] Consequently, two signaling pathways were considered as core mechanisms directly related to PAF in OB.

PSTC network construction

We constructed the PFA-signaling pathways-targets-compounds (PSTC) network to identify the relationships between each component. The bioactive compounds of PAF were retrieved by the NPASS database via input "*Persea americana*". In the merged networks, PAF, the signaling pathways, the targets, and the compounds were used as nodes (orange square: signaling pathway; red triangle: target; and green circle: compound), and the relationships were depicted as edges (gray line).

Molecular docking assay (MDA) with the targets

MDA was performed to obtain the most stable conformer between ligand(s) and target(s). The cut-off of binding energy (or the lowest Gibbs energy) was set to −6.0 kcal/mol from each pair.[26] The chemical compounds of PAF were downloaded in .sdf format from PubChem and transformed into .pdb format with PyMOL. The obtained .pdb files were transformed into .pdbqt format for the MDA. The typical structure of each protein was selected via the RCSB PDB (https://www.rcsb.org/) (accessed on 18 December 2022). MDA was conducted with AutoDockTools-1.5.6 to validate the stability of the binding between the targets and ligands.

The dimensions for docking were $x=40$ Å, $y=40$ Å, and $z=40$ Å. The active region of each target was configured in a cubic box in the center of the area: AKT Serine/Threonine Kinase 1 (AKT1) ($x=6.313$, $y=-7.926$, $z=17.198$), Fatty Acid Binding Protein 4 (FABP4) ($x=7.693$, $y=9.921$, $z=14.698$), Integrin-linked kinase (ILK) ($x=-2.921$, $y=-8.642$, $z=15.470$), Nuclear receptor subfamily 1 group H member 3 (NR1H3) ($x=48.735$, $y=39.677$, $z=77.096$), Peroxisome proliferator-activated receptor alpha (PPARA) ($x=8.006$, $y=-0.459$, $z=23.392$), Peroxisome proliferator-activated receptor delta (PPARD) ($x=39.265$, $y=-18.736$, $z=119.392$), and Peroxisome proliferator-activated receptor gamma (PPARG) ($x=2.075$, $y=31.910$, $z=18.503$). Detailed information on the hydrophilic and hydrophobic interactions in the conformers was obtained via LigPlot+2.2. (https://www.ebi.ac.uk/thornton-srv/software/LigPlus/download2.html) (accessed on 19 December 2022).

Identifying of frontier molecular orbitals (FMO)

The .sdf file was input into GaussView software to visualize the molecule structure, based on the Lee Yang Parr (B3LYP-D3) theory, which was employed to measure LUMO (Lowest Occupied Molecular Orbital), and HOMO (Highest Occupied Molecular Orbital). The setup of Gaussian16 program package was followed as below.

- Gaussian Calculation Setup: Job Type; optimization.
- Method: DFT; B3LYP; Basis set: 6-31G.
- Link 0: Memory Limit; 1500 MB, Shared Processors; 4.
- MO Editor: Visualize; Add Type: HOMO, LUMO.

The Energy gap (HOMO – LUMO), hardness (η), softness (S), and electronegativity (χ) have been considered as significant factors to explore its chemical reactiveness on pharmacological space.[27,28] Depending on the Energy gap, other parameters can be computed by the below formulae.

- Energy gap = (HOMO – LUMO)
- η = (LUMO – HOMO)/2
- $S = 1/\eta$
- $\chi = -(1/\eta)$

Identification of the toxic parameters of the key compounds

Toxicity parameters (hERG, human hepatotoxicity, carcinogenicity, cytotoxicity, and eye corrosion) were validated by the ADMETlab platform (https://admetmesh.scbdd.com/) (accessed on 20 December 2022). The verification and assessment of toxicity are significant factors to provide pharmaceutical value without any interventions.

RESULTS

Profiling of the chemical compounds in PAF

A total of 41 chemical compounds in PAF were identified by NPASS and literature sources. Then, all compounds were confirmed to adhere to Lipinski's rule via SwissADME platform analysis. These 41 chemical compounds were found to be associated with 286 targets by the SEA database and 387 targets by the STP database. Overall, 52 overlapping targets were identified as significant targets in the chemical space (Figure 2a) (Table S3).

Identification of the OB-related targets and crucial targets

The OB-related targets (3028) were retrieved by DisGeNET and OMIM and compared with the 52 intersecting targets (Table S2). The final 31 overlapping targets between them were considered as crucial targets (Figure 2b). Among the 31 crucial targets, APH1A, OXER1, GSTK1, and ADH1B had no connectivity with one another. The final 27 targets had strong connectivity centered on AKT1 with the highest degree value (19), followed by PPARG (13), ESR1 (9), PPARA (9), and FABP4 (7) (Table S3).

Construction of PPI network and bubble plot

The crucial 31 targets were employed to construct a PPI network (Figure 3a). AKT1 was determined as the top protein-coding target. Then, the constructed bubble chart showed that there were six signaling pathways related to OB, indicating that the PAF components might function as effector(s) in these pathways. Of the six signaling

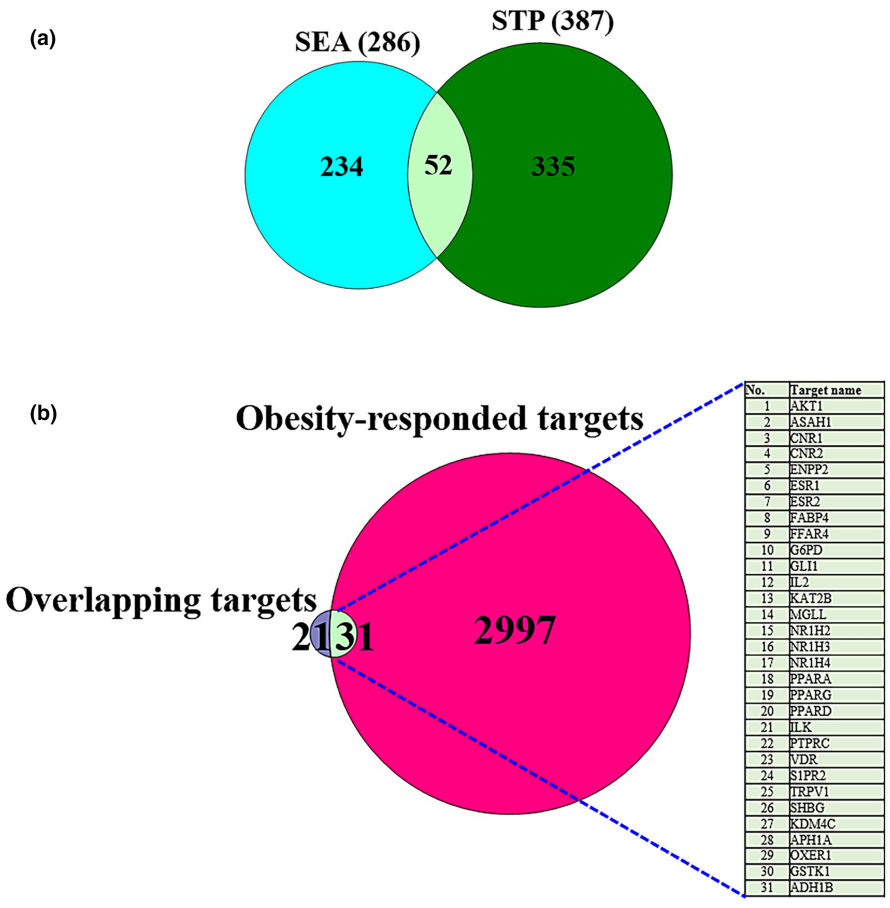

FIGURE 2 (a) The number of 52 overlapping targets from SEA (286 targets) and STP (387 targets). (b) The final 31 targets between obesity-responded targets (3028 targets) and 52 targets from (a).

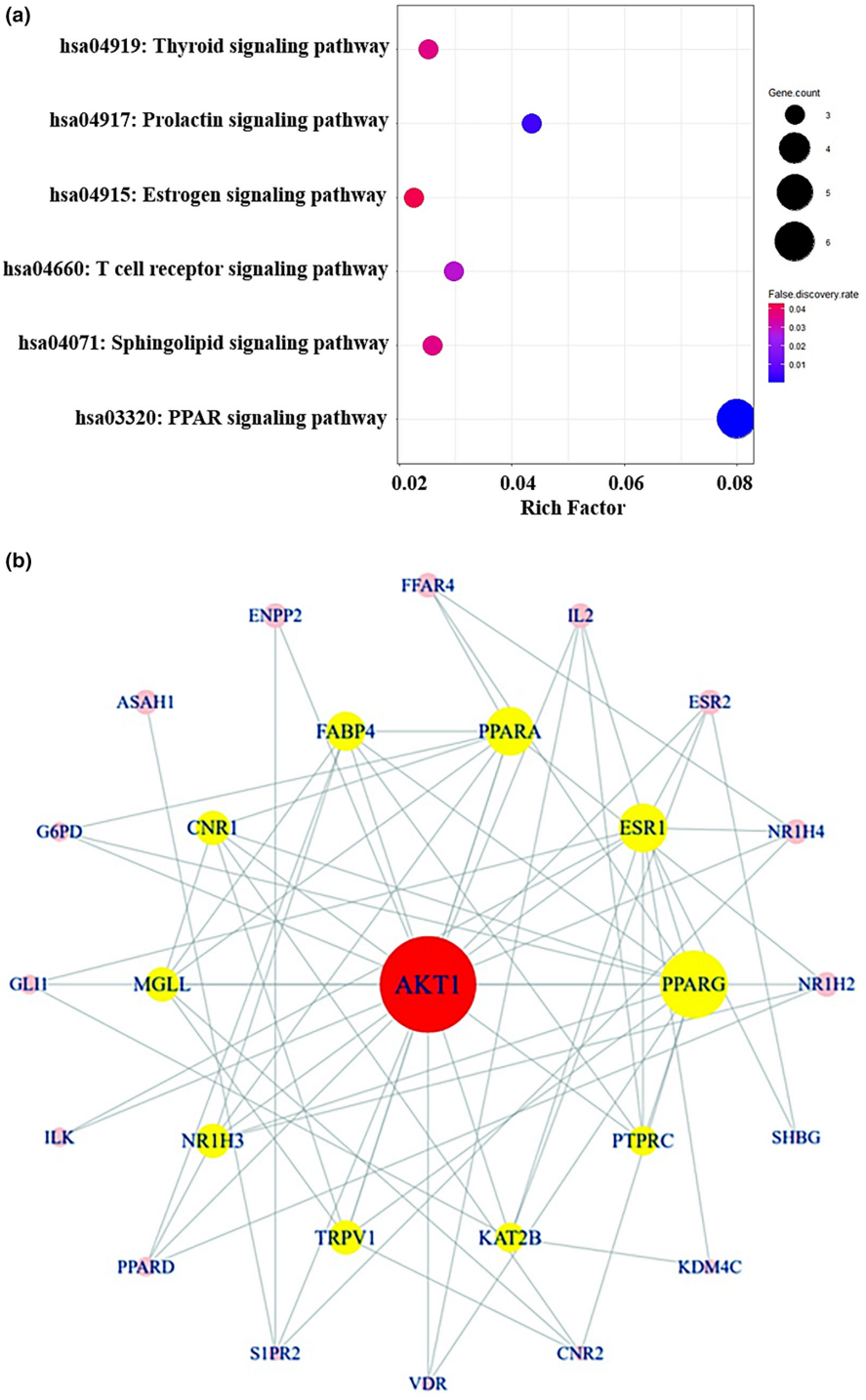

FIGURE 3 (a) The protein–protein interaction networks to identify a key target. (b) The bubble chart of six signaling pathways related to the development of obesity. (c) *Persea americana* fruit-signaling pathways-targets-compounds networks to represent the relationships between the four components.

pathways, the PPAR signaling pathway had the greatest rich factor, and the estrogen signaling pathway had the lowest rich factor, and these two mechanisms were considered as the key mechanisms of action (Figure 3b). Higher rich factors indicate that a greater number of genes are expressed in the pathway, suggesting that a pathway with a higher rich factor is more activated than the pathways with lower rich factors.[29,30] Thus, the PPAR signaling pathway functioned as agonism in OB; in contrast, the estrogen signaling pathway worked as antagonism. The

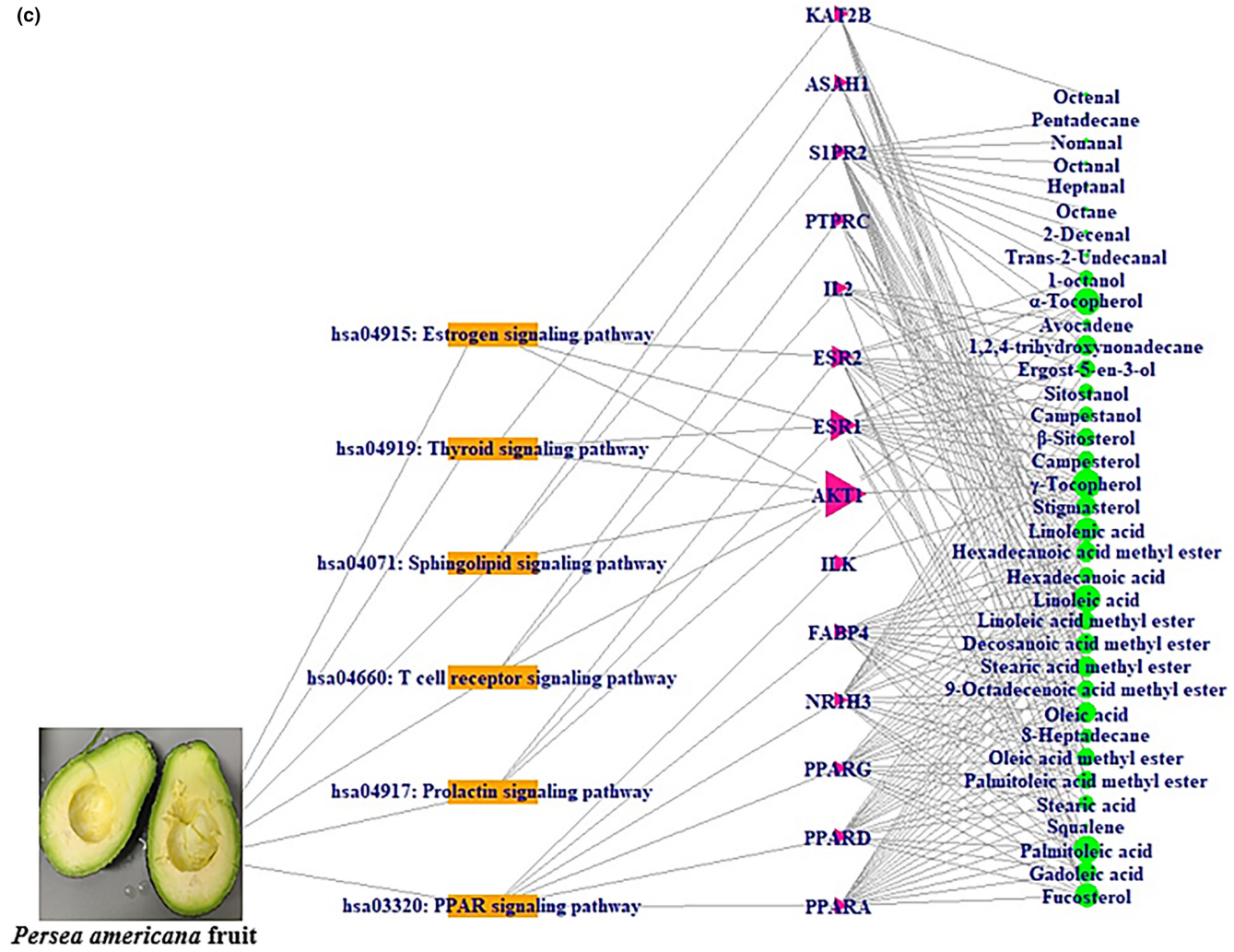

FIGURE 3 (Continued)

descriptions of the six signaling pathways are listed in Table S4.

PSTC network analysis

The PSTC network shows strong interactions between PAF, 6 signaling pathways, 14 targets, and 36 compounds (Figure 3c). Interestingly, the number of 36 out of 41 bioactive compounds browsed by NPASS were interconnected with 6 signaling pathways. The 2,4-decadienal, 2-heptenal, 3,5-octadien-2-ol, avocadyne, and cycloartenol acetate were no relationships on the 6 signaling pathway (Table 1). Notably, AKT1 had the greatest connectivity to the PSTC network, suggesting that AKT1 might be a promising target to regulate the OB. The incorporated network comprised 57 nodes and 189 edges. The nodes indicate the total number of each component (PAF, signaling pathway, target, and compound), while the edges represent the relationships between the four elements. The PSTC network showed that 38 compounds in PAF connect to 6 signaling pathways and 14 targets to relieve OB.

MDA analysis

Among the components of PAF, the compound that bound most rigidly to AKT1 was gamma-tocopherol with a binding energy of −6.7 kcal/mol. In the PPAR signaling pathway, three active compounds (fucosterol, stigmasterol, campesterol, and alpha-tocopherol) and six potential targets (PPARA, PPARD, PPARG, NR1H3, FABP4, and ILK) were identified by network investigation based on molecular docking assay (MDA). MDA was performed to examine the interactions between the compounds from PAF and the OB-related targets at the atomic level. AutoDockTools-1.5.6 software was adopted for 3D simulations and LIGPLOT^{+} software was employed for 2D simulations of ligand–protein docking. The docking

TABLE 1 The physicochemical properties of phytochemicals of *Persea americana* fruit.

No.	Compound name	PubChem CID	MW	HBA	HBD	MLogP	Lipinski's violations	Bioavailability score
			<500	<10	≤5	≤4.15	≤1	>0.1
1	Octane	356	114.23	0	0	4.20	1	0.55
2	Heptanal	8130	114.19	1	0	1.74	0	0.55
3	2-heptenal	5,283,316	112.17	1	0	1.63	0	0.55
4	Octanal	454	128.21	1	0	2.07	0	0.55
5	3,5-octadien-2-ol	5,364,580	126.20	1	1	1.97	0	0.55
6	Octenal	5,283,324	126.20	1	0	1.97	0	0.55
7	1-octanol	957	130.23	1	1	2.22	0	0.55
8	Nonanal	31,289	142.24	1	0	2.39	0	0.55
9	Trans-2-undecanal	5,283,356	168.28	1	0	2.88	0	0.55
10	2-decenal	5,283,345	154.25	1	0	2.59	0	0.55
11	2,4-decadienal	5,283,349	152.23	1	0	2.49	0	0.55
12	Pentadecane	12,391	212.41	0	0	6.19	1	0.55
13	8-heptadecane	520,230	238.45	0	0	6.54	1	0.55
14	Hexadecanoic acid	985	256.42	2	1	4.19	1	0.85
15	Oleic acid	445,639	282.46	2	1	4.57	1	0.85
16	9-octadecanoic acid methyl ester	5,280,590	296.49	2	0	4.80	1	0.55
17	Hexadecanoic acid methyl ester	8181	270.45	2	0	4.44	1	0.55
18	Ergost-5-en-3-ol	18,660,356	400.68	1	1	6.54	1	0.55
19	Palmitoleic acid methyl ester	643,801	268.43	2	0	4.33	1	0.55
20	Linoleic acid methyl ester	5,284,421	294.47	2	0	4.70	1	0.55
21	Oleic acid methyl ester	5,364,509	296.49	2	0	4.80	1	0.55
22	Stearic acid methyl ester	8201	298.50	2	0	4.91	1	0.55
23	Decosanioc acid methyl ester	13,584	354.61	2	0	5.79	1	0.55
24	Palmitoleic acid	445,638	254.41	2	1	4.09	0	0.85
25	Stearic acid	5281	284.48	2	1	4.67	1	0.85
26	Linoleic acid	5,280,450	280.45	2	1	4.47	1	0.85
27	Linolenic acid	5,280,934	278.43	2	1	4.38	1	0.85
28	Gadoleic acid	5,282,767	310.51	2	1	5.03	1	0.85
29	Avocadene	158,573	286.45	3	3	2.74	0	0.55
30	Avocadyne	3,015,189	284.43	3	3	2.74	0	0.55

TABLE 1 (Continued)

No.	Compound name	PubChem CID	MW	HBA	HBD	MLogP	Lipinski's violations	Bioavailability score
31	1,2,4-trihydroxynonadecane	10,567,452	316.52	3	3	3.36	0	0.55
32	α-Tocopherol	14,985	430.71	2	1	6.14	1	0.55
33	Squalene	638,072	410.72	0	0	7.93	1	0.55
34	Campesterol	173,183	400.68	1	1	6.54	1	0.55
35	Cycloartenol acetate	13,023,741	468.75	2	0	7.08	1	0.55
36	β-Sitosterol	521,199	456.74	2	0	7.57	1	0.55
37	γ-Tocopherol	92,729	416.68	2	1	5.94	1	0.55
38	Fucosterol	5,281,326	412.69	1	1	6.62	1	0.55
39	Stigmasterol	5,280,794	412.69	1	1	6.62	1	0.55
40	Sitostanol	241,572	416.72	1	1	6.88	1	0.55
41	Campestanol	119,394	402.70	1	1	6.68	1	0.55

Note: No. 3, 5, 11, 30, and 35 had no relationships on the 6 signaling pathway.

scores and interacting residues are listed in Table S5. The active docking site was formatted in a cubic box at the center of each ligand for MDA. The lower the binding energy (the higher the negative value), the greater the affinity is between the compound and target. In the estrogen signaling pathway, the conformations of AKT1-gamma-tocopherol (−6.7 kcal/mol), ESR1-campesterol (−9.6 kcal/mol), and ESR2-campesterol (−10.5 kcal/mol) had the best binding affinities among the pairs, indicating that gamma-tocopherol and campesterol may show therapeutic effects by inactivating the estrogen signaling pathway. These molecular conformations are shown in Figure 4(a–c).

The MDA showed that the conformations of the PPARA-fucosterol (−7.1 kcal/mol), PPARD-stigmasterol (−7.3 kcal/mol), PPARG-fucosterol (−7.4 kcal/mol), NR1H3-campesterol (−10.6 kcal/mol), FABP4-gadoleic acid (−5.6 kcal), and ILK-alpha-tocopherol (−7.8 kcal/mol) pairs had the best binding affinities in the PPAR signaling pathway, suggesting that multiple compounds may exert therapeutic efficacy by activating the PPAR signaling pathway. Of the seven targets, the FABP4-gadoleic acid (−5.6 kcal) complex was not selected as an effector because the common binding energy threshold in AutoDockTools-1.5.6 is −6.0 kcal/mol.[26] The conformers are shown in Figure 4(d–h).

DFT confirmation of key molecules and a standard drug

The DFT of five key molecules (campesterol, stigmasterol, fucosterol, alpha-tocopherol, and gamma-tocopherol) and a standard drug (orlistat) was investigated to identify the chemical reactivity in aspects of orbital chemistry. In a general sense, HOMO and LUMO levels are important parameters to determine how a molecule moiety can be a donator or an acceptor via its valence electrons. The five key molecules with "orlistat" as a representative drug were analyzed with six parameters: LUMO, HOMO, Egap, η, S, and χ (Table S6). As a result, parameter values of the five key molecules had no big difference by comparison with orlistat. Of these, alpha-tocopherol (Egap = −0.197 eV) and gamma-tocopherol (Egap = −0.196 eV) had better chemical reactiveness than orlistat (Egap = −0.239 eV) (Figure 5).

Validation of the toxicities on the five key compounds

Finally, the toxicities of the five key compounds (fucosterol, stigmasterol, campesterol, alpha-tocopherol, and

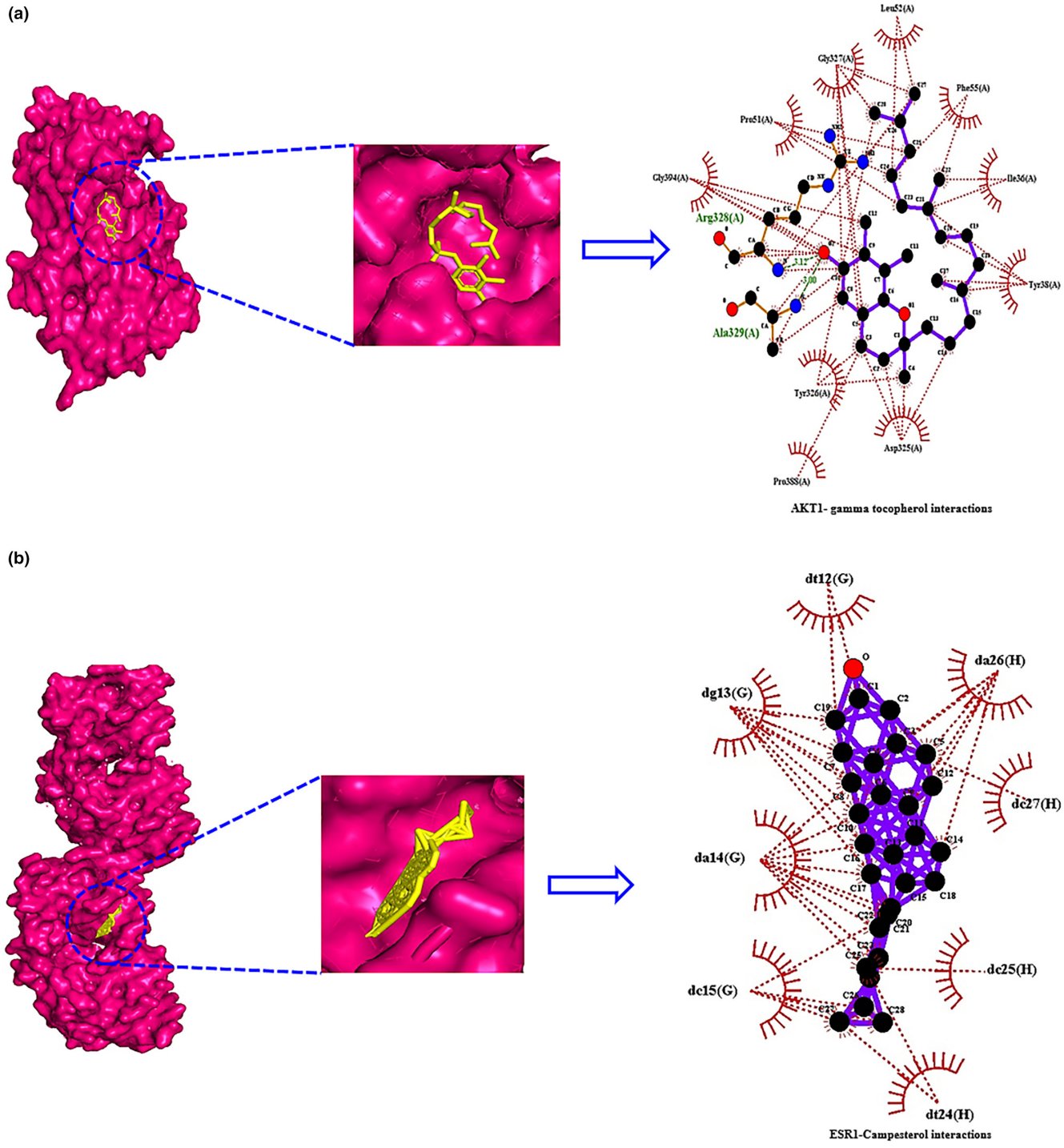

FIGURE 4 (a) Molecular conformation of AKT1-gamma-tocopherol. (b) Molecular conformation of ESR1-campesterol. (c) Molecular conformation of ESR2-campesterol. (d) Molecular conformation of PPARA-fucosterol. (e) Molecular conformation of PPARD-stigmasterol. (f) Molecular conformation of PPARG-stigmasterol. (g) Molecular conformation of NR1H3-campesterol. (h) Molecular conformation of ILK-alpha-tocopherol.

gamma-tocopherol) were assessed in silico, and each did not display any noticeable toxicity (Table S7). As a result, these five compounds from PAF did not have hurdles to develop as new agents.

DISCUSSION

Reportedly, the UFAs found in PAF are widespread in various herbal plants and seeds. Still, its therapeutic potency

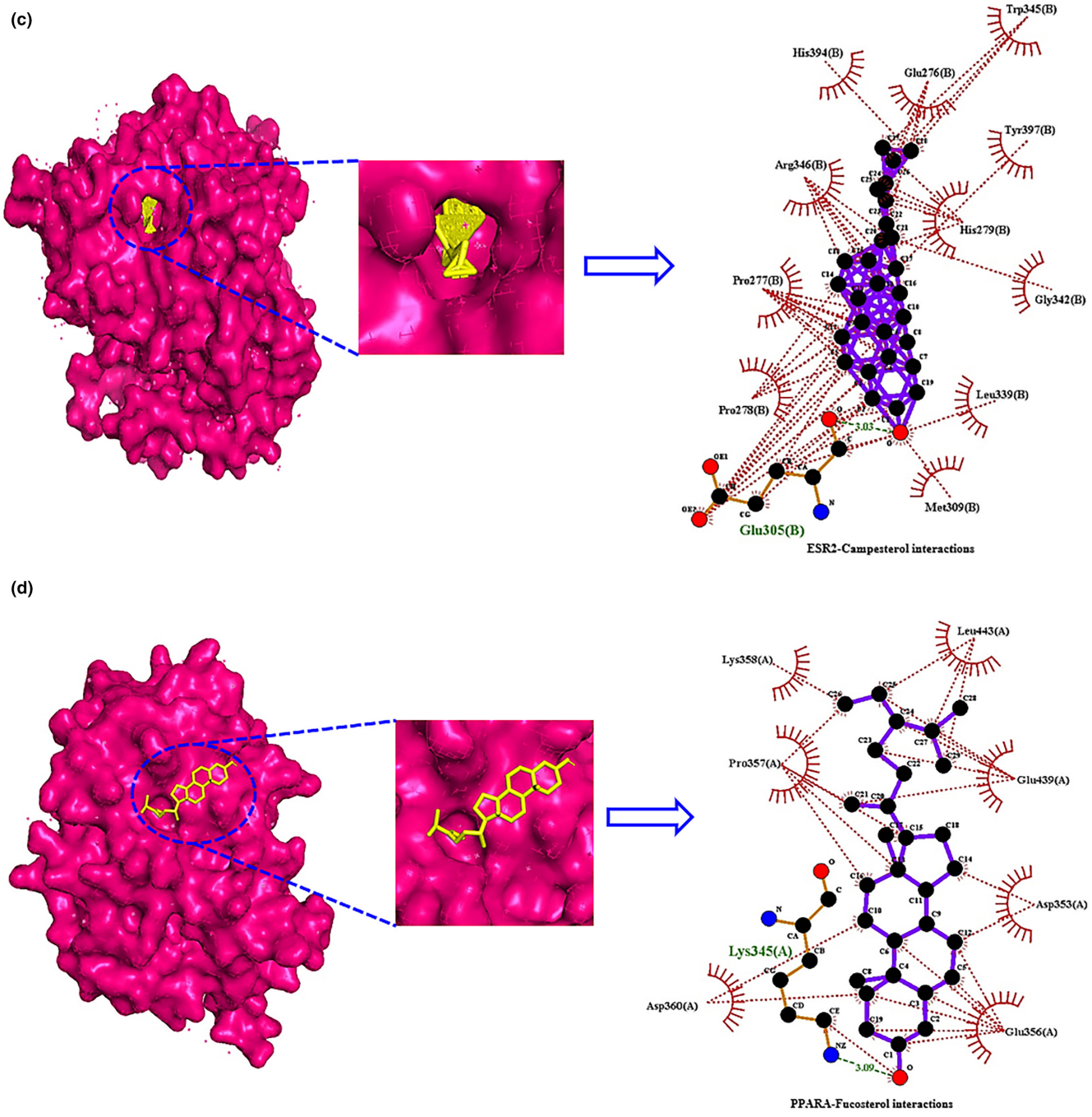

FIGURE 4 (Continued)

is questionable and yet to be revealed clearly. Thus, we postulated that other compounds in PAF might exert therapeutic effectors against OB.

The PPI network showed that AKT1 is a key target involved in the regulation of other adjacent targets (26 targets). AKT1 is related directly to the estrogen signaling pathway, which can have antagonistic effects by binding to gamma-tocopherol. A previous report showed that gamma-tocopherol can diminish oxidative stress induced by estrogen.[31] Additionally, an animal experiment demonstrated that deletion of AKT1 induces the activation of PPARA, leading to anti-OB effects.[32] These suggestions are consistent with our results. The relationships between the 6 signaling pathways and OB are concisely described as follows.

- Thyroid signaling pathway: The occurrence and progression of OB can be exacerbated by hypothyroidism, which is linked to weight gain.[33]
- Prolactin signaling pathway: An increase in circulating prolactin counteracts energy metabolism in OB.[34] It has

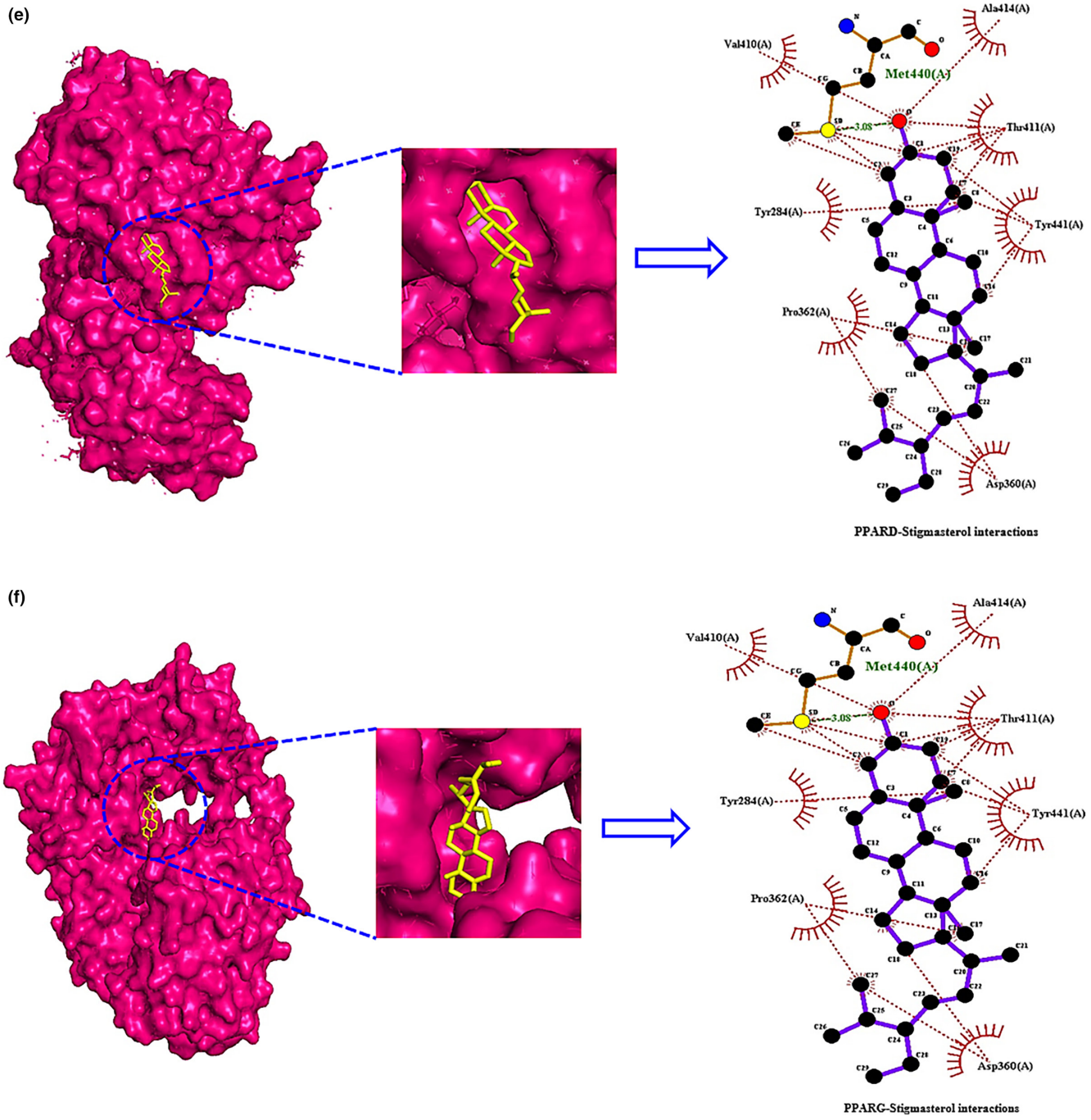

FIGURE 4 (Continued)

been suggested that prolactin might be a positive effector of OB.

- T-cell receptor signaling pathway: OB commonly activates adipose tissue T cells, which can potentiate inflammatory responses.[35]
- Sphingolipid signaling pathway: Sphingolipids increase the absorption of free fatty acids in obese individuals and are implicated in the development of related comorbidities such as atherosclerosis and hypertension.[36,37]
- Estrogen signaling pathway: Normally, OB drives adipocyte hyperplasia and aggravates macrophage infiltration, resulting in an increase in estrogen.[38] It has been indicated that elevation of estrogen levels might be a signal to adipocytes to secrete proinflammatory cytokines.
- PPAR signaling pathway: PPARA and PPARG agonists are independently significant relievers of OB-related inflammation; furthermore, dual PPARA/PPARG agonists might be more effective agents for the treatment of OB.[39]

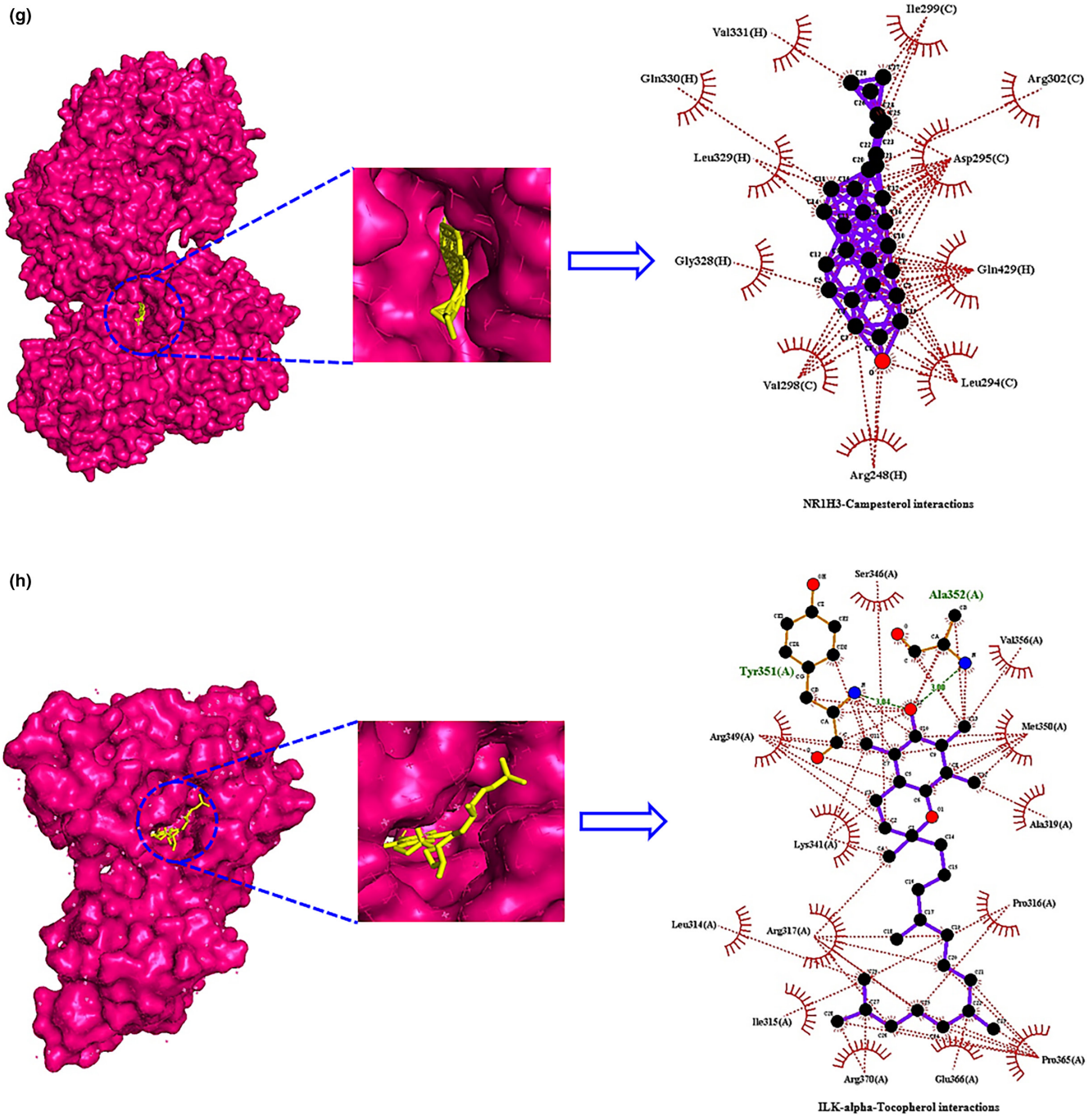

FIGURE 4 (Continued)

Certain PPARA/PPARG dual agents in clinical trials have been developed as new OB medications; however, most of them (aleglitazar, ragaglitazar, imiglitazar, tesaglitazar, and peliglitazar) have failed due to unexpected toxicity.[40] Hence, this study was focused on uncovering natural organic compounds, not synthetic chemicals, from PAF. Distinctively, PAF has been used as a dietary supplement to maintain body weight due to its excellent anti-inflammatory and antioxidant effects.[41]

The hydroquinone lipids (1) alpha-tocopherol and (2) gamma-tocopherol in PAF alleviate the metabolic disorders, including OB.[42,43] Additionally, the stigmastane derivatives (3) fucosterol and (4) stigmasterol are significant natural compounds used to treat OB.[44,45] (5) The ergostane steroid campesterol is also an important anti-OB agent with beneficial efficacy.[46] Accordingly, we utilized the network pharmacology method to elucidate the synergistic effects of the PAF compounds

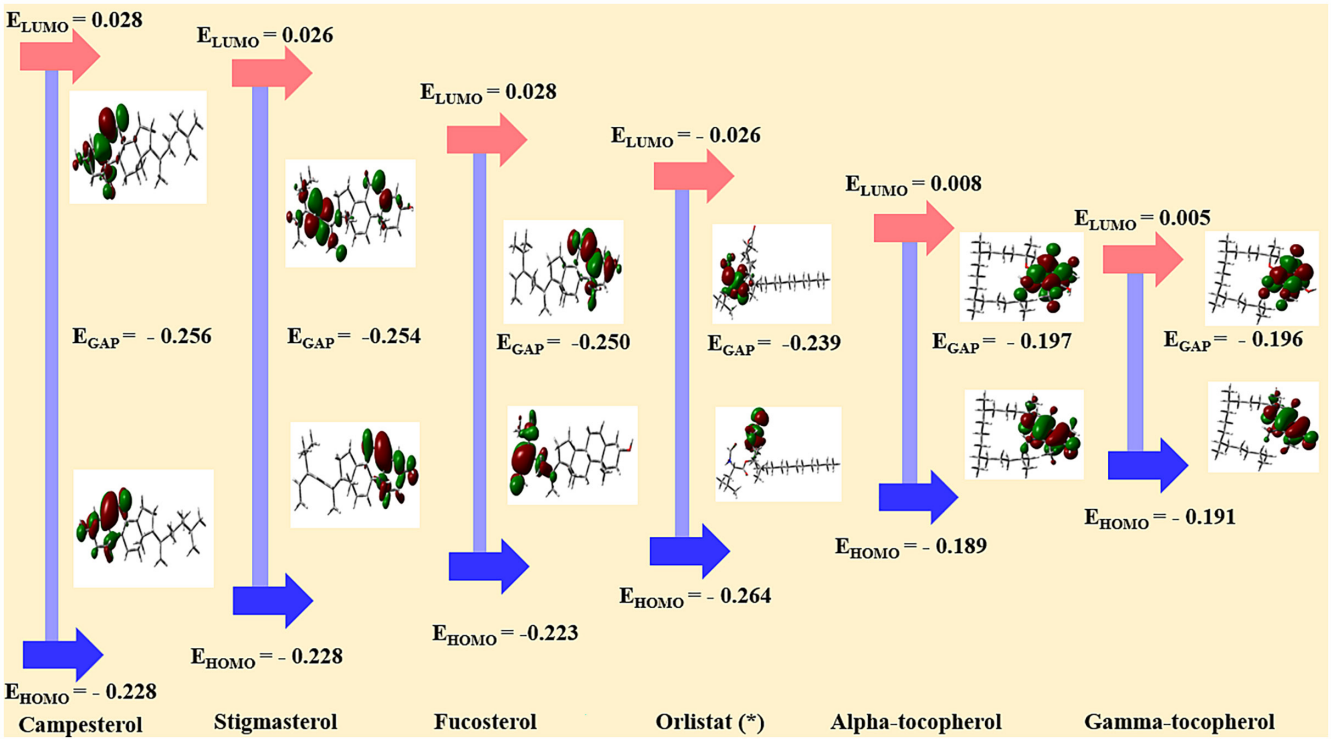

FIGURE 5 The highest occupied molecular orbital – lowest occupied molecular orbital energy gap plots to identify the reactivity level between five key molecules and a standard drug. (*) standard drug.

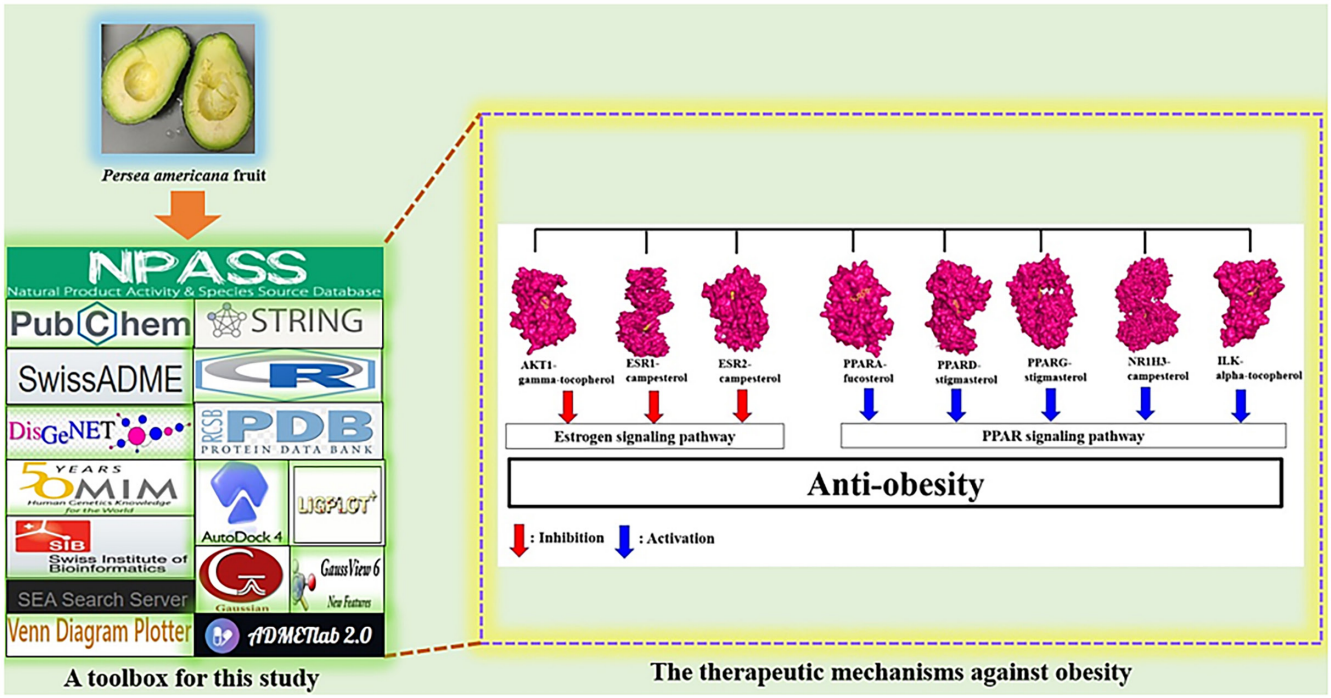

FIGURE 6 The key findings of this study.

against OB. Of interest, the abovementioned five bioactives (alpha-tocopherol, gamma-tocopherol, fucosterol, stigmasterol, and campesterol) formed the most stable complexes with their target proteins despite the presence of many fatty acids in PAF. Based on these results, AKT1-gamma-tocopherol, ESR1-campesterol, and

ESR2-campesterol act on the estrogen signaling pathway in an antagonistic mode, and PPARA-fucosterol, PPARD-stigmasterol, PPARG-fucosterol, NR1H3-campesterol, and ILK-alpha-tocopherol function as agonistic mode on the PPAR signaling pathway thereby, which form stable conformers to exert favorable effects against OB. Additionally, DFT theory can be characterized by chemical reactiveness via the HOMO-LUMO gap, which assists to expound the chemical index, including its hardness and softness.[47,48] Considering current disclosure, the captured five potent molecules can be promising candidates as inhibitors of Estrogen signaling mechanism and pan-PPAR activators for anti-OB. The key findings of this study are displayed in Figure 6.

The limitations of this study

The revealed workflow might be a platform to elucidate the pharmacological effects on diverse natural products, including PAF. In the current version, our platform can be accessible to add up easily new data and is the extensive format to apply different natural herbal resources. In addition, our study provides a concept about how to enhance clinical precision, prior to clinical tests. Noticeably, the platform integrated significant theory: bioinformatics, cheminformatic, systems biology, and computer biology, its convergence gives significant hint to shed light on the therapeutic value of PAF. Through this study, we have established a new therapeutic perspective on NFAs in PAF. However, in spite of highlighting important therapeutic clues, it is required to clarify the bona fide efficacy of the key molecules via clinical trials.

CONCLUSION

In conclusion, this study shows that the combinatorial application of five NFAs (alpha-tocopherol, gamma-tocopherol, fucosterol, stigmasterol, and campesterol) in PAF might have potency for anti-OB effects. The findings indicated that two signaling pathways (Estrogen signaling pathway, and PPAR signaling pathway) might function as an antagonism, and an agonism to treat OB. This work provides significant evidence for clinical efficacy against OB and a pharmacological basis for further elucidating the bioactive compounds and mechanisms of PAF against OB.

AUTHOR CONTRIBUTIONS

K.-T.S., D.J.K., K.-K.O., and M.-G.C. wrote the manuscript. S.-J.Y., S.-B.L., and S.Y.L. designed the research. H.G., R.G., and S.P.S. performed the research. S.-M.W. and J.-J.J. analyzed the data.

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CONFLICT OF INTEREST STATEMENT

The authors declared no competing interests for this work.

DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are included in this published article (and its Supplementary Information files).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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