

The orchestration of Cornus kousa fruit and gut microbiota for anti- obesity via integrated pharmacological analysis based on metabolomics

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Abstract

Background: *Cornus kousa* fruit (CKF) has been utilized as anti-obesity (AOB) supplementation in East Asia, including Korea, and gut microbiota (GM) might have positive effects on obesity (OB) via its interplay. We aimed to decode molecule(s), mechanism(s), and target(s) on interactive association between CKF and GM via network pharmacology (NP) analysis. The chemical constituents from ethanolic CKF (ECKF) were detected by Gas Chromatography-Mass Spectrometry (GC-MS).

Results: The 31 compounds in ECKF were accepted by Lipinski's rule and the overlapping targets (45) were identified by SEA and STP. The final targets (22) were selected on OB-responded targets, indicating that IL6 was the most crucial protein-coding target on PPI networks. A bubble plot and CKF, GM, signaling pathways, targets, and metabolites (CGSTM) networks suggested that AGE-RAGE signaling pathway in diabetic complications inhibited by CKF, and PPAR signaling pathway activated by GM. As the most stable conformers, the IL6-equal complex was attributed to GM, and PPARA-linoleic acid, PPARD-stearic acid, and FABP4-dimethyl 2,3-bis(1,3-dimethylindol-2-yl) fumarate complex were attributed to CKF. Noticeably, stearic acid was removed by Density Functional Theory (DFT) analysis, all other three molecules were proposed as good electron donors with the higher electronegativity, compared with a standard drug (Orlistat).

Conclusion: This study shows that integrated pharmacological analysis can enable to decode the unknown relationships between CKF and GM. For the extensive viewpoints, our approach can obtain scientific evidence to verify the food-GM-host interactions by introducing several fields: bioinformatics, cheminformatics, pharmacology, microbiology, natural products chemistry, and quantum chemistry. Overall, this study reveals that the combination of CKF and favorable GM might exert dual therapeutic effects on OB.

Background

Obesity (OB) influences negatively on diverse metabolic disorders such as cardiovascular diseases (CVD), diabetes, hypertension, heart failure, which accounts for around one-fifth of cancer types [1, 2]. In 2014, more than 2 billion people, approximately one-third of the world population were overweight or around 5% of mortality is liable to OB [3]. OB is represented by excessive accumulation of fat in the tissue, leading unbalanced energy, inappropriate secretion of appetite hormones, and insulin resistance [4]. BMI (Body Mass Index) is a criteria to assess OB, its clinical standard is more than 30.0 or higher [5]. The most significant therapeutics for anti-obesity (AOB) is to block the absorption of lipid in the body tissue, furthermore, to dampen the decomposition and transportation of fat via metabolic pathways [6]. Up to now, five representative medications (Orlistat, Phentermine/topiramate, Naltrexon/bupropion, Liraglutide, and Lorcaserin) against OB have been taken to alleviate its pathophysiological mechanism in spite of some side effects (insomnia, anxiety, depression, diarrhea, and vomiting) [7]. At present, a therapeutic strategy to diminish the adverse effects might be an optimal selective for the obese. For the past decade years, natural products have been increasingly significant therapeutic resource for the alleviation in OB due to its low negative side effects [8]. In recent years, the ethanolic fraction of *Cornus kousa* leaves (CKLs) exerted potent AOB efficacy by downregulating adipogenesis and lipogenesis in 3T3-L1 cells [9]. Commonly, leaves and fruits possess in the majority of chemical constituents, however, fruits comprised more notable chemical compounds and contained higher chemo-diversity [10]. Thus, we aimed to elucidate the therapeutic value on *Cornus kousa* fruit (CKF) in the treatment of OB. The AOB effects of herbal organic compounds are mediated by synchronizing lipolysis, and lipogenesis [11]. However, during the past decades, bona fide efficacy of the natural products has been controversial although noticeable progression has been advanced in treating OB on herbal remedy [12]. Also, its exact pharmacological mechanisms against OB are yet to be determined.

From another viewpoint, GM played important role to alleviate OB and therefore commensal bacteria modulate numerous genes to improve immune system [13, 14]. Most recently, the diversity of GM had favorable therapeutic effects on humans and animal models in OB [15]. A report demonstrated that GM-derived metabolites (amino acids, lipid-like compounds, and bile acid derivatives, or byproducts of carnitine, choline, polyphenols, and purines) involved in metabolic disorders of OB, along with insulin resistance, hyperglycemia, and dyslipidemia [16]. According to study done to date, it elicits that metabolites from GM could be useful agents in ameliorating OB.

Collectively, we firstly integrated chemical constituents of CKF and metabolites of GM to investigate the combination efficacy in the treatment on OB. With a bird's eye view, we postulated that integrative application of natural compounds of CKF, and metabolites of GM can facilitate the alleviative efficacy for AOB.

It is imperative to utilize the network pharmacology (NP) concept to elucidate its combinatorial effects concerning OB. NP can unravel the biological mechanisms(s) of key effectors with extensive concept, which defined as the paradigm shift from "one target, one ligand, one disease" to "network-integrated-multi component therapeutics" [17, 18]. Additionally, the progressive development of bioinformatics, system biology, and cheminformatics is an emerging trend to discover new agents or repurpose existing drugs [19]. Noticeably, NP methodology might be a powerful tool to decode promising new drugs to dampen metabolic disorders, including AOB [20]. From this perspective, we devised new method and concept to reveal AOB effects via combination of compounds from CKF and metabolites from GM. Firstly, chemical constituents from CKF were detected by Gas Chromatography-Mass Spectrum (GC-MS) and evaluated the drug-resemblance properties by Lipinski's rule. The targets related to the chemical compounds were retrieved by Similarity Ensemble Approach (SEA) and SwissTargetPrediction (STP), the intersecting targets of which were identified by Venn diagram plotter. Secondly, OB-responded targets were sorted by public bioinformatics and filtered the final targets from the intersecting targets. The final overlapping targets are elements to analyze protein protein interaction (PPI) networks and a bubble plot. Thirdly, we utilized the GM database as to its metabolites and constructed relationships of CKF or GM, signaling pathways, targets, and molecules (CGSTM). Then, we performed molecular docking assay (MDA)

to verify key compound(s) on key target(s) in key signaling pathway(s). Finally, Density Functional Theory (DFT) was conducted to verify the chemical stability and reactivity of the key targets in this study. The stepwise processing of this study is described in Fig. 1.

Methods

The collection and extraction of CKF

Cornus kousa fruit (CKF) (Fig. 2A) were collected from (latitude: 37.52, longitude:128.29) Kangwon-do, Korea, in October 2022. The CKF was dried in a shaded room with air ventilator maintaining 20–22 °C for 3 weeks, and its seeds were removed from the dried CKF. The CKF removed seeds pulverized with blender. Around 30 g of CKF powder was extracted with 500ml ethyl alcohol (Samchun, Korea, Seoul) for 2 weeks and repeated three times to obtain higher yield rate. The ethyl alcohol extract was collected, used Whatman filter paper No. 1 (Whatman, Model no. WF1-1850, UK Maidstone), and evaporated with a vacuum evaporator (IKA- RV8, Staufen city, Germany) at 40°C. The extraction amount after evaporating was 7.78 g (yield rate: 25.9%), which was calculated as below formula:

Yield rate (%) = (Evaporated extraction weight/Dried CKF weight) × 100

The GC-MS analysis condition

We employed Agilent 7890A (Agilent, Santa Clara, CA, USA) to conduct for GC–MS, its column was selected as DB-5 (30 m × 0.25 mm × 0.25 μm) (Agilent, Santa Clara, CA, USA) with reverse phase. The GC–MS was kept at 100°C for 2.1 min, rising gradually to 300°C at a rate of 25°C/min and was maintained for 20 min. Injection port temperature was 250°C and helium flow rate was 1.5 mL/min, and the ionization voltage was 70 eV. The split mode of sample injection was at 10:1 and the Mass Spectra (MS) detection range consisted of 35–900 (m/z). The fragmentation modes of MS were compared with the W8N05ST Library MS database (analyzed 13 January 2023).

The utilization of the web-accessible public databases

The rapid development of public databases' technologies, including bioinformatics, and cheminformatics, provides a great amount of data produced that is in gradual improvement in diverse research fields. To be utilized the significant bio databases in drug discovery or development is a powerful analytical technology. Specifically, we merged the important databases to cull the valuable components in intricate microbiome research. We categorized the accessible database repositories in Table 1.

Identification of chemical compounds in CKF and filtering of drug-resemblance biomolecules

The chemical compounds in CKF were identified by GC-MS analysis, converting into Simplified Molecular Input Line Entry System (SMILES) format via PubChem (<https://pubchem.ncbi.nlm.nih.gov/>) (accessed on 19 February 2023). Then, we employed Lipinski's rule to screen drug-resemblance biomolecules (DRBs) through SwissADME (<http://www.swissadme.ch/>) (accessed on 19 February 2023). As a criterion of cell permeability, we adopted topological polar surface area (TPSA) value with less than 140 Å² (a threshold) [21].

Targets associated with DRBs from CKF or OB

The targets related to DRBs were browsed by Similarity Ensemble Approach (SEA) [22], SwissTargetPrediction (STP) [23] on "Homo Sapiens" mode. The relationship between targets and DRBs was constructed by utilizing the two databases, which confirmed its effectiveness through experimentation: Approximately 80% out of drug candidates line up with SEA database, additionally, the significant targets on Cudraflavone C were sorted by STP, its bioactivities were confirmed by experiment [24, 25]. The targets related to DRBs were identified by DisGeNET (<https://www.disgenet.org/>) (accessed on 20th February 2023) and OMIM (<https://www.omim.org/>) (accessed on 20th February 2023). The final intersecting targets between sorted DRBs-associated targets and OB-responded targets were established and visualized via Venn diagram plotter.

The protein-protein-interaction (PPI) networks and a bubble chart

The PPI networks were constructed to discern what the most significant target is among intersecting targets. We adopted STRING (<https://string-db.org/>) (accessed on 21th February 2023) database for the PPI construction and a bubble chart was plotted by R package. The bubble chart was built by rich factor defined as the number of differentially expressing targets annotated in each pathway [26]. Based on the bubble chart, we could identify an activating signaling pathway and an inhibitive signaling pathway. Hence, we considered as key mechanisms in the treatment of OB.

The CKF or GM–Signaling pathways–Targets–Metabolites (CGSTM) networks

We employed gutMGene database to incorporate the metabolites of GM, thereby, assembled merged networks, defining as CKF or GM–Signaling pathways–Targets–Metabolites (CGSTM) networks. The CGSTM networks were constructed as a map, based on degree of value (DV). In the incorporated networks, taking CKF or GM, signaling pathways, targets, and metabolites as nodes, its relationships were indicated as edges. The green circles represented GM, red circles stood for signaling pathways, orange circles indicated targets, and purple circles described metabolites. Then, the gray line indicated its relationships and the metabolites from GM represented with red color text. The integrated CGSTM networks were plotted by R Package.

The verification for molecular docking assay (MDA)

We performed the MDA to select the uppermost biomolecule(s) from two key signaling pathways. Based on MDA, we can identify what the most significant metabolite(s) are stemmed from either GM or CKF. The targets were retrieved by RCSB PDB (<https://www.rcsb.org/>) (accessed on 22th February 2023). The extracted .pdb format was transformed into .pdbqt format via Autodock tool (<https://autodock.scripps.edu/>) (accessed on 22th July 2023) to perform the MDA. Then, the DRBs related to the targets were determined .sdf format by PubChem, and the .sdf configuration was altered into .pdb file via Pymol.

MDA of biomolecules on targets related to key signaling pathways

The MDA was analyzed to elucidate its affinity of the biomolecules and targets linked to two key signaling pathways to be functioned as effector(s). The center of targets on the two key signaling pathways was $x = 11.213$, $y = 33.474$, $z = 11.162$, and $x = 8.006$, $y = -0.459$, $z = 23.392$, respectively. The cubic box size of active site was $x = 40 \text{ \AA}$, $y = 40 \text{ \AA}$, $z = 40 \text{ \AA}$. The LigPlot + 2.2 was utilized to describe the interaction of 2D binding on hydrophobic and hydrophilic bonds (<https://www.ebi.ac.uk/thornton-srv/software/LigPlus/>) (accessed on 23th February 2023) [27].

The reactivity of key biomolecules via frontier molecular orbitals (FMO)

Based on the Lee-Yang-Parr (LYP) correlation functional theory at the 6-31G++ (d,p), the key biomolecules including positive controls were completely formatted utilizing GaussView 6.0 and Gaussian 16W software. This setup methodology was to obtain molecular orbitals (MO): lowest occupied molecular orbital (LUMO), and highest occupied molecular orbital (HOMO). To assess the reactivity of key molecules, energy gap (eV), hardness (χ), softness (S), and electronegativity (χ) were calculated by below formulae.

Energy gap = HOMO – LUMO

$$\Delta = (\text{LUMO} - \text{HOMO})/2$$

$$S = 1/\Delta$$

$$\chi = (\text{LUMO} - \text{HOMO})/2$$

Results

Profiling of biomolecules from CKF

A total of 31 biomolecules were recorded by Gas Chromatography Mass Spectrum (GC-MS) (Fig. 2B), which was listed up biomolecule name, PubChem ID, retention time (RT), area, and chemical taxonomy (Table 2). The 31 biomolecules were accepted by Lipinski's rule (Molecular Weight $\leq 500 \text{ g/mol}$; Moriguchi octanol–water partition coefficient ≤ 4.15 ;

Number of Nitrogen or Oxygen ≤ 10 ; Number of NH or OH ≤ 5). The TPSA values as the cell permeability standard are less than $140 \text{ \AA}^2$, the chemical constituents confirmed by the filtering were considered as DRBs (Table 3).

Recognition of intersecting targets between SEA and STP databases

The targets associated with DRBs were identified by SEA and STP databases, indicating that targets identified by SEA (343), STP (458), each other (Supplementary Table S1). The Venn diagram plotter represented the intersecting targets (45) as important targets linked to DRBs (Supplementary Table S1) (Fig. 3A).

Identification of the final overlapping targets between OB-responded targets and the intersecting targets (45)

The number of 3028 targets connected to occurrence and progression of OB were browsed by DisGeNET and OMIM databases. Also, the Venn diagram plotter exhibited that a total of 22 targets was the uppermost targets between CKF-related and OB-responded targets (Supplementary Table S1) (Fig. 3B). However, among the number of 22 targets, the number of four targets (PDCD4, ADH1B, GSTK1, and OXER1) was not correlated with one another.

PPI networks and key signaling pathways via a bubble plot

The PPI networks were identified the most significant target via STRING database with assistance of R Package, suggesting that IL6 had the greatest degree of value (12) in 18 targets (18 nodes, and 37 edges) (Table 4), (Fig. 3C). A bubble plot is according to targets of PPI networks, demonstrating that PPAR signaling pathway as an agonistic mode, and AGE-RAGE signaling pathway in diabetic complications as an antagonistic function might exert therapeutic efficacy on OB (Table 5) (Fig. 3D).

The CGSTM analysis with GM, and CKF

The CGSTM networks consist of CKF, 10 GMs, 2 signaling pathways, 7 targets, and 21 metabolites, representing as 41 nodes, and 59 edges. The CKF, *Enterococcus faecalis*, *Lactobacillus paracasei*, *Streptococcus salivarius* JIM8772, and *Streptococcus salivarius* K12 related to PPAR signaling pathway on FABP4, PPARA, PPARG, and PPARG with 10 metabolites. The *Eubacterium limosum*, *Lactobacillus paracasei* JS1, *Enterococcus durans* EP1, *Enterococcus durans* EP2, *Enterococcus durans* EP3, and *Enterococcus durans* M4-5 associated with AGE-RAGE signaling pathway in diabetic

complications on CDC42, VEGFA, and IL6 with 11 metabolites (Fig. 4). Hence, GM's metabolites and CKF biomolecules show that they have strong relationships to exert the therapeutic effects in treating OB.

Molecular docking assay (MDA)

In the PPAR signaling pathway, the MDA unveiled that both linoleic acid and stearic acid on PPARA (PDB ID: 3SP6) and PPARD (PDB ID: 5U3Q) had valid docking scores with -6.0 kcal/mol, and -6.8 kcal/mol, respectively (Fig. 5A, 5B). On VEGFA (PDB ID: 3P9W) and PPARG (PDB ID: 3E00), there were no potent metabolites from CKF or GM. Noticeably, dimethyl 2,3-bis(1,3-dimethylindol-2-yl) fumarate had the most stable binding score (-6.7 kcal/mol) on FABP4 (PDB ID: 3P6D) (Fig. 5C). The potential ligands on the PPAR signaling pathway were originated from CKF.

In the AGE-RAGE signaling pathway in diabetic complications, IL6 (PDB ID: 4NI9) was associated with nine metabolites out of DRBs from CKF or GM: (1) equol (PubChem ID: 91469), (2) 3-indolepropionic acid (PubChem ID: 3744), (3) 3-deoxy-d-mannonic lactone (PubChem ID: 541561), (4) linoleic acid (PubChem ID: 5280450), (5) 9,12-octadecadienoic acid (PubChem ID: 3931), (6) butyrate (PubChem ID: 104775), (7) acetate (PubChem ID: 175), (8) trimethylamine oxide (PubChem ID: 1145), (9) propionate (PubChem ID: 104745). In nine metabolites, the most stable conformer was equol converted from *Lactobacillus paracasei JS1*, which was -7.2 kcal/mol with valid binding energy (Fig. 5D). Commonly, the active value of binding energy is less than -6.0 kcal/mol on AutoDock Vina [28].

Next, on CDC42 (PDB ID: 3GCG), (1) 2-amino-8-[3-d-ribofuranosyl]imidazo[1,2-a]-s-triazin-4-one (PubChem ID: 15954620) produced by CKF formed the most stable complex with -6.8 kcal/mol (Fig. 5E), followed by (2) guanosine (PubChem ID: 135398635). The significant metabolites were originated from CKF and GM on the AGE-RAGE signaling pathway in diabetic complications. The detailed information of MDA represented in Table 6.

DFT verification of key molecules and positive controls

The Density Functional Theory (DFT) of key molecules and positive controls were compared with their chemical reactivity factors: energy gap (eV), hardness (χ), softness (S), and electronegativity (χ). Commonly, a compound to be either an acceptor or a donator can be determined by the LUMO and HOMO levels. The two key compounds suggested by this study (Equol, Dimethyl 2,3-bis(1,3-dimethylindol-2-yl) fumarate, and 2-amino-8-[3-d-ribofuranosyl]imidazo[1,2-a]-s-triazin-4-one) represented noticeable softness values (10.025 eV, 18.248 eV, and 9.706 eV) and seems to be stable molecules, compared to a standard medicine (Orlistat). A molecule stability is determined to HOMO-LUMO energy gap and the molecule with lower energy gap is linked to its softer property, the compound(s) with the characters can be considered as the most significant agent(s) [29]. Specifically, stearic acid with positive values on both LUMO, and HOMO was removed because a positive HOMO would suggest that the physicochemical property can be ionized easily [30]. The detailed representation was depicted in Table 7, Fig. 6.

Discussion

The CKF is known as herbal source to ameliorate OB; nevertheless, its effectors are yet to be reported completely, including its promising targets, and mechanism(s). In this study, we employed GC-MS analysis to select the DRBs to be permeable in the cells.

In parallel, our aim was to establish the merged representation with biomolecules from CKF and GM, which will be a new insight to clarify the specific relationships between the diet supplementation and GM. Up to date, the perspective viewpoints to demonstrate scientific evidences have been developed with data repository [31]. Thereby, we analyzed the interplay between CKF and GM in the treatment on OB with assistance of rigorous bioinformatics: SEA [32], STP [33], STRING [34], and gutMGene [35]. Moreover, based on the quantum chemical analysis, we adopted DFT theory to confirm the chemical reactivity of key molecules. The reactivity was determined by energy gap, hardness, softness, and electronegativity through LUMO and HOMO values. The stability and reactivity of molecules may be attributed to E_{GAP} , which is a significant description to explain such as hardness, softness and electronegativity [36–39]. The greater of the electronegativity, the more greatly that atom attracts the shared electrons, which is an index to measure the charge transfer in a molecule [40]. DFT was employed to establish more accurate theory, suggesting that the three molecules had more stability, softness, and reactivity than a standard drug (Orlistat).

In this study, we have shown the combinatorial efficacy to relieve OB on PPAR signaling pathway and AGE-RAGE signaling pathways in diabetic complications. For example, dietary mixture with 1% (weight/weight) linoleic acid for 4 weeks reduced body weight (BW) and white adipose tissue (WAT) density in mice experiment [41]. Likewise, a 1% and 1.5% (weight/weight) composition of linoleic acid for 21 days to 28 days diminished BW and WAT amount in obese mice [42]. It elicits that optimal treatment of linoleic acid might be an ameliorator for AOB. As an indole derivative, pharmacological activity of dimethyl 2,3-bis(1,3-dimethylindol-2-yl) fumarate is yet to be confirmed. Our study suggests that dimethyl 2,3-bis(1,3-dimethylindol-2-yl) fumarate might exert therapeutic effect on FABP4. As aforementioned above, linoleic acid, and dimethyl 2,3-bis(1,3-dimethylindol-2-yl) fumarate are effectors to activate PPAR signaling pathway due to the highest rich factor on a bubble plot. Accordingly, the biomolecules from CKF were associated with activation on PPAR signaling pathway.

Next, in AGE-RAGE signaling pathway in diabetic complications, equol from GM is the most stable ligand on IL6, considering that equol might be a potent agent against OB. Our previous study clarified that equol converted by *Lactobacillus paracasei JS1* can bind firmly to the active site on IL6 [43]. Additionally, our result has been shown that 2-Amino-8-[3-d-ribofuranosyl]imidazo[1,2-a]-s-triazin-4-one from CKF exhibited good binding score on CDC42. Both CKF and GM were involved in dampening of AGE-RAGE signaling pathway in diabetic complications.

The epidemiological evidence demonstrated that infants are subjected to obese category during breastfeeding had a decreased pattern of *Enterococcus* compared with observed control groups [44]. It implies that *Enterococcus* is a significant microbe to modulate weight, which lines up with our CGSTM networks analysis. *Eubacterium limosum* can covert natural polyphenols into favorable metabolites, which is a beneficial microbe to prevent OB in polyphenols-microbiota interconnections [45]. *Lactobacillus paracasei* to be taken $10^7 \sim 10^8$ Colony Forming Unit (CFU)/day for 10 weeks exerts AOB effects in an obese mice model [46]. Above mentioned, CKF, and GM is capable to alleviate OB via its orchestration in pharmacological space. The key findings are represented in Fig. 7.

The intention of utilizing biological network module was to decrypt the intricated relationships between CKF and GM. NP is a powerful methodology to identify the relationships between targets and metabolites from CKF, and GM. The application of the combined methodology has already been confirmed to diminish the error rate between interpretation of great amount of data in biological fields and its results [47]. Correspondingly, this study shows that the integrated pharmacological analysis with CKF and GM provides significant clues to uncover its nuanced relationships in the treatment on OB.

The pros and cons of this study

With the integrated pharmacological concept, we confirmed the therapeutic value of CKF and beneficial GM in the treatment of OB. The layout has been devised to decrypt interrelationships between CKF and GM, which is a platform mode to input new information. Also, with exactness and rigorousness, we introduced quantum chemistry theory to realize the drug-resemblance level of key compounds by comparison with a standard drug. In a way, this scenario shows that combinatorial study (bioinformatics, cheminformatics, pharmacology, microbiology, natural products chemistry, and quantum chemistry) can be beneficial to develop more significant effectors via extensive microbiome and nutrition against human disease. This setting might be a hallmark to advance further microbiome research as well as would be a new inflection point to unravel the unclarified relationships of exogenous species (diet) – endogenous species (GM) – host (human).

In this report, the output obtained by the platform are significant elements to confirm the synergistic efficacy of CKF and GM against OB. On the entire viewpoint, the platform can be a significant tool to discover new findings on human diseases. In the context of it, more experimental data and its mechanistic studies are needed to obtain better interpretation of the complicated matrix between CKF and GM.

Conclusion

To summarize, we revealed that PPARA-linoleic acid; and FABP4-dimethyl 2,3-bis(1,3-dimethylindol-2-yl) fumarate from CKF on PPAR signaling pathway as agonistic mode, and IL6-equal from *Lactobacillus paracasei JS1*; and CDC42-2-amino-8-[3-d-ribofuranosyl] imidazo[1,2-a]-s-triazin-4-one from CKF on AGE-RAGE signaling pathway in diabetic complications as antagonistic mode for relieving OB. This study revealed that CKF and GM might lead to orchestrated effects against OB. In spite of the crucial findings, more pharmacokinetic and mechanistic approach are necessary to untangle the intricated microbiome atlas based on our scientific evidence that unified the therapeutic efficacy of CKF on OB, as characterized in this study.

Abbreviations

Anti-OBesity	AOB
Body Mass Index	BMI
Body Weight	BW
Colony Forming Unit	CFU
CKF or GM, Signaling pathways, Targets, and Molecules	CGSTM
<i>Cornus kousa</i> Fruit	CKF
<i>Cornus kousa</i> Leaves	CKLs
CardioVascular Diseases	CVD
Density Functional Theory	DFT
Drug-Resemblance Biomolecules	DRBs
Degree of Value	DV
Ethanollic CKF	ECKF
Gas Chromatography-Mass Spectrometry	GC-MS
Gut Microbiota	GM
Highest Occupied Molecular Orbital	HOMO
Hydrogen Bond Acceptor	HBA
Hydrogen Bond Donor	HBD
Lowest Unoccupied Molecular Orbital	LUMO
Molecular Docking Assay	MDA
<i>Moriguchi</i> octanol-water partition coefficient	MLog P
Mass Spectra	MS
Molecular Weight	MW
Network Pharmacology	NP
Obesity	OB
Protein Protein Interaction	PPI
Protein Protein Interaction	PPI
Retention Time	RT
Similarity Ensemble Approach	SEA
Similarity Ensemble Approach	SEA
Saturated Fatty Acid	SFA
Simplified Molecular Input Line Entry System	SMILES
SwissTargetPrediction	STP
SwissTargetPrediction	STP
Topological Polar Surface Area	TPSA
Unsaturated Fatty Acid	UFA
White Adipose Tissue	WAT

Declarations

Supplementary Information

Supplementary Table S1: The number of 343 targets from SEA; The number of 458 targets from STP; The number of 3028 targets responded to obesity; The number of 22 final targets.

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Author Contributions

DJK and KTS investigated the project. KTS and KKO designed the study. SBL, SYL, HG, RG, SPS, SMW, and JJJ contributed the data acquisition. KKO, and SJY performed the visualization of the data. KKO, SJY, SBL, and SYL analyzed and interpreted the data. KTS and KKO drafted the manuscript. All authors have read and agreed to the published version of the manuscript.

Author's information

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

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1. The profiles of available databases for the study.

No.	Databases	Specification	URL
1	AutoDock 1.5.6	A program for virtual screening of molecules to targets	https://mybiosoftware.com/autodock-4-2-3-autodocktools-1-5-6-suite-automated-docking-tools.html
2	DisGeNET	The collective repository related to human diseases	https://www.disgenet.org/
3	Gaussian 16W	Computational chemistry software for DFT	no available: commercial item
4	GaussView 6.0	Chemical interface program	https://gaussview.software.informer.com/6.0/
5	gutMGene	The database of gut microbiota and its metabolites	http://bio-annotation.cn/gutmgene
6	LigPlot++	A program to generate 2D ligand-protein interaction diagrams	https://www.ebi.ac.uk/thornton-srv/software/LigPlus/download.html
7	Online Mendelian Inheritance in Man (OMIM)	Human disease database associated with targets	https://www.omim.org/
8	PubChem	A database of chemical information	https://pubchem.ncbi.nlm.nih.gov/
9	RCSB PDB	The protein-structure based database	https://www.rcsb.org/
10	R package	A software for networking visualization	https://www.r-project.org/
11	Similarity Ensemble Approach (SEA)	A database to identify targets on compounds	https://sea.bkslab.org/
12	STRING	A database for protein-protein interaction (PPI) networks	https://string-db.org/
13	SwissADME	A database to confirm the drug-like properties	http://www.swissadme.ch/
14	SwissTargetPrediction (STP)	A database to estimate the targets on biomolecules	http://www.swisstargetprediction.ch/
15	Venn Diagram Plotter	Venn diagram proportional drawing tool	http://www.softsea.com/review/Venn-Diagram-Plotter.html

Table 2. The profiling of chemical constituents from CKF via GC-MS.

No.	Compounds	PubChem ID	RT(mins)	Area(%)	Classification
1	alpha.-d-Mannofuranoside, isopropyl-	537903	5.337	0.99	Carbohydrates and carbohydrate conjugates
2	2-Furanone, 3,4-dihydroxytetrahydro	536124	5.395	0.86	Gamma butyrolactones
3	Glyceraldehyde (propanal, 2,3-dihydroxy-)	751	5.712, 6.174	0.95	Carbohydrates and carbohydrate conjugates
4	Dihydromaltol (2,3-dihydro-5-hydroxy-6-methyl-4H- pyran-4-one)	6429306	6.250	0.66	Pyranones and derivatives
5	Pyrimidine-4,6-diol, 5-methyl-	581007	6.375	1.46	Pyrimidines and pyrimidine derivatives
6	2,3-Dihydro-3,5-dihydroxy-6-methyl-4h-pyran-4-one	119838	6.799, 7.068	10.86	Pyranones and derivatives
7	5-Hydroxymethylfurfural	237332	7.404	30.08	Carbonyl compounds
8	2-propyl-5- oxohexanoic acid	538225	7.500, 7.606	4.30	Fatty acids and conjugates
9	6-Acetyl-.beta.-d-mannose	91691338	7.808, 8.058	1.13	Carbohydrates and carbohydrate conjugates
10	4-methylthiazol-2-ol	220936	7.866	2.24	Thiazoles
11	9-Acetoxynonanal	537145	8.154	1.09	Fatty alcohol esters
12	1,3-Dioxolane-4-methanol, alpha-ethynyl-2,2-dimethyl-, acetate	268060	8.375	1.41	Ethers
13	3,7-Dimethyl-1-octene	21085	8.481	0.80	Unsaturated aliphatic hydrocarbons
14	4-Methoxy-2,3-dimethylbutyraldehyde	102239823	8.875	5.13	Ethers
15	Heptose	219662	8.980	2.82	Carbohydrates and carbohydrate conjugates
16	N-Acetyl-d-cycloserine	3246575	9.106	0.90	Amino acids, peptides, and analogues
17	2-Amino-8-[3-d-ribofuranosyl]imidazo[1,2-a]-s-triazin-4-one	15954620	9.164	1.16	Carbohydrates and carbohydrate conjugates
18	2-Ethyl-2-hexenal	5354264	9.222	1.05	Carbonyl compounds
19	Acetamide, N-(3-methylbutyl)-	98643	9.481	2.47	Carboxylic acid derivatives
20	3-Deoxy-d-mannoic lactone	541561	9.895	13.93	Delta valerolactones
21	1-Deoxy-d-altritol	18667263	10.164	3.17	Carbohydrates and carbohydrate conjugates
22	Guanosine	135398635	10.549	0.80	Purine nucleosides
23	Palmitic Acid	985	10.722	1.78	Fatty acids and conjugates
24	(Z,Z)-Gossyplure	5363265	11.039	0.75	Fatty alcohol esters
25	Linoleic Acid	5280450	11.433	0.35	Lineolic acids and derivatives
26	Stearic Acid	5281	11.520	1.90	Fatty acids and conjugates
27	7-Pentadecyne	549063	12.116, 12.173, 12.279	0.32	Acetylenes
28	9,12-octadecadienoic acid	3931	12.693, 19.164	0.88	Lineolic acids and derivatives
29	Z,E-7,11-Hexadecadien-1-yl acetate	5363282	12.847, 12.962	1.20	Fatty alcohol esters
30	2-Chloroethyl linoleate	5365671	13.164, 13.250, 13.289, 13.548, 13.654	3.80	Lineolic acids and derivatives
31	Dimethyl 2,3-bis(1,3-dimethylindol-2-yl)fumarate	129854768	16.683	0.28	Indoles

RT: Retention Time

Table 3. The physicochemical properties of chemical constituents.

No.	Compounds	Lipinski Rules			Lipinski's Violations		Bioavailability Score	TPSA(Å²)
		MW	HBA	HBD	MLog P			
		< 500	< 10	≤ 5	≤ 4.15	≤1		
1	alpha.-d-Mannofuranoside, isopropyl-	222.24	6	4	-1.75	0	0.55	99.38
2	2-Furanone, 3,4-dihydroxytetrahydro	118.09	4	2	-1.64	0	0.55	66.76
3	Glyceraldehyde (propanal, 2,3-dihydroxy-)	90.08	3	2	-1.66	0	0.55	57.53
4	Dihydromaltol (2,3-dihydro-5-hydroxy-6-methyl-4H-pyran-4-one)	128.13	3	1	-0.92	0	0.85	46.53
5	Pyrimidine-4,6-diol, 5-methyl-	126.11	3	2	-0.85	0	0.55	65.98
6	2,3-Dihydro-3,5-dihydroxy-6-methyl-4h-pyran-4-one	144.13	4	2	-1.77	0	0.85	66.76
7	5-Hydroxymethylfurfural	126.11	3	1	-1.06	0	0.55	50.44
8	2-propyl-5- oxohexanoic acid	172.22	3	1	1.29	0	0.85	54.37
9	6-Acetyl-.beta.-d-mannose	222.19	7	4	-2.2	0	0.55	116.45
10	4-methylthiazol-2-ol	115.15	1	1	-0.16	0	0.55	61.10
11	9-Acetoxy-nonanal	200.27	3	0	1.88	0	0.55	43.37
12	1,3-Dioxolane-4-methanol, alpha-ethynyl-2,2-dimethyl-, acetate	198.22	4	0	0.76	0	0.55	44.76
13	3,7-Dimethyl-1-octene	140.27	0	0	4.68	1	0.55	0.00
14	4-Methoxy-2,3-dimethylbutyraldehyde	130.18	2	0	0.81	0	0.55	26.30
15	Heptose	210.18	7	6	-3.36	1	0.55	138.45
16	N-Acetyl-d-cycloserine	144.13	3	2	-1.35	0	0.55	67.43
17	2-Amino-8-[3-d-ribofuranosyl]imidazo[1,2-a]-s-triazin-4-one	283.24	7	4	-2.76	0	0.55	148.13
18	2-Ethyl-2-hexenal	126.20	1	0	1.97	0	0.55	17.07
19	Acetamide, N-(3-methylbutyl)-	129.20	1	1	1.22	0	0.55	29.10
20	3-Deoxy-d-mannoic lactone	162.14	5	3	-1.68	0	0.55	86.99
21	1-Deoxy-d-altritol	166.17	5	5	-1.95	0	0.55	101.15
22	Guanosine	283.24	7	5	-2.76	0	0.55	159.51
23	Palmitic Acid	256.42	2	1	4.19	1	0.85	37.30
24	(Z,Z)-Gossyplure	280.45	2	0	4.47	1	0.55	26.30
25	Linoleic Acid	280.45	2	1	4.47	1	0.85	37.30
26	Stearic Acid	284.48	2	1	4.67	1	0.85	37.30
27	7-Pentadecyne	208.38	0	0	6.04	1	0.55	0.00
28	9,12-octadcadienoic acid	280.45	2	1	4.47	1	0.85	37.30
29	Z,E-7,11-Hexadecadien-1-yl acetate	280.45	2	0	4.47	1	0.55	26.30
30	2-Chloroethyl linoleate	342.94	2	0	5.15	1	0.55	26.30
31	Dimethyl 2,3-bis(1,3-dimethylindol-2-yl)fumarate	430.50	4	0	3.01	0	0.55	62.46

MW: Molecular Weight; HBA: Hydrogen Bond Acceptor; HBD: Hydrogen Bond Donor; MLog P: *Moriguchi octanol-water partition coefficient*; TPSA: *Topological Polar Surface Area*.

Table 4. The degree of value (DV) in PPI networks.

No.	Target name	Degree of value
1	IL6	12
2	PPARG	8
3	PPARA	7
4	VEGFA	7
5	CNR1	6
6	AKR1B1	5
7	TRPV1	5
8	CDC42	4
9	FABP4	4
10	CNR2	3
11	FFAR4	3
12	EHMT1	2
13	RAC1	2
14	PPARD	2
15	ADA	1
16	ENPP2	1
17	BCAT2	1
18	KDM4C	1

Table 5. The targets of key signaling pathways on OB.

KEGG ID & Description	Target	False discovery rate
hsa03320: PPAR signaling pathway	PPARA, PPARD, PPARG, FABP4	0.00057
hsa04933: AGE-RAGE signaling pathway in diabetic complications	IL6, VEGFA, CDC42	0.03210

Table 6. The detailed information of MDA.

Protein	Ligand	PubChem ID	Binding energy (kcal/mol)	Grid box		Hydrogen Bond Interactions	Hydrophobic Interactions
				Center	Dimension		
PPARA (PDB ID: 3SP6)	linoleic acid	5280450	-6.0	x=8.006	size_x = 40	Met320, Met220	Phe218,Asn219,Gly335
				y=-0.459	size_y = 40		Tyr334,Leu331,Val332
				z=23.392	size_z = 40		Il2901,Thr279,Val324
	stearic acid	5281	-5.7	x=8.006	size_x = 40	n/a	Leu321,Thr283
				y=-0.459	size_y = 40		Gln442,Asp353,Leu413
				z=23.392	size_z = 40		His440,Glu439,Leu436
	2-propyl-5-oxohexanoic acid	538225	-5.6	x=8.006	size_x = 40	Tyr334,Thr283,Asn219	Asp432,Phe361,Lys358
				y=-0.459	size_y = 40		Pro357,Ile446
				z=23.392	size_z = 40		Gly335,Leu331,Thr279
	heptose	219662	-5.6	x=8.006	size_x = 40	Thr283,Met220,Tyr334	Val324,Met220,Leu321
				y=-0.459	size_y = 40		Met320,Val332
				z=23.392	size_z = 40		Phe218,Glu286,Thr279
	3,7-dimethyl-1-octene	21085	-5.2	x=8.006	size_x = 40	n/a	Val332
				y=-0.459	size_y = 40		Met320,Leu321,Val324
				z=23.392	size_z = 40		Met220,Leu331,Il2901
	palmitic acid	985	-4.9	x=8.006	size_x = 40	n/a	Thr283,Ile317
				y=-0.459	size_y = 40		Val332,Ile241,IL2901
				z=23.392	size_z = 40		Ala333,Thr279,Val255
	9,12-octadecadienoic acid	3931	-4.0	x=8.006	size_x = 40	n/a	Tyr334,Leu258,Cys275
				y=-0.459	size_y = 40		Ala250,Leu254,Glu251
				z=23.392	size_z = 40		Thr697,Thr285,Lys692
PPARG (PDB ID: 3E00)	10-keto-12z-octadecenoic acid	24970825	-5.7	x=39.265	size_x = 40	Glu343	His457,Val281,Leu459
				y=-18.736	size_y = 40		Leu689,Leu693,Glu289
				z=119.392	size_z = 40		Ala696
	linoleic Acid	5280450	-5.3	x=39.265	size_x = 40	Thr162,Leu167	Ser342,Ile341,Leu228
				y=-18.736	size_y = 40		Met329,Pro227,Glu295
				z=119.392	size_z = 40		Glu291,Arg288
	palmitic Acid	985	-5.1	x=39.265	size_x =	Glu291,Arg288	Asp166,Lys336,Glu369
				y=-18.736	size_y = 40		Val372,Arg350,Glu351
				z=119.392	size_z = 40		Gln193,Lys354,Arg202
							Glu343,Leu333,Leu330

				40			
				y=-18.736	size_y = 40	Leu228, Met329, Ala292	
				z=119.392	size_z = 40	Ile326, Glu295	
2-chloroethyl linoleate	5365671	-5.0	x=39.265	size_x = 40	Gln451	Phe437, Leu433, Lys367	
				y=-18.736	size_y = 40	Leu452, Pro366, Asp362	
				z=119.392	size_z = 40	His49, Glu448, Glu434	
stearic acid	5281	-5.0	x=39.265	size_x = 40	n/a	Lys157, Glu207, Gln206	
				y=-18.736	size_y = 40	Arg164, Val163, Ala376	
				z=119.392	size_z = 40	Arg234, Val372, Asn375	
						Lys336, Arg202, Glu203	
9,12-octadecadienoic acid	3931	-5.0	x=39.265	size_x = 40	Leu430, Gln451	Glu434, Phe437, Leu452	
				y=-18.736	size_y = 40	Asp362, Glu448, Val455	
				z=119.392	size_z = 40	Leu433	
FABP4 (PDB ID: 3P6D)	dimethyl 2,3-bis(1,3-dimethylindol-2-yl)fumarate	129854768	-6.7	x=7.693	size_x = 40	Lys120	Gly121, Thr103, Val94
				y= 9.921	size_y = 40	Ser101, Thr102, Val118	
				z=14.698	size_z = 40		
heptose	219662	-5.6	x=7.693	size_x = 40	Ile51, Thr60, Arg106	Gln95, His93, Ala75	
				y= 9.921	size_y = 40	Thr74, Lys52, Ser53	
				z=14.698	size_z = 40	Met40, Glu72, Ile104	
stearic acid	5281	-5.5	x=7.693	size_x = 40	n/a	Gly46, Cys1, Leu86	
				y= 9.921	size_y = 40	Asp47, Met0, Ser1	
				z=14.698	size_z = 40	Leu66	
2-chloroethyl linoleate	5365671	-5.2	x=7.693	size_x = 40	Gly88	Gly46, Leu66, Ile49	
				y= 9.921	size_y = 40	Met0, Asp87, Leu86	
				z=14.698	size_z = 40	Ser1, Cys1, Asp47	
linoleic Acid	5280450	-4.9	x=7.693	size_x = 40	Leu86	Thr85, Leu66, Asp47	
				y= 9.921	size_y = 40	Cys1, Gly46, Ser1	
				z=14.698	size_z = 40	Met0	
palmitic Acid	985	-4.4	x=7.693	size_x = 40	Glu72, Val80	Lys79, Asp71, Val73	
				y= 9.921	size_y = 40	Glu61, Thr60	
				z=14.698	size_z = 40		
2-propyl-5-oxohexanoic acid	538225	-4.4	x=7.693	size_x = 40	Thr56	Phe57, Met35, Val32	
				y= 9.921	size_y = 40		
				z=14.698	size_z = 40		
9,12-octadecadienoic acid	3931	-4.3	x=7.693	size_x = 40	Asp47	Met0, Cys1, Ser1	
				y= 9.921	size_y = 40	Leu86, Thr85, Leu66	

IL6 (PDB ID: 4NI9)	equol	91469	-7.4	z=14.698	size_z = 40	Glu110,Asp34,Tyr31	Gln111,Gly35,Ala114
				x=11.213	size_x = 40		
				y=33.474	size_y = 40		
	3-indolepropionic acid	3744	-7.2	z=11.162	size_z = 40	Arg16	Pro18,Gln17
				x=11.213	size_x = 40		
				y=33.474	size_y = 40		
	3-deoxy-d-mannonic lactone	541561	-6.3	z=11.162	size_z = 40	n/a	n/a
				x=11.213	size_x = 40		
				y=33.474	size_y = 40		
	linoleic acid	5280450	-5.0	z=11.162	size_z = 40	Lys39	Glu81,Pro80,Ser168
				x=11.213	size_x = 40		
				y=33.474	size_y = 40		
	9,12-octadecadienoic acid	3931	-4.8	z=11.162	size_z = 40	Glu110	Gln166,Lys103,Glu165
				x=11.213	size_x = 40		
				y=33.474	size_y = 40		
	butyrate	104775	-4.4	z=11.162	size_z = 40	n/a	n/a
				x=11.213	size_x = 40		
				y=33.474	size_y = 40		
	acetate	175	-3.8	z=11.162	size_z = 40	n/a	Arg16
				x=11.213	size_x = 40		
				y=33.474	size_y = 40		
	trimethylamine oxide	1145	-3.6	z=11.162	size_z = 40	n/a	n/a
				x=11.213	size_x = 40		
				y=33.474	size_y = 40		
	propionate	104745	-3.5	z=11.162	size_z = 40	Ser194,Ser114,Ser193	Pro192,Asn137,Ser141
				x=11.213	size_x = 40		
				y=33.474	size_y = 40		
VEGFA (PDB ID:3P9W)	alpha.-d-mannofuranoside, isopropyl-	537903	-5.9	z=-40.286	size_z = 40	Asp63,Asn62,Glu64	Ile83,Asp63,His86
				x=-12.652	size_x = 40		
				y=70.481	size_y = 40		
	6-acetyl-.beta.-d-mannose	91691338	-5.8	z=-40.286	size_z = 40	Glu64,Asn62,Asp63	Pro85,Ile46,Ile83
				x=-12.652	size_x = 40		
				y=70.481	size_y = 40		Asp63,Ile46,Asn62
				z=-40.286	size_z = 40		

	2-chloroethyl linoleate	5365671	-5.4	x=-12.652	size_x = 40	n/a	Glu64,Ile83,Ile46
				y=70.481	size_y = 40		His86,Phe36,Glu64
				z=-40.286	size_z = 40		Pro85
CDC42 (PDB ID: 3GCG)	2-amino-8-[3-d-ribofuranosyl]imidazo[1,2-a]-s-triazin-4-one	15954620	-6.8	x=-12.652	size_x = 40	Lys107,Lys104,Pro34	Val33,Gly15,Lys16
				y=70.481	size_y = 40		Ala59,Gly12,Glu62
				z=-40.286	size_z = 40		Ala13,Thr35,Asn125
	guanosine	135398635	-6.7	x=-12.652	size_x = 40	Lys104,Lys107,Gly60	Thr35,Asn125,Ala59
				y=70.481	size_y = 40		Lys16,Gly12,Ala13
				z=-40.286	size_z = 40		Pro34,Val33

Table 7. The parameters of quantum kinetics of key compounds and a standard drug.

Molecules	LUMO	HOMO	E _{GAP} (eV)	μ (eV)	S (eV)	χ (eV)
Orlistat (*)	- 0.0257	- 0.2645	- 0.2388	0.1194	8.3766	- 0.1194
Linoleic acid	- 0.0082	- 0.2355	- 0.2272	0.1136	8.8017	- 0.1136
2-amino-8-[3-d-ribofuranosyl]imidazo[1,2-a]-s-triazin-4-one	- 0.0257	- 0.2318	- 0.2061	0.1030	9.7059	- 0.1030
Equol	- 0.0097	- 0.2092	- 0.1995	0.0998	10.0246	- 0.0998
Dimethyl 2,3-bis(1,3-dimethylindol-2-yl)fumarate	- 0.0811	- 0.1907	- 0.1096	0.0548	18.2482	- 0.0548

(*): Anti-obesity standard drug

Supplementary Files

Supplementary Table S1 is not available with this version.

Figures

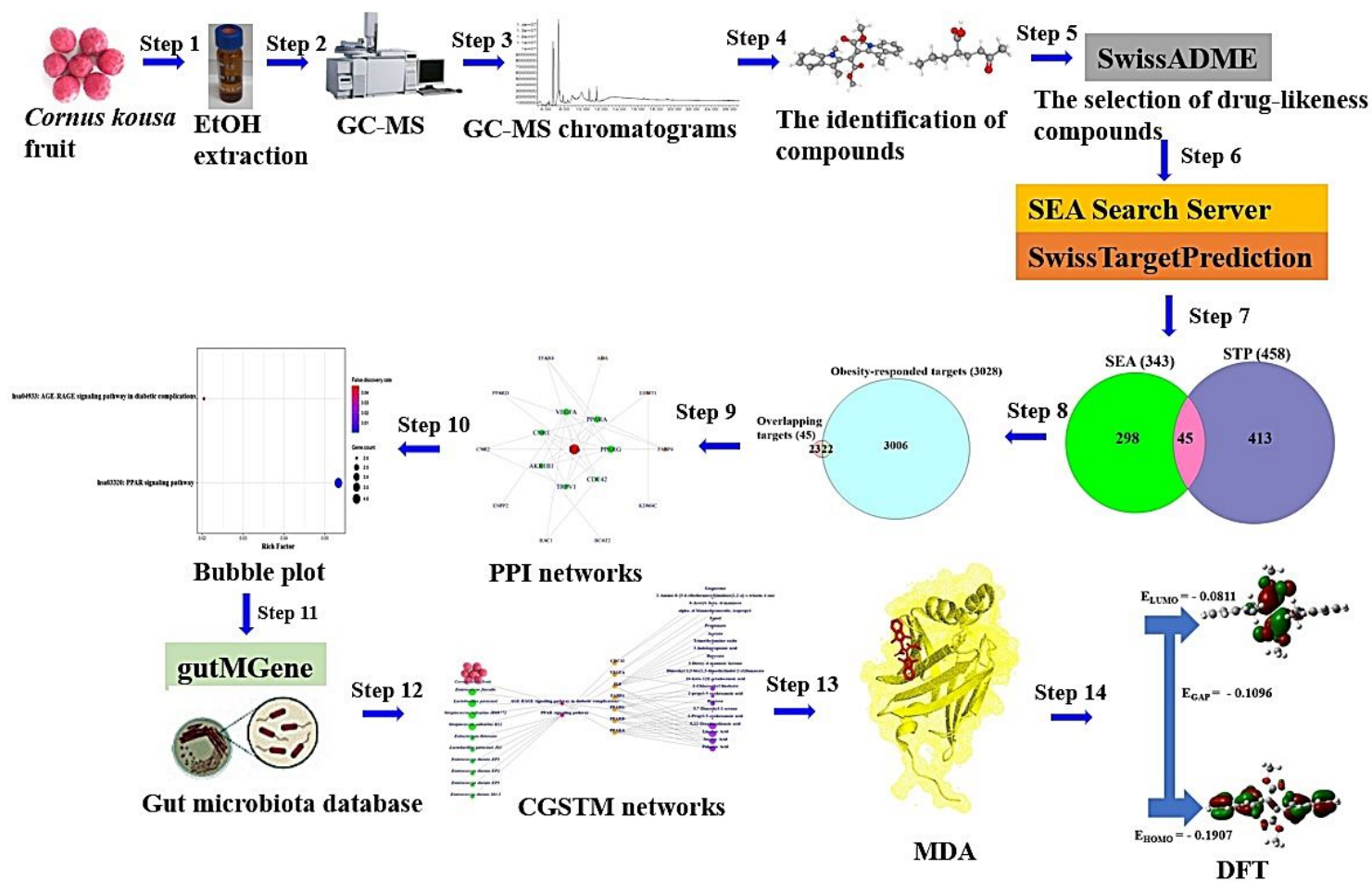


Figure 1

The workflow of this study.

(A)



(B)

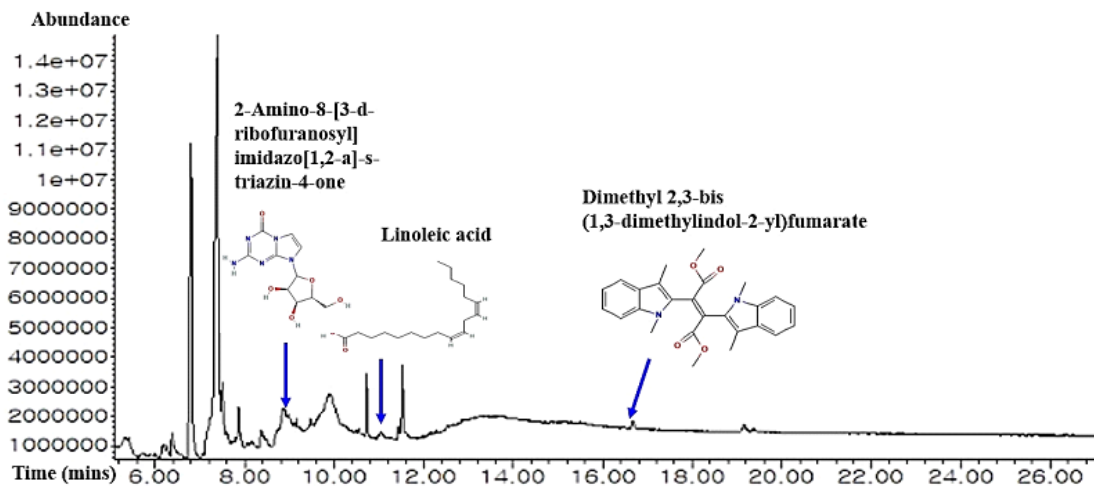


Figure 2

(A) The picture of CKF. (B) GC-MS chromatography of CKF.

(D) CDC42-2-amino-8-[3-d-ribofuranosyl]imidazo[1,2-a]-s-triazin-4-one.

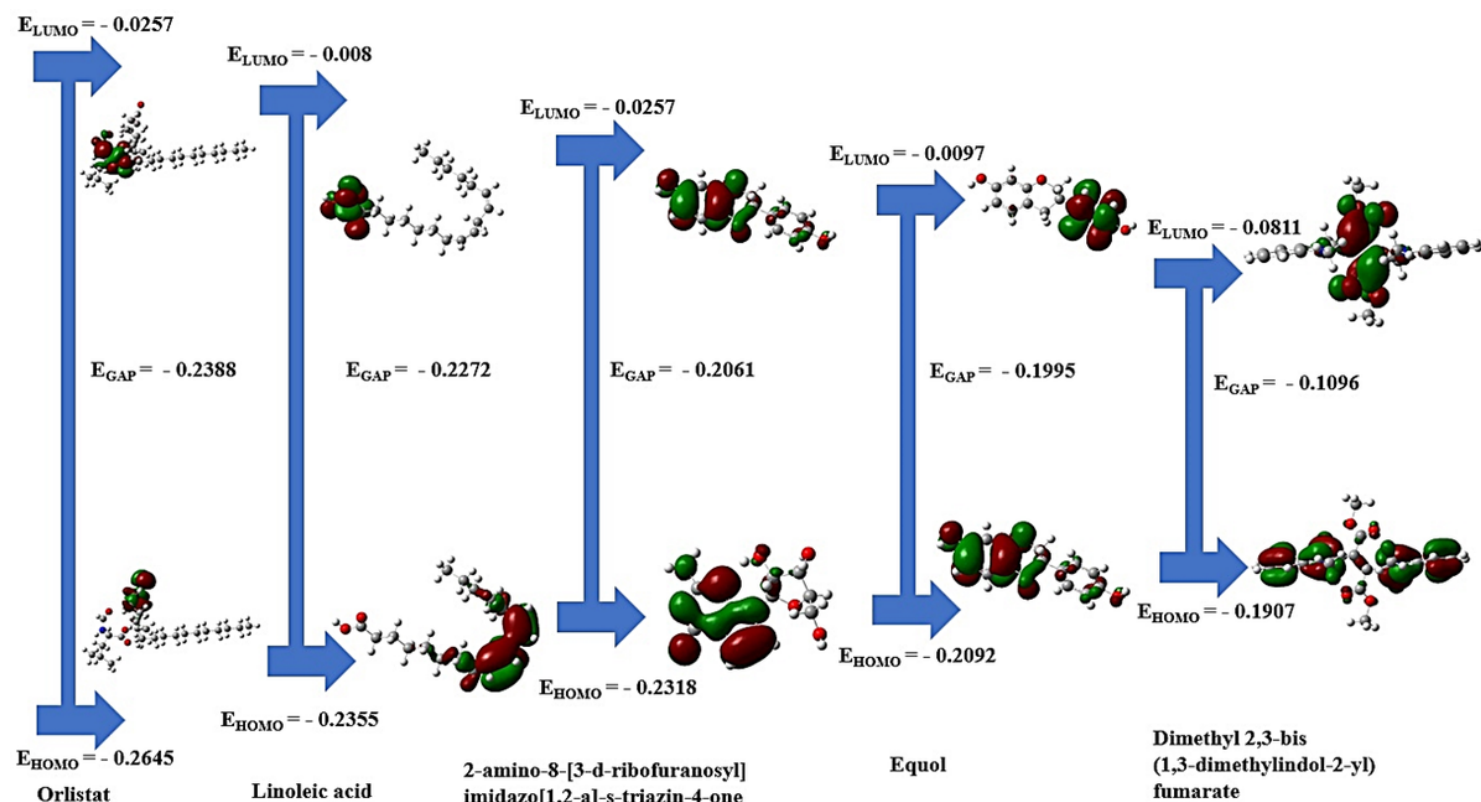


Figure 6

Molecular orbital energy description and their energy gap of key molecules from CKF, GM and a standard drug (Orlistat). Green color orbitals: electrophilic groups; Red color orbitals: nucleophilic groups.

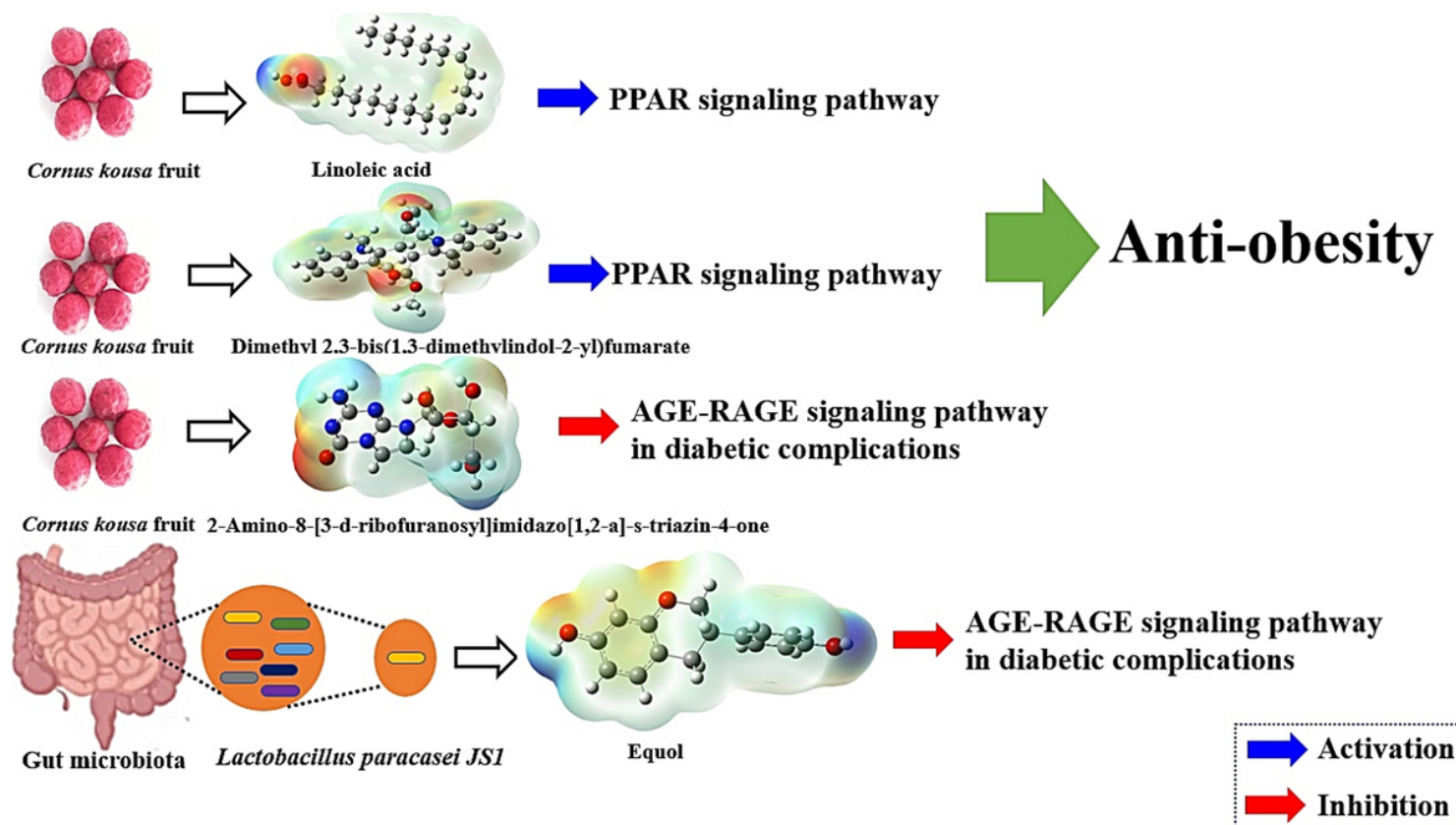


Figure 7

The key findings of this study.