PHARMACIA

Pharmacia 71: 1–12
DOI 10.3897/pharmacia.71.e116794

Research Article

Free radical scavenging, α-amylase, α-glucosidase, and lipase inhibitory activities of metabolites from strawberry kombucha: Molecular docking and in vitro studies

Adriyan Pramono[1,2], William Ben Gunawan[1], Fahrul Nurkolis[3], Darmawan Alisaputra[4], Gilbert Ansell Limen[5], Muhammad Subhan Alfaqih[6], Martha Ardiaria[1,2]

1 *Department of Nutrition Science, Faculty of Medicine, Universitas Diponegoro, Semarang, Indonesia*
2 *Center of Nutrition Research (CENURE), Universitas Diponegoro, Semarang, Indonesia*
3 *Biological Sciences, Faculty of Science and Technology, State Islamic University of Sunan Kalijaga, Yogyakarta, Indonesia*
4 *Chemistry Programme, Biological Sciences, Faculty of Science and Technology, State Islamic University of Sunan Kalijaga, Yogyakarta, Indonesia*
5 *Medical Programme, Faculty of Medicine, Sam Ratulangi University, Manado, Indonesia*
6 *Faculty of Medicine, President University, Bekasi, Indonesia*

Corresponding author: Adriyan Pramono (adriyanpramono@fk.undip.ac.id)

Received 5 December 2023 ♦ **Accepted** 19 January 2024 ♦ **Published** 7 May 2024

Citation: Pramono A, Gunawan WB, Nurkolis F, Alisaputra D, Limen GA, Alfaqih MS, Ardiaria M (2024) Free radical scavenging, α-amylase, α-glucosidase, and lipase inhibitory activities of metabolites from strawberry kombucha: Molecular docking and in vitro studies. Pharmacia 71: 1–12. https://doi.org/10.3897/pharmacia.71.e116794

Abstract

Obesity, a global issue, is linked to cardiometabolic syndrome. Dietary modification is one of the recommended modes for managing cardiometabolic syndrome. Strawberries, a functional food, and kombucha, a fermented tea beverage, have gained attention for their health benefits.

This study investigated the bioactive components of strawberry kombucha drink (SKD) and their effects on antioxidant activities and improving metabolic disorder markers.

An in vitro experiment was performed to determine the effect of SKD on enzymatic parameters: lipase, α-glucosidase, and α-amylase activities. In addition, antioxidant activity using the DPPH method and quantification of the radical scavenging activity were also measured. Furthermore, untargeted metabolomic profiling of SKD and molecular docking simulation were conducted.

The findings suggest that SKD, rich in secondary metabolites, can inhibit lipase, α-glucosidase, and α-amylase activities. It demonstrated in vitro anti-obesity, anti-diabetic, and antioxidant properties, potentially reducing metabolic and inflammatory issues.

Thus, SKD could be a therapeutic beverage to alter metabolic issues associated with obesity. Nevertheless, further preclinical study is warranted to determine SKD's potential in vivo.

Keywords

Antioxidant, fermentation, metabolite, molecular docking, strawberry kombucha

Introduction

Fermented beverages are gaining popularity due to their potential as probiotic beverages, enriched with bioactive compounds, antioxidants, and significant health benefits (Selvaraj and Gurumurthy 2023). One prominent example is kombucha, which has become a focal point in functional food research. Kombucha is a fermented beverage produced using black or green tea as a substrate, along with sucrose and a symbiotic culture of bacteria and yeast (SCOBY) (Jakubczyk et al. 2020). The microorganisms present in SCOBY consist of a mixture of acetic bacteria, lactic acid bacteria, and osmophilic yeast, which, during the fermentation process, generate new compounds from the substrate, leading to the production of various metabolites (Miranda et al. 2022).

Kombucha, made from tea, contains abundant catechins, theaflavins, flavonoids, and polyphenols (Abaci et al. 2022). The bioactive components in kombucha encompass not only tea-derived polyphenols but also various metabolite compounds produced during fermentation, including organic acids, vitamins, organic nitrogen, enzymes, minerals, and other substances (Kitwetcharoen et al. 2023). Throughout the fermentation process, the nutrient content of the beverage increases, stimulating the probiotic and prebiotic functions of kombucha. Several microorganisms found in kombucha include *Komagataeibacter xylinus*, *Brettanomyces bruxellensis*, *Acetobacter pasteurianus*, *Acetobacter xylinum*, *Acetobacter aceti*, *Saccharomyces cerevisiae*, *Zygosaccharomyces bailii*, *Zygosaccharomyces* spp., and *Gluconacetobacter* (Cuamatzin-García et al. 2022).

Several studies have demonstrated that kombucha exhibits significant therapeutic effects as an antioxidant, anti-inflammatory, anticancer, and antimicrobial agent. Moreover, it possesses the ability to bolster the immune system and prevent various diseases, including diabetes, hypertension, and cardiovascular diseases (Kitwetcharoen et al. 2023). As a fermented beverage, kombucha is a rich source of nutrients and phytonutrients, and thus, it holds the potential for further development (Ferruzzi et al. 2020). The exploration of kombucha's health-beneficial effects as a beverage is ongoing. Additionally, it is noteworthy that kombucha is no longer exclusively derived from tea; it can also be produced using other basic ingredients, such as strawberries. In a systematic review study, strawberries have several functional metabolites as a functional food (Basu et al. 2014). In addition, strawberries also contain various biologically active non-nutrient compounds, mainly represented by polyphenolic phytochemicals (Giampieri et al. 2017). These strawberry phenolics have wide clinical potential for humans as an antioxidant, anti-inflammatory action, and inhibition of metabolic enzymes and receptors, alleviating oxidative stress-related conditions (Afrin et al. 2016).

Recently, kombucha is well-known as a beverage made through the fermentation process of tea and sugar with SCOBY (Villarreal-Soto et al. 2018). Recent studies have shown that because of fermentation, kombucha drink has anti-inflammatory, antioxidant, antidiabetic, cholesterol-lowering, and hepatoprotective effects (Kapp and Sumner 2019; Júnior et al. 2022). In addition, multiple studies have consistently shown that the chemical properties of fermented beverages are enhanced compared to unfermented beverages (Jafari et al. 2020; Zofia et al. 2020; Değirmencioğlu et al. 2021). Incorporating strawberries (*Fragaria ananassa*) into a kombucha using the SCOBY fermentation method is expected to increase its bioactive properties. This study aimed to investigate bioactive compounds of strawberry kombucha using liquid chromatography high-resolution mass spectrometry (LC-HRMS) and its effect on the modulation of the immune system related anti-oxidative (DPPH and ABTS), markers of metabolic disorders (enzymes activity: lipase, alpha-amylase, alpha-glucosidase,) through *in vitro* enzymatic studies and biochemical analysis, as well as molecular docking approach.

Materials and methods

Formulation of strawberry kombucha drink (SKD)

Strawberry (*Fragaria ananassa*; *Fragaria* X *ananassa* ssp. *ananassa*; Integrated Taxonomic Information System – Report and Taxonomic Serial Number: 837344) collected from Sarangan Strawberry Garden, Raya Sarangan Street No.47, Plaosan III, Sarangan, Plaosan District, Magetan Regency, East Java 63361, Indonesia (Google Maps Coordinates = -7.6743967, 111.2308193). Plant species were identified at the Biochemistry and Biomolecular Laboratory, Brawijaya University, Malang, Indonesia. Specimens were collected for future validation. The characteristic of strawberry fruit harvested and used is the fruit derived from the strawberry plant aged 2.5 months and has a chewy-tender texture when held; the skin is dark red, and the stalk is yellowish brown. To maintain its quality, strawberry is kept in a refrigerator with a temperature of 4–8 °C before being fermented into a kombucha using SCOBY. This method adopted the well-established protocol published in other papers (Permatasari et al. 2021, 2022).

Furthermore, the SKD drink was formulated using 2,000 mL of water, 24 g of strawberry pulp, 300 g of white sugar, 166 g v/v of SCOBY starter solution, and 10 g of SCOBY gel, all contributing to a total volume of 2,500 mL. The production was initiated by boiling 2 L of water (±80 °C) followed by the addition of 300 g of table sugar. The mixture was stirred until homogenous and then added with 24 g of strawberry flesh. After that, the water was stirred until the color turns dark brownish red, turn off the stove heat, cover the pot, and let it cool. The solution was then poured into a sterile 3 L jar along with the SCOBY starter solution and SCOBY gel. A clean gauze was placed over and tied to the bottle; then the bottle was kept in anaerobic conditions at 20–25 °C for 12 days. Right after the fermentation process finished, all beverage samples were kept in a 4–8 °C refrigerator for further studies.

In vitro enzymatic parameters

Assay for lipase inhibition (%)

First, 1 mg/mL crude porcine pancreatic lipase (PPL) was solubilized in a 50 mM phosphate buffer, followed by the removal of insoluble materials through centrifugation (12,000 g). The process was then continued with the addition of buffer to the supernatants, resulting in a 10-times dilution.

The potential inhibition of lipase was determined using the method utilized by Permatasari et al. 2022. As much as 100 µL of SKD at all concentrations (50, 100, 150, 200, and 250 µg/mL along with 20 µL 10 mM p-nitrophenyl butyrate were added into the reaction buffer in a clear 96-well microplate and incubated for 10 min at 37 °C. The result was contrasted with the positive control (orlistat). The absorbance values were determined using a microplate reader at 405 nm. The unit of activity was calculated using the yield resulting from a one-minute reaction rate of 1 mol p-nitrophenol at 37 °C. When PPL activity was incubated in the test combination, the reduction percentage was used to calculate the inhibition activity of lipase inhibition. To ensure that the findings of the study are accurate, each sample was verified three times (in triplicate). The inhibitory data were obtained using the equation below:

$$Inhibition\ of\ lipase\ activity = 100 - \frac{(B - B_c)}{(A - A_c)} \times 100\%$$

A = Lipase inhibition activity without any inhibitor; Ac = Negative control without any inhibitor; B = Lipase inhibition activity with inhibitor; Bc = Negative control with inhibitor.

Determining the α-amylase inhibition (%)

Diluted SKD (at all concentrations) were incubated for 10 min at room temperature with 500 L of 0.02 M pH 6.9 Na_3PO_4 buffer, NaCl 0.006 M, and porcine pancreatic amylase 0.5 mg/mL. Then, each mixture in the assay buffer received 500 L of a 1% starch solution. After 10 minutes of incubation at 25 °C, 3,5-dinitrosalicylic acid was added to complete the process (1.0 mL). After 5 minutes in a 100 °C water bath, the test was continued and allowed to cool at 22 °C. The measurements were diluted with distilled water (10 mL) to make them readable within the permissible range (540 nm). Acarbose served as the positive control in this investigation. Enzymes and reagents are included in the reference sample but not the sample itself. This method referred to a similar methodology in Permatasari et al. 2022.

Investigation of α-glucosidase inhibition (%)

1.52 UI/mL α-glucosidase solution was created by combining 1 mg (76 UI) of the enzyme with 50 mL pH 6.9 of phosphate buffer, which was proceeded to be kept at -20 °C. Then, 0.35 mL each of the sucrose (65 mM), maltose solution (65 mM), and SKD (0.1 mL) at 50, 100, 150, 200, and 250 g/mL were added. Prior to adding the α-glucosidase solution (0.2 mL), the system was heated to 37 °C for 5 min and maintained at that temperature for 15 min. In a 100 °C water bath, the system was warmed up for 2 minutes. Acarbose was employed in this investigation as the positive control under identical conditions as SKD. The testing solution (0.2 mL), coloring reagent (3 mL), and α-glucosidase inhibitory test solution were then sequentially combined. After 5 minutes of system warming at 37 °C, the system was then evaluated at 505 nm absorbance. The amount of glucose produced during the response served as a sign of inhibitory activity.

DPPH inhibition activity assay (%)

The determination of antioxidant activity was based on the inhibition of DPPH, referring to Kaur et al. (Kaur et al. 2021). Glutathione (Sigma-Aldrich) was chosen as a positive control. The samples and the control (at all concentrations) were poured into the testing vials, followed by the addition of DPPH reagent (3 mL). The resulting DPPH extract mixture was then left undisturbed (30 min; dark cycle). The change was determined on 517 nm absorbance. The proportion of inhibition of DPPH was expressed and calculated using the following formula:

$$\%DPPH\ inhibition = \frac{A0 - A1}{A0} \times 100\%$$

A0 = Absorbance value of blank; A1 = Absorbance value of standard or sample.

The half maximal effective concentration (EC_{50}), a concentration measurement of a sample that results in a 50% reduction in the starting radical concentration, expressed the radical scavenging ability of SKD and GSH.

Quantification of the radical scavenging activity of ABTS (%)

The scavenging capability of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) was assessed with the protocol adhering to (Sancho et al. 2013). Potassium persulfate (2.4 mM) and 7 mM ABTS were mixed in a 1:1 ratio to form the stock solution. Aluminum foil was used to block the light from the combination, and it was then left to react for 14 hours at 22 °C. Afterward, 1 mL of ABST stock solution was added with 60 mL of ethanol to get an absorbance of 0.706 at 734 nm. For each test, a brand-new functioning solution was created. After allowing the samples at all concentrations to react for 7 minutes with 1 mL of the ABTS working solution, the absorbance at 734 nm was measured. As a positive control, Trolox was employed.

$$\%ABTS\ radical\ scavenging\ activity = \frac{A0 - A1}{A0} \times 100\%$$

A0 = Absorbance value of blank; A1 = Absorbance value of standard or sample.

The half maximal effective concentration (EC_{50}) is the amount of sample concentration that reduces the concentration of radical levels to 50% reduction in the starting radical concentration, which expressed the radical scavenging ability of SKD and Trolox.

Untargeted metabolomic profiling of strawberry kombucha drink

Testing service at the Central Laboratory of Life Sciences, Brawijaya University, Malang, Indonesia) was utilized to analyze an untargeted metabolomic profile test on SKD using the combination of a high-performance liquid chromatography system with a high-resolution mass spectrometer (LC-HRMS) as described according to the manufacturer's specification (Suppl. material 1). The concentration of 50 µl of SKD was reduced by 30 times using ethanol (96%) and vortexed (at 2,000 rpm for 2 min), followed by centrifugation (at 6,000 rpm for 2 min). The supernatants were accumulated and then filtrated using a 0.22 µm syringe filter prior to analysis.

Thermo Scientific Dionex Ultimate 3000 RSLC Nano High-Performance Liquid Chromatography (HPLC) and a micro-flow meter made up the LC-HRMS system. The analytical column was a Hypersil GOLD aQ with a particle size of 50 × 1 mm × 1.9 maintained at 30 °C, and the solvents A and B are 0.1% formic acid and 0.1% formic acid dissolved in water and acetonitrile, respectively. Next, they were kept apart with a 40 uL/min linear gradient for 30 minutes. HRMS utilized Thermo Scientific Q Exactive which has 70,000 resolution at its full scan capacity, a 17,500 resolution data-dependent MS2, and a 30 minute operating period in positive and negative modes. The successfully identified compounds were then analyzed *in silico* against the human pancreatic lipase (1LPB), α-glucosidase (2QV4), and α-amylase (3L4Y) enzymes.

Molecular docking simulation

Hardware and software

ASUS Vivobook M413ia – Ek502t with AMD Ryzen 5 4500u (2.3 GHz) processor, 8GB DDR4 memory, 512 GB SSD M.2 storage, and Windows 10 Home operating system was equipped with ChemDraw Ultra 12.0, AutoDock tools (version 4.2), and BIOVIA Discovery software. The website of Protein Data Bank (https://www.rcsb.org) and PubChem structure database (https://pubchem.ncbi.nlm.nih.gov) was also used in this study.

Preparing ligands and targets

The compounds that were identified as a constituent of the SKD metabolomic profile were used as test ligands. ChemDraw Ultra 12.0 was used to sketch the whole structure in 2D, which was then transformed to 3D and optimized using the MM2 algorithm. The selected target proteins were human pancreatic lipase (PDB ID: 1LPB), α-amylase (PBD ID: 2QV4), and α-glucosidase (PDB ID: 3L4Y). All proteins were acquired from the website of Protein Data Bank (https://www.rcsb.org). Kollman charges were applied to the receptors while the ligands were added with a Gasteiger charge.

Validation of molecular docking

Redocking was used as the molecular docking validation approach. By utilizing AutoDock tools version 4.2, the original ligand was transferred to the target pocket with specific coordinates. After the re-docking method, the ligand position's RMSD (root-mean-square deviation) must be less than 2.0 Å.

Simulation of molecular docking

The docking parameters were developed using the findings of docking validation (Table 1). The outcome was recorded in a *dlg file for each docking's final conformation structure. Analysis was done on the ligand-receptor interaction using Discovery Studio 2016.

Statistical analysis

In the early phase of the study, *in vitro* data regarding antioxidants (DPPH and ABTS) were analyzed using an unpaired T-test CI 95% with the Windows version of GraphPad Prism 9.4.1 software (San Diego, California USA, www.graphpad.com). EC_{50} datasets were each acquired from nonlinear regression models. GraphPad Prism 9.4.1 was used to present the graphic visualizations.

Results and discussion

Metabolite profile of strawberry kombucha drink (SKD)

A total of 45 compounds were identified in SKD (Table 2). The majority of these compounds exhibited several health benefits, ranging from antioxidant, neuroprotective, and hepatoprotective to hypolipidemic and protection against cardiometabolic risk factors. The identification produced a spectrum that may be compared to those in the database by combining electrospray ionization and Fourier processing (Fig. 1A). An electrospray positive weak peak with 1.80×10^6 counts is transformed into an appropriate spectrum (m/z 50–750 Da) (Fig. 1B). Table 1 lists the detected chemicals in detail based on the findings of non-targeted metabolomic profiling using LC-HRMS.

In silico study of α-amylase, α-glucosidase, and lipase inhibitory activities

As shown in Table 2, identified compounds of SKD were validated by computational *in silico* or molecular docking assays on the enzymes lipase, a-glucosidase, and a-amylase. After the validation process was done, the molecular docking tests against lipase, α-amylase, and α-glucosidase enzymes were performed on 47 compounds (due to the availability of the databases), acarbose (as the control for α-amylase and α-glucosidase), and orlistat (as a control for lipase). This examination found that 1-Methyl-N-{[(2R,4S,5R)-5-(2-methyl-6-phenyl-4-pyrimidinyl)-1-azabicyclo[2.2.2]oct-2-yl]methyl}-4-piperidinamine had the best result based on the ligand test in each receptor when compared with other compounds and controls. This comparison is based on the value of ΔG (kcal/mol), and the results of molecular docking tests were listed in Table 3.

Table 1. Identified Compounds from Untargeted Metabolomic Profiling of SKD.

No	Name	Formula	Calculated MW	RT (min)	Area (Max)	mzCloud Best Match
1	D-(+)-Maltose	$C_{12}H_{22}O_{11}$	364.09682	0.926	7,110,242,090.90	91.4
2	5-({[3-chloro-5-(trifluoromethyl)-2-pyridyl]methyl}thio)-4-pentyl-4H-1,2,4-triazol-3-ol	$C_{14}H_{16}ClF_3N_4OS$	380.07057	0.946	5,481,884,554.95	64.2
3	2-[3-methyl-2-(methylimino)-4-oxo-1,3-thiazolan-5-yl]acetic acid	$C_7H_{10}N_2O_3S$	202.0445	0.904	5,110,631,469.12	70.5
4	Caffeine	$C_8H_{10}N_4O_2$	194.07955	4.698	2,002,924,546.31	99.4
5	Diisobutylphthalate	$C_{16}H_{22}O_4$	278.15074	17.77	924,753,800.01	99.2
6	5-Hydroxymethyl-2-furaldehyde	$C_6H_6O_3$	126.0313	1.137	431,873,514.06	92.3
7	Pentane-1,2,3,4,5-pentol	$C_5H_{12}O_5$	174.04979	0.903	376,514,195.88	97.3
8	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390.2755	23.05	299,992,693.45	99.7
9	NP-020014	$C_{15}H_{26}O_3$	276.17153	13.32	281,454,788.30	68.9
10	Isobutyraldehyde	C_4H_8O	72.0577	1.358	157,345,987.19	72.7
11	Dibenzylamine	$C_{14}H_{15}N$	197.11978	7.282	132,075,688.98	99.0
12	DL-Stachydrine	$C_7H_{13}NO_2$	143.09406	0.953	126,807,825.53	93.4
13	Monobutyl phthalate	$C_{12}H_{14}O_4$	222.08857	17.79	97,834,005.78	97.0
14	Theobromine	$C_7H_8N_4O_2$	180.06409	2.037	71,557,968.75	99.0
15	D-Lactose monohydrate	$C_{12}H_{22}O_{11}$	342.11505	0.826	33,730,046.47	81.7
16	3-hydroxy-3-methylpentanedioic acid	$C_6H_{10}O_5$	184.03409	0.927	63,255,525.11	77.3
17	D-Raffinose	$C_{18}H_{32}O_{16}$	526.14952	0.836	59,525,278.77	89.6
18	(-)-Epicatechin	$C_{15}H_{14}O_6$	290.07784	4.541	53,638,661.97	99.1
19	Mevalonolactone	C6 H10 O3	130.06259	1.488	49,822,362.80	64.3
20	(1S,4aS,7aS)-7-({[(2E)-3-phenylprop-2-enoyl]oxy}methyl)-1-{[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy}-1H,4aH,5H,7aH-cyclopenta[c]pyran-4-carboxylic acid	$C_{25}H_{28}O_{11}$	542.1234	0.846	48,709,783.20	93.1
21	NP-000358	$C_{15}H_{14}O_7$	306.07302	4.063	29,746,512.44	99.2
22	Tributyl phosphate	$C_{12}H_{27}O_4P$	266.16385	16.37	39,966,286.39	99.9
23	NP-013538	$C_{12}H_{16}O_8$	288.08342	1.126	36,462,233.46	68.9
24	Methylimidazoleacetic acid	$C_6H_8N_2O_2$	140.05799	1.061	35,722,013.31	67.2
25	4-Coumaric acid	$C_9H_8O_3$	164.04686	4.786	34,660,580.43	99.0
26	DEET	$C_{12}H_{17}NO$	191.13039	11.61	32,410,930.57	98.5
27	2,2,6,6-Tetramethyl-1-piperidinol (TEMPO)	$C_9H_{19}NO$	157.1462	12.23	30,810,168.71	91.9
28	Bis(3,5,5-trimethylhexyl) phthalate	$C_{26}H_{42}O_4$	418.30702	16.9	30,141,921.96	98.3
29	n-Pentyl isopentyl phthalate	$C_{18}H_{26}O_4$	323.20864	17.77	29,880,782.76	87.0
30	3,5-di-tert-Butyl-4-hydroxybenzaldehyde	$C_{15}H_{22}O_2$	234.16115	16.81	28,572,320.75	99.6
31	Caprolactam	$C_6H_{11}NO$	113.08388	3.472	25,998,433.02	96.4
32	3,5-di-tert-Butyl-4-hydroxybenzoic acid	$C_{15}H_{22}O_3$	250.15625	14.79	19,609,117.25	95.6
33	Sulcatol	$C_8H_{16}O$	128.11986	14.1	19,080,856.60	67.7
34	N,N-Diisopropylethylamine (DIPEA)	$C_8H_{19}N$	129.15138	4.608	18,332,420.83	76.4
35	4-(2,3-dihydro-1,4-benzodioxin-6-yl)butanoic acid	$C_{12}H_{14}O_4$	244.07048	13.01	17,707,359.41	71.1
36	Tetranor-12(S)-HETE	$C_{16}H_{26}O_3$	248.17677	16.82	17,690,476.85	72.3
37	3-(2-Hydroxyethyl)indole	$C_{10}H_{11}NO$	161.08358	8.054	17,099,586.02	91.2
38	(2R,3R)-5,7-dihydroxy-2-(3,4,5-trihydroxyphenyl)-3,4-dihydro-2H-1-benzopyran-3-yl 3,4,5-trihydroxybenzoate	$C_{22}H_{18}O_{11}$	458.08388	5.77	16,420,630.11	99.3
39	Rutin	$C_{27}H_{30}O_{16}$	610.15239	6.861	16,318,729.84	99.4
40	Choline	$C_5H_{13}NO$	103.09958	1.04	16,193,389.87	97.0
41	α-Pyrrolidinopropiophenone	$C_{13}H_{17}NO$	203.13042	16.48	14,091,680.31	88.7
42	Hesperidin	$C_{28}H_{34}O_{15}$	610.18846	7.79	13,481,790.12	96.8
43	benzyl N-(1-{[(3,4-dimethoxyphenethyl)amino]carbonyl}-2-methylpropyl)carbamate	$C_{23}H_{30}N_2O_5$	452.17772	8.209	13,379,568.24	94.6
44	(2E)-3-(2-{[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy}phenyl)prop-2-enoic acid	$C_{15}H_{18}O_8$	348.08101	4.757	12,724,287.81	93.3
45	Vanillin	$C_8H_8O_3$	152.04688	6.072	11,781,792.86	89.0
46	3,4-Dihydroxybenzaldehyde	$C_7H_6O_3$	138.03116	5.544	5,298,795.15	79.2
47	6-Methyl-2-pyridinemethanol	C_7H_9NO	123.0681	1.26	10,839,550.92	80.6
48	3,4-Dihydroxyphenylpropionic acid	$C_9H_{10}O_4$	164.04688	13.31	10,660,470.60	94.5
49	N-Cyclohexyl-N-methylcyclohexanamine	$C_{13}H_{25}N$	195.19823	7.233	10,459,159.69	90.4
50	7-Oxobenz[de]anthracene	$C_{17}H_{10}O$	230.07582	1.272	10,388,291.21	75.3
51	Levalbuterol	$C_{13}H_{21}NO_3$	261.13573	17.34	10,251,911.95	93.1
52	N-Octyl-2-pyrrolidone	$C_{12}H_{23}NO$	197.17739	15.25	10,113,937.91	73.5
53	Catechin gallate	$C_{22}H_{18}O_{10}$	442.08901	6.98	10,030,569.60	99.4
54	Hexamethylenetetramine	$C_6H_{12}N_4$	140.10565	26.42	9,238,125.20	92.3
55	1-Methyl-N-{[(2R,4S,5R)-5-(2-methyl-6-phenyl-4-pyrimidinyl)-1-azabicyclo[2.2.2]oct-2-yl]methyl}-4-piperidinamine	$C_{25}H_{35}N_5$	427.27715	2.762	9,038,413.78	90.3

No	Name	Formula	Calculated MW	RT (min)	Area (Max)	mzCloud Best Match
56	4-Aminophenol	C_6H_7NO	109.05258	1.251	8,177,405.86	77.9
57	3-{[(2S,3R,4S,5R,6R)-3,5-dihydroxy-6-(hydroxymethyl)-4-{[(2S,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methyloxan-2-yl]oxy}oxan-2-yl]oxy}-5,7-dihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one	$C_{27}H_{30}O_{15}$	594.15752	7.399	7,731,006.18	98.2
58	(2S)-7-{[(2S,3R,4S,5S,6R)-4,5-dihydroxy-6-(hydroxymethyl)-3-{[(2S,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methyloxan-2-yl]oxy}oxan-2-yl]oxy}-2-(3,4-dihydroxyphenyl)-5-hydroxy-3,4-dihydro-2H-1-benzopyran-4-one	$C_{27}H_{32}O_{15}$	596.17308	6.736	7,698,163.80	96.6
59	L-(+)-Citrulline	$C_6H_{13}N_3O_3$	394.15033	4.327	7,590,020.04	77.2
60	Sunitinib	$C_{22}H_{27}FN_4O_2$	420.19603	6.077	7,501,984.66	84.0
61	(-)-Caryophyllene oxide	C15 H24 O	220.1819	9.887	6,306,915.32	68.6
62	Nicotinamide	C6 H6 N2 O	122.04772	1.247	4,680,656.48	61.4

Table 2. Validation of molecular docking simulation.

No	Drug Target	PDB ID	Docking Site (x;y;z)	Docking Area (x.y.z)	RMSD (Å)	ΔG (kcal/mol)	Number in Cluster (/100)	Judgement (<2Å)
1	Human Pancreatic ***Lipase***	1LPB	4.448, 27.955, 49.675	40×40×40	1.89	-6.70	25	Valid
2	Human Pancreatic ***α-Amylase***	2QV4	12.942, 47.170, 26.200	42×40×40	1.77	-9.60	22	Valid
3	Human Pancreatic ***α-Glucosidase***	3L4Y	-1.542, -19.201, -21.043	42×40×40	1.53	-5.23	33	Valid

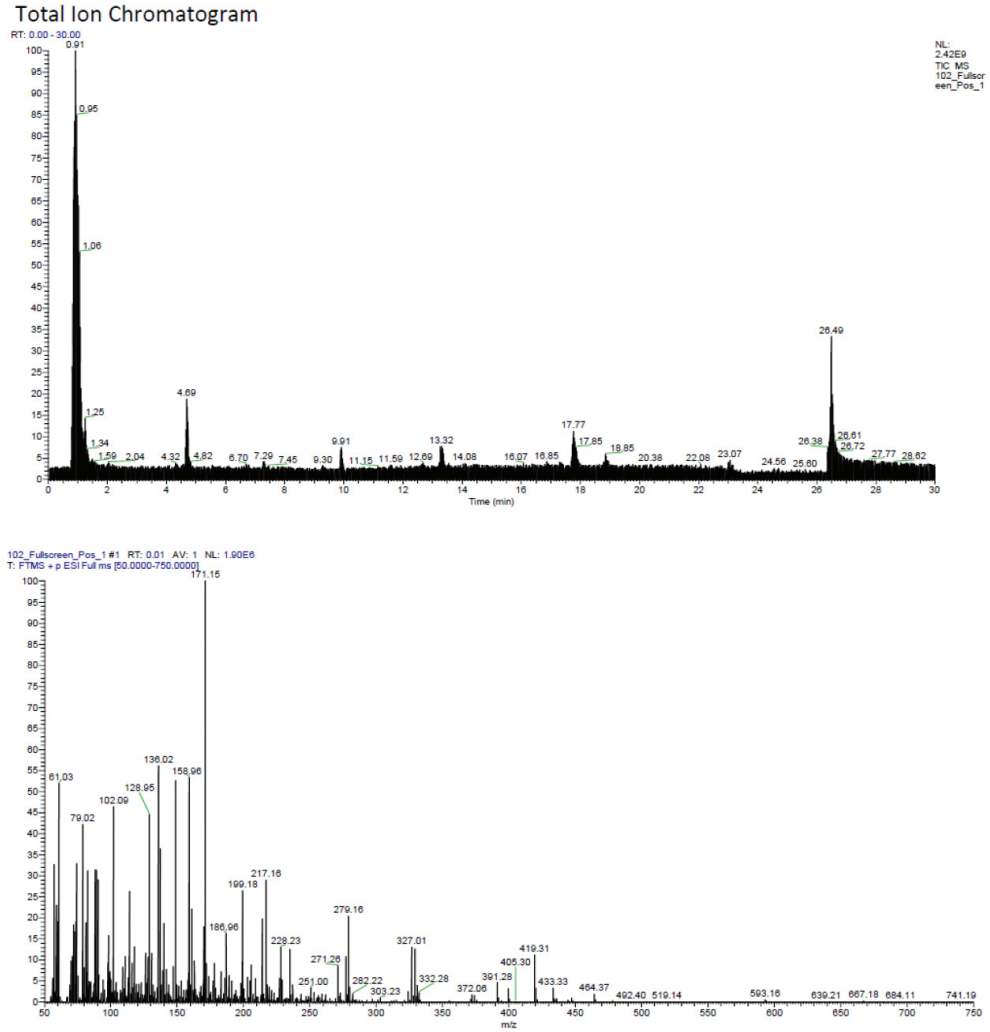

Figure 1. Total Ion Chromatogram LC-MS of Strawberry Kombucha Drink. Total ion chromatogram (ESI +) and the LC-MS metabolite profiles of SKD (**A**). Positive ion mass spectra (FTMS-ESI (+)) of the m/z range 50–750 of SKD (**B**). S#: Number of scans; RT: Retention time; AV: Averaged number of scans; SB: Subtracted (followed by subtraction information); NL: Neutral loss; T: Scan type; F: Scan filter.

Table 3. Molecular Docking Parameter of Identified Compounds of SKD.

No.	Substance	Number in Cluster (/100)			1G (kcal/mol)			Ki		
		1LPB	2QV4	3L4Y	1LPB	2QV4	3L4Y	1LPB	2QV4	3L4Y
1	Orlistat	5			–2.42			5.44 mM		
2	Acarbose		13	13		–4.22	–1.01		38.46 µM	3.76 mM
3	D-(+)-Maltose	90	23	24	-3,44	-3,22	-2,43	246.63 uM	210.11 uM	725.66 uM
4	5-({[3-chloro-5-(trifluoromethyl)-2-pyridyl]methyl}thio)-4-pentyl-4H-1,2,4-triazol-3-ol	67	81	9	-7,94	-6,66	-5,18	492.27 nM	4.04 uM	97.00 uM
5	2-[3-methyl-2-(methylimino)-4-oxo-1,3-thiazolan-5-yl] acetic acid	50	40	94	-4,45	-3,3	-3,71	287.10 uM	2.65 mM	1.51 mM
6	Caffeine	100	100	100	-5,07	-4,17	-4,25	185.64 uM	883.60 uM	731.38 uM
7	Diisobutylphthalate	38	99	88	-5,89	-4,37	-4,09	7.59 uM	378.61 uM	366.15 uM
8	Dibenzylamine	98	79	69	-6,11	-7,09	-7,48	28.29 uM	3.44 uM	1.94 uM
9	DL-Stachydrine	92	62	73	-4,25	-3,37	-4,08	687.26 uM	2.55 mM	916.46 uM
10	Monobutyl phthalate	40	43	49	-4,95	-3,14	-2,76	86.18 uM	2.92 mM	3.46 mM
11	Theobromine	90	100	93	-4,92	-4,33	-3,99	246.05 uM	667.24 uM	1.16 mM
12	D-Lactose monohydrate	80	35	42	-3,11	-2,9	-1,65	466.97 uM	716.25 uM	9.15 mM
13	3-hydroxy-3-methylpentanedioic acid	77	84	47	-1,79	-1,17	-0,63	20.80 mM	25.49 mM	65.57 mM
14	D-Raffinose	95	17	18	1,91	-2,5	-0,29	21.32 mM	1.11 mM	51.82 mM
15	(-)-Epicatechin	100	100	59	-8,68	-6,58	-5,72	350.40 nM	6.17 uM	25.33 uM
16	(1S,4aS,7aS)-7-({[(2E)-3-phenylprop-2-enoyl]oxy}methyl)-1-{[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy}-1H,4aH,5H,7aH-cyclopenta[c]pyran-4-carboxylic acid	20	16	28	-0,75	-4,41	-3,62	64.12 uM	80.72 uM	74.13 uM
17	Tributyl phosphate	96	45	40	-4,6	-2,78	-2,71	122.83 uM	3.27 mM	3.93 mM
18	Methylimidazoleacetic acid	77	46	82	-3,44	-2,17	-2,56	2.23 mM	23.10 mM	6.16 mM
19	4-Coumaric acid	78	100	64	-4,5	-4,06	-3,09	497.84 uM	905.62 uM	5.08 mM
20	2,2,6,6-Tetramethyl-1-piperidinol (TEMPO)	100	75	50	-5,57	-4,76	-5,48	81.88 uM	325.17 uM	89.09 uM
21	Bis(3,5,5-trimethylhexyl) phthalate	41	12	13	-4,98	-4,68	-4,04	1.60 uM	95.96 uM	151.73 uM
22	3,5-di-tert-Butyl-4-hydroxybenzaldehyde	48	96	80	-6,5	-5,54	-5,32	15.49 uM	73.08 uM	98.98 uM
23	3,5-di-tert-Butyl-4-hydroxybenzoic acid	72	59	54	-6,11	-4,56	-3,68	26.28 uM	289.18 uM	1.56 mM
24	Sulcatol	49	100	35	-4,61	-3,94	-4,49	278.89 uM	1.04 mM	256.04 uM
25	N,N-Diisopropylethylamine (DIPEA)	100	100	100	-3,74	-4,24	-6,00	1.55 mM	680.93 uM	23.19 uM
26	4-(2,3-dihydro-1,4-benzodioxin-6-yl)butanoic acid	62	88	32	-5,4	-4,18	-3,38	89.41 uM	605.65 uM	2.40 mM
27	Tetranor-12(S)-HETE	40	11	20	-4,56	-2,97	-3,01	55.34 uM	628.46 uM	296.13 uM
28	3-(2-Hydroxyethyl)indole	82	75	56	-5,95	-4,96	-5,26	39.70 uM	212.49 uM	84.40 uM
29	(2R,3R)-5,7-dihydroxy-2-(3,4,5-trihydroxyphenyl)-3,4-dihydro-2H-1-benzopyran-3-yl 3,4,5-trihydroxybenzoate	52	40	25	-5,48	-6,75	-6,7	1.09 uM	2.27 uM	222.88 nM
30	Rutin	11	20	16	27,59	-4,87	-5,94	–	43.93 uM	2.77 uM
31	Choline	86	100	56	-3,41	-3,91	-5,43	2.00 mM	1.15 mM	72.44 uM
32	α-Pyrrolidinopropiophenone	53	66	91	-6,59	-6,61	-7,3	13.40 uM	12.51 uM	2.22 uM
33	Hesperidin	49	23	8	37,35	-6,93	-3,95	–	189.65 nM	144.03 uM
34	benzyl N-(1-{[(3,4-dimethoxyphenethyl)amino]carbonyl}-2-methylpropyl)carbamate	31	25	22	-5,74	-4,86	-5,61	8.47 uM	66.17 uM	9.42 uM
35	(2E)-3-(2-{[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy}phenyl)prop-2-enoic acid	27	58	23	-3,66	-4,06	-1,86	1.00 mM	268.73 uM	30.25 mM
36	Vanillin	52	61	58	-4,72	-3,85	-5,1	304.63 uM	1.30 mM	124.12 uM
37	3,4-Dihydroxybenzaldehyde	100	89	60	-5,73	-4,7	-5,21	59.00 uM	319.73 uM	115.44 uM
38	3,4-Dihydroxyphenylpropionic acid	74	81	49	-4,93	-3,9	-2,9	78.86 uM	634.27 uM	4.99 mM
39	Levalbuterol	55	43	45	-5,06	-6,41	-7,89	63.50 uM	6.38 uM	298.18 nM
40	Catechin gallate	75	43	35	-5,32	-6,98	-7,58	6.83 uM	1.10 uM	382.96 nM
41	1-Methyl-N-{[(2R,4S,5R)-5-(2-methyl-6-phenyl-4-pyrimidinyl)-1-azabicyclo[2.2.2]oct-2-yl]methyl}-4-piperidinamine	94	32	60	-8,73	-10,74	-13,6	186.36 nM	2.45 nM	61.95 pM
42	4-Aminophenol	100	67	78	-4,09	-3,36	-4,36	984.59 uM	3.32 mM	534.15 uM
43	3-{[(2S,3R,4S,5R,6R)-3,5-dihydroxy-6-(hydroxymethyl)-4-{[(2S,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methyloxan-2-yl]oxy}oxan-2-yl]oxy}-5,7-dihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one	28	20	17	43,81	-4,91	-5,3	–	55.56 uM	12.10 uM
44	(2S)-7-{[(2S,3R,4S,5S,6R)-4,5-dihydroxy-6-(hydroxymethyl)-3-{[(2S,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methyloxan-2-yl]oxy}oxan-2-yl]oxy}-2-(3,4-dihydroxyphenyl)-5-hydroxy-3,4-dihydro-2H-1-benzopyran-4-one	17	19	18	33,13	-5,61	-5,04	–	7.58 uM	41.59 uM
45	L-(+)-Citrulline	53	56	47	-2,87	-4,11	-4,72	1.35 mM	99.10 uM	94.18 uM
46	(-)-Caryophyllene oxide	100	68	74	-6,99	-6,09	-5,49	7.51 uM	34.23 uM	86.43 uM
47	Nicotinamide	99	99	55	-4,56	-4,27	-4,86	435.81 uM	682.74 uM	262.66 uM

Lipase inhibition activity by SKD

The inhibition of lipase by SKD and orlistat are detailed in Fig. 2. Statistical analysis revealed that orlistat showed a significantly higher inhibition against lipase than SKD at the dose of 50 µg/mL (Fig. 2A). Interestingly, the lipase inhibition activity of SKD at 100, 150, 200, and 250 µg/mL were equal to orlistat. However, further validation through the analysis of EC_{50} values of SKD and orlistat concluded that the lipase inhibition of orlistat is without a doubt stronger than SKD (Fig. 2B).

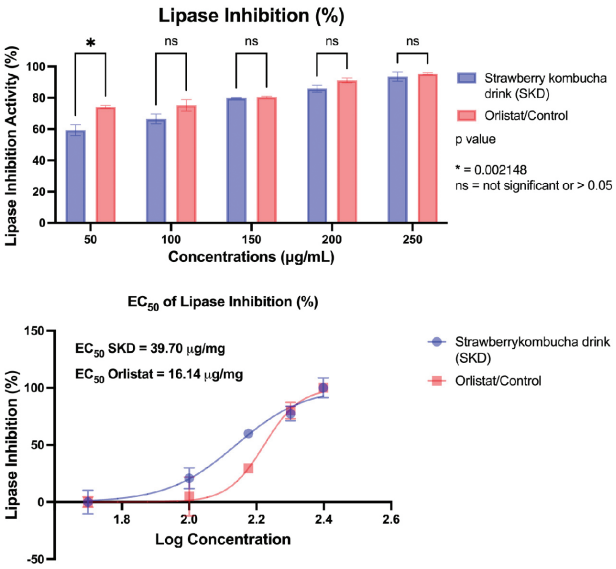

Figure 2. Lipase Inhibition Activity Test of SKD and Orlistat. The inhibition of lipase was presented in % activity (**A**) and EC_{50} value (**B**).

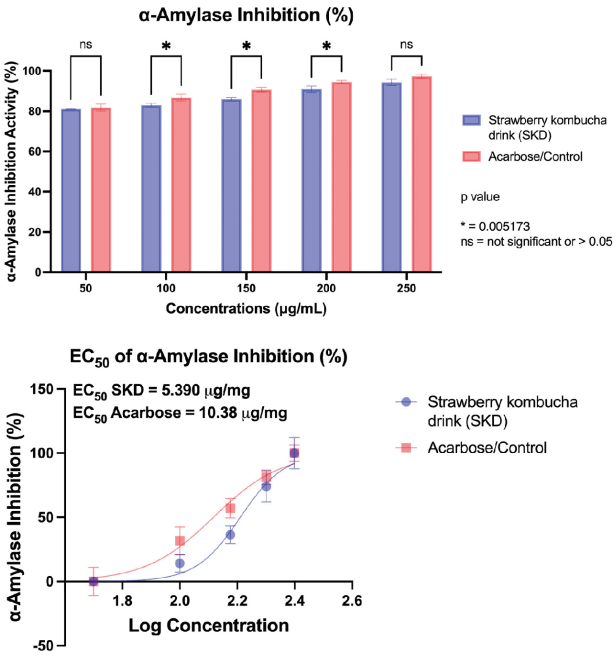

Figure 3. α-Amylase Inhibition Activity Test of SKD and Acarbose. The inhibition of α-amylase was presented in % activity (**A**) and EC_{50} value (**B**).

α-Amylase inhibition activity of SKD

Fig. 3 showed the α-amylase inhibition activity by SKD and acarbose. An α-amylase inhibition activity that was equal to acarbose was observed in SKD at 50 and 250 µg/mL, which are the lowest and highest doses (Fig. 3A). However, at 100, 150, and 200 µg/mL, the α-amylase inhibition property of SKD differs significantly from orlistat. The EC_{50} value of SKD was also lower than orlistat, suggesting greater α-amylase inhibition than the positive control (Fig. 3B).

α-Glucosidase inhibition activity by SKD

The antidiabetic property of SKD was compared to acarbose, which was shown in Fig. 4. The potential of SKD in inhibiting α-glucosidase was identical to acarbose at 50, 150, and 250 µg/mL while on the other doses, they differ notably from acarbose (Fig. 4A). The EC_{50} value of SKD and acarbose were 15.03 µg/mg and 10.62 µg/mg, respectively (Fig. 4B).

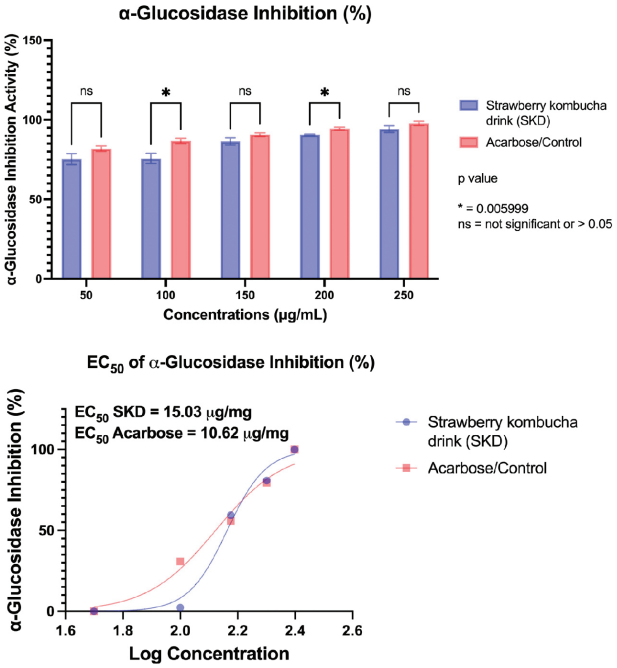

Figure 4. α-Glucosidase Inhibition Activity Test of SKD and Acarbose. The inhibition of α-glucosidase was presented in % activity (**A**) and EC_{50} value (**B**).

Free radical scavenging activity of SKD

The free radical scavenging activity of SKD was observed through the inhibition of DPPH and ABTS, which were compared against glutathione (Figs 5, 6). The statistical approach found that SKD showed a significantly lower DPPH inhibition than glutathione at the dose of 50 and 100 µg/mL (Fig. 5A). Interestingly, the free radical scavenging activity of SKD at 150, 200, and 250 µg/mL was equal to the control. On the other side, the ABTS inhibition activity of all doses of SKD still fell short of the

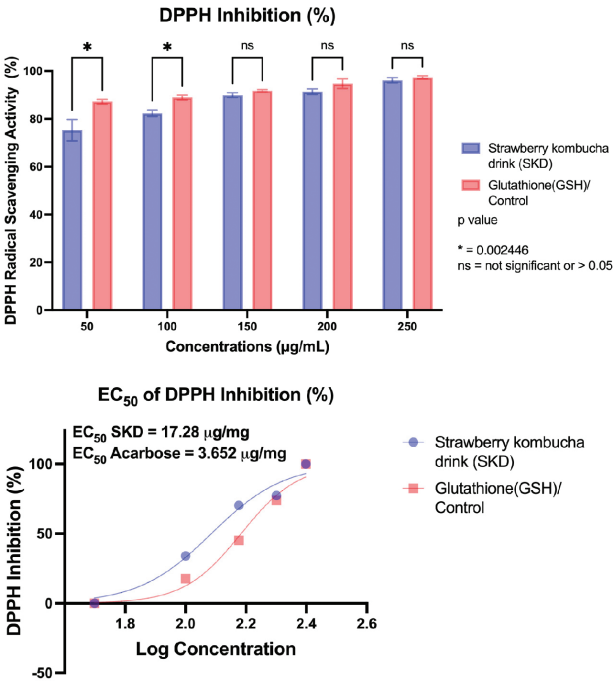

Figure 5. DPPH Inhibition Activity Test of SKD and Glutathione. The inhibition of DPPH was presented in % activity (**A**) and EC_{50} value (**B**).

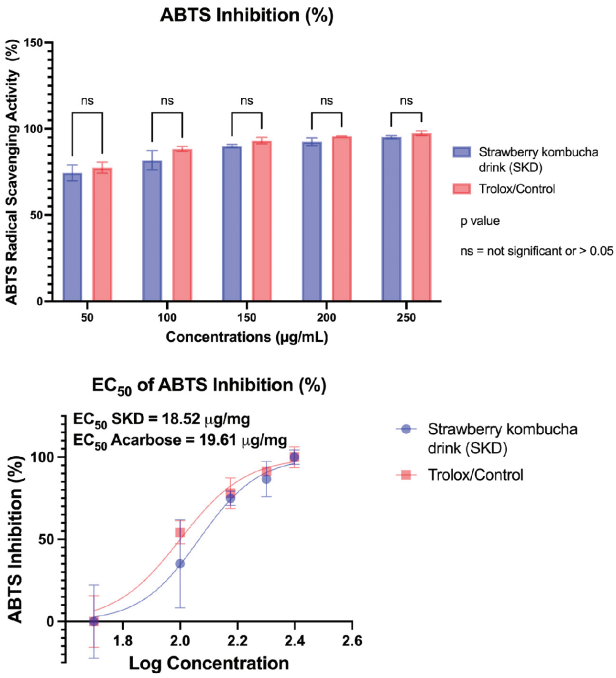

Figure 6. ABTS Inhibition Activity Test of SKD and Trolox. The inhibition of ABTS was presented in % activity (**A**) and EC_{50} value (**B**).

control, significantly (Fig. 6A). The determination of EC_{50} values also revealed that DPPH inhibition by glutathione is notably stronger than SKD (Fig. 5B). The EC_{50} values of SKD and glutathione regarding the inhibition of ABTS were 18.52 µg/mg and 19.61 µg/mg, respectively (Fig. 6B).

Discussion

In this study we demonstrated that SKD had 62 potential secondary metabolites that may be beneficial for metabolic health. In addition, we also showed inhibition activity of these metabolites on the activity of α-amylase, α-glucosidase, and lipase at least from *in silico* modelling. Furthermore, SKD had the ability to inhibit of α-amylase, α-glucosidase, and lipase *in vitro*. Interestingly, we also showed the free radical scavenging activity of SKD *in vitro*.

Clinical evidence showed that strawberries promoted health and prevented diseases due to several nutritive and non-nutritive bioactive compounds (Afrin et al. 2016; Miller et al. 2019). This work incorporated strawberries into a kombucha probiotic drink and evaluated its activity *in silico* and *in vitro*. The initial work successfully identified 62 secondary metabolites in the strawberry kombucha drink (SKD) (Table 1). These results also highlighted that SKD contained far more metabolites from the strawberry in the absence of fermentation, with a comparison of 62 to 12–20 metabolites (Zhang et al. 2011; Antunes et al. 2019). This is in line with other studies that the fermentation process – especially using kombucha or SCOBY – can increase bioactive compounds in a food product (Leonard et al. 2021; Permatasari et al. 2022). Furthermore, we confirmed the potential biological significance of these metabolites to the biological properties of SKD using molecular docking (Table 3). Finally, *in vitro* examination also revealed the potential antiobesity, antidiabetic, and antioxidant activity of SKD.

Based on molecular docking simulation, the docking protocol was valid as shown by the RMSD <2.0 Å (Table 2). From the identified bioactive compounds of SKD, 1-Methyl-N-{[(2R,4S,5R)-5-(2-methyl-6-phenyl-4-pyrimidinyl)-1-azabicyclo[2.2.2]oct-2-yl]methyl}-4- was the best ligand that can interact with all target proteins, namely human pancreatic lipase, a-glucosidase, and a-amylase. 1-Methyl-N-{[(2R,4S,5R)-5-(2-methyl-6-phenyl-4-pyrimidinyl)-1-azabicyclo[2.2.2]oct-2-yl]methyl}-4-piperidinamine – as the best metabolite – demonstrated lowest binding affinity against lipase (ΔG = -8.73 kcal/mol), a-amylase (ΔG = -10.74 kcal/mol), and a-glucosidase (ΔG = -13.60 kcal/mol) compared to orlistat (1LPB, ΔG = -8.73 kcal/mol) and acarbose (2QV4, ΔG = -4.22 kcal/mol; 3L4Y; ΔG = -1.01 kcal/mol) (Table 3). Following the best metabolite, other compounds such as dibenzylamine, (-)-epicatechin, α-pyrrolidinopropiophenone, levalbuterol, 5-({[3-chloro-5-(trifluoromethyl)-2-pyridyl]methyl} thio)-4-pentyl-4H-1,2,4-triazol-3-ol, catechin gallate, (2R,3R)-5,7-dihydroxy-2-(3,4,5-trihydroxyphenyl)-3,4-dihydro-2H-1-benzopyran-3-yl 3,4,5-trihydroxybenzoate, and (-)-caryophyllene oxide also showed an overall lowest binding energy to target proteins. From these metabolites, (-)-epicatechin and catechin gallate have been revealed to improve obesity and its comorbidities (Wu et al. 2018; Cremonini et al. 2020). Possible therapeutic actions of caryophyllene have also been reviewed (Hashiesh et al. 2020). Overall, 39 compounds were identified as potential antiobesity, followed by 27 and 44 compounds

based on a-amylase and a-glucosidase inhibitions, respectively. These results concluded that SKD consisted of various bioactive substances that contribute to the antiobesity, antidiabetic, and antioxidant activities of SKD.

The capability of SKD in inhibiting lipase was examined in this study. On a weight basis, lipase inhibition activity of SKD at doses of 100, 150, 200, and 250 µg/mL was similar to orlistat, a control that inhibits lipase in obesity (Son and Kim 2020). SKD had an EC_{50} value of lipase inhibition of 39.70 µg/mg (Fig. 2B) while strawberry extract had a reported EC_{50} of around 5 µg/mL (McDougall et al. 2009). Lipase inhibition will improve lipid metabolism in obese individuals by reducing the accumulation of fatty acids, maintaining the HDL-to-LDL ratio, and preventing adipocyte growth (Liu et al. 2020). On the other hand, strawberry supplementation also demonstrated health benefit implications in obese adults (Basu et al. 2016). Sustaining a metabolically healthy condition in obesity may also decrease the risk of diabetes, NAFLD, and metabolic syndrome (Godoy-Matos et al. 2020).

The α-amylase and α-glucosidase inhibition activities of SKD were observed. SKD at doses of 50 and 250 µg/mL showed similar α-amylase inhibition to acarbose while inhibition of α-glucosidase of SKD did not differ significantly from acarbose at doses of 50, 150, and 250 µg/mL. Acarbose is an α-amylase and α-glucosidase inhibitor with the potential as a calorie restriction mimetic and weight-loss agent (Smith et al. 2021). SKD showed an EC_{50} value of α-amylase inhibition of 5.39 µg/mg while strawberry extract had a reported EC_{50} of 96.82–398.46 µg/mL (Huneif et al. 2022). The same trend was observed in the inhibition of α-glucosidase, where SKD exhibited an EC_{50} value of α-glucosidase inhibition of 15.03 µg/mg while strawberry extract had a reported EC_{50} of 117.54–429.39 µg/mL (Huneif et al. 2022). The health-promoting effects of strawberries may be contributed to the phenolics and antioxidant compounds contained in strawberries (Giampieri et al. 2012, 2017). Dietary strawberry was also shown to decrease the risk factors of obesity-related disorders (Zunino et al. 2012). These findings also support the fact that kombucha showed anti-diabetic potential based on the inhibition of α-amylase and α-glucosidase (Permatasari et al. 2021, 2022).

The antioxidant activity of plant-based fermented drinks was widely studied since fermentation will result in a high-quality beverage with enhanced antioxidant, total phenolic, and bioactive compounds (Hur et al. 2014; Yang et al. 2018). In this study, SKD showed DPPH inhibition similar to glutathione at 150, 200, and 250 µg/mL doses. Interestingly, at all doses, SKD had a similar ABTS inhibition to Trolox. The EC50 value of DPPH inhibition of SKD was also greater than strawberry crude extract (17.28 µg/mg to 59.55–349.35 µg/mL (Huneif et al. 2022). A diverse range of aspects, such as total phenolic, organic acids, vitamins, and microbial hydrolysis in fermentation can influence the antioxidant activity of kombucha drink (Massoud et al. 2022). Subsequently, the identified phenolic compounds (catechin and epicatechin) and flavonoids (such as rutin) have been proven to improve metabolic function and inflammation while also acting as an antioxidant (Simos et al. 2012; Muvhulawa et al. 2022). On the other hand, dietary strawberry supplementation has induced beneficial effects on lipid profile, antioxidant, and inflammatory markers in obese adults (Zunino et al. 2012; Basu et al. 2016). Inflammation and oxidative stress themselves have been linked with the pathophysiology of obesity and metabolic syndrome (Ruiz-Ojeda et al. 2018). Therefore, SKD may become a nutraceutical with antiobesity and antidiabetic activities along with antioxidant and anti-inflammatory properties.

Conclusion

Strawberry or *Fragaria ananassa* can be processed or innovated into a functional probiotic drink (SKD) with several secondary metabolites that inhibit the activity of lipase, α-glucosidase, and α-amylase as proved by *in silico* study. SKD also exhibited potential antiobesity, antidiabetic, and antioxidant properties which may play a role in attenuating metabolic and inflammatory disorders *in vitro*, further reinforcing the potential health benefits of SKD. However, our study has not studied the potential of SKD using cell lines, hence the limitation of our study. These findings suggest that SKD can be a promising therapeutic functional food in preventing metabolic disorders and obesity.

Author contributions

Concept and design: AP, WBG, FN. Analysis and interpretation: AP, WBG, FN, DA. Data collection: FN. Writing the article: AP, WBG, FN, GAL, MSA. Critical revision of the article: AP, MA. Final approval of the article: all authors. Statistical analysis: WBG, FN. Obtained funding: AP. Overall responsibility: AP.

Acknowledgements

We want to thank the faculty and technical staff for their administrative support. AP would like to thank RPI fellowship no. 225-18/UN7.D2/PP/IV/2023 from LPPM Universitas Diponegoro.

References

Abaci N, Senol Deniz FS, Orhan IE (2022) Kombucha – An ancient fermented beverage with desired bioactivities: A narrowed review. Food Chemistry X 14: 100302. https://doi.org/10.1016/j.fochx.2022.100302

Afrin S, Gasparrini M, Forbes-Hernandez TY, Reboredo-Rodriguez P, Mezzetti B, Varela-López A, Giampieri F, Battino M (2016) Promising health benefits of the strawberry: A focus on clinical studies.

Journal of Agricultural and Food Chemistry 64(22): 4435–4449. https://doi.org/10.1021/acs.jafc.6b00857

Antunes AC, Acunha TDS, Perin EC, Rombaldi CV, Galli V, Chaves FC (2019) Untargeted metabolomics of strawberry (*Fragaria* × *ananassa* 'Camarosa') fruit from plants grown under osmotic stress conditions. Journal of the Science of Food and Agriculture 99(15): 6973–6980. https://doi.org/10.1002/jsfa.9986

Basu A, Morris S, Nguyen A, Betts NM, Fu D, Lyons TJ (2016) Effects of dietary strawberry supplementation on antioxidant biomarkers in obese adults with above optimal serum lipids. Journal of Nutrition and Metabolism 2016: 1–7. https://doi.org/10.1155/2016/3910630

Basu A, Nguyen A, Betts NM, Lyons TJ (2014) Strawberry as a functional food: An evidence-based review. Critical Reviews in Food Science and Nutrition 54(6): 790–806. https://doi.org/10.1080/10408398.2011.608174

Cremonini E, Iglesias DE, Kang J, Lombardo GE, Mostofinejad Z, Wang Z, Zhu W, Oteiza PI (2020) (-)-Epicatechin and the comorbidities of obesity. Archives of Biochemistry and Biophysics 690: 108505. https://doi.org/10.1016/j.abb.2020.108505

Cuamatzin-García L, Rodríguez-Rugarcía P, El-Kassis EG, Galicia G, Meza-Jiménez ML, Baños-Lara MDR, Zaragoza-Maldonado DS, Pérez-Armendáriz B (2022) Traditional fermented foods and beverages from around the world and their health benefits. Microorganisms 10(6): 1151. https://doi.org/10.3390/microorganisms10061151

Değirmencioğlu N, Yıldız E, Sahan Y, Güldas M, Gürbüz O (2021) Impact of tea leaves types on antioxidant properties and bioaccessibility of kombucha. Journal of Food Science and Technology 58(6): 2304–2312. https://doi.org/10.1007/s13197-020-04741-7

de Miranda JF, Ruiz LF, Silva CB, Uekane TM, Silva KA, Gonzalez AGM, Fernandes FF, Lima AR (2022) Kombucha: A review of substrates, regulations, composition, and biological properties. Journal of Food Science 87(2): 503–527. https://doi.org/10.1111/1750-3841.16029

Ferruzzi MG, Tanprasertsuk J, Kris-Etherton P, Weaver CM, Johnson EJ (2020) Perspective: The role of beverages as a source of nutrients and phytonutrients. Advances in Nutrition 11(3): 507–523. https://doi.org/10.1093/advances/nmz115

Giampieri F, Forbes-Hernandez TY, Gasparrini M, Afrin S, Cianciosi D, Reboredo-Rodriguez P, Varela-Lopez A, Quiles JL, Mezzetti B, Battino M (2017) The healthy effects of strawberry bioactive compounds on molecular pathways related to chronic diseases. Annals of the New York Academy of Sciences 1398(1): 62–71. https://doi.org/10.1111/nyas.13373

Giampieri F, Tulipani S, Alvarez-Suarez JM, Quiles JL, Mezzetti B, Battino M (2012) The strawberry: Composition, nutritional quality, and impact on human health. Nutrition 28(1): 9–19. https://doi.org/10.1016/j.nut.2011.08.009

Godoy-Matos AF, Silva Júnior WS, Valerio CM (2020) NAFLD as a continuum: From obesity to metabolic syndrome and diabetes. Diabetology and Metabolic Syndrome 12(1): 1–20. https://doi.org/10.1186/s13098-020-00570-y

Hashiesh HM, Meeran MFN, Sharma C, Sadek B, Kaabi JA, Ojha SK (2020) Therapeutic potential of β-caryophyllene: A dietary cannabinoid in diabetes and associated complications. Nutrients 12(10): 1–30. https://doi.org/10.3390/nu12102963

Huneif MA, Alqahtani SM, Abdulwahab A, Almedhesh SA, Mahnashi MH, Riaz M, Ur-Rahman N, Jan MS, Ullah F, Aasim M, Sadiq A (2022) α-Glucosidase, α-Amylase and Antioxidant Evaluations of Isolated Bioactives from Wild Strawberry. Molecules 27(11): 1–13. https://doi.org/10.3390/molecules27113444

Hur SJ, Lee SY, Kim YC, Choi I, Kim GB (2014) Effect of fermentation on the antioxidant activity in plant-based foods. Food Chemistry 160: 346–356. https://doi.org/10.1016/j.foodchem.2014.03.112

Jafari R, Naghavi NS, Khosravi-Darani K, Doudi M, Shahanipour K (2020) Kombucha microbial starter with enhanced production of antioxidant compounds and invertase. Biocatalysis and Agricultural Biotechnology 29: 101789. https://doi.org/10.1016/j.bcab.2020.101789

Jakubczyk K, Kałduńska J, Kochman J, Janda K (2020) Chemical profile and antioxidant activity of the kombucha beverage derived from white, green, black and red tea. Antioxidants 9(5): 447. https://doi.org/10.3390/antiox9050447

Júnior JCDS, Meireles Mafaldo Í, de Lima Brito I, Tribuzy de Magalhães Cordeiro AM (2022) Kombucha: Formulation, chemical composition, and therapeutic potentialities. Current Research in Food Science 5: 360–365. https://doi.org/10.1016/j.crfs.2022.01.023

Kapp JM, Sumner W (2019) Kombucha: a systematic review of the empirical evidence of human health benefit. Annals of Epidemiology 30: 66–70. https://doi.org/10.1016/j.annepidem.2018.11.001

Kaur P, Sandhu KS, Bangar SP, Purewal SS, Kaur M, Ilyas RA, Asyraf MRM, Razman MR (2021) Unraveling the bioactive profile, antioxidant and dna damage protection potential of rye (*Secale cereale*) flour. Antioxidants 10(8): 1–14. https://doi.org/10.3390/antiox10081214

Kitwetcharoen H, Phung LT, Klanrit P, Thanonkeo S, Tippayawat P, Yamada M, Thanonkeo P (2023) Kombucha healthy drink-recent advances in production, chemical composition and health benefits. Fermentation 9(1): 48. https://doi.org/10.3390/fermentation9010048

Leonard W, Zhang P, Ying D, Adhikari B, Fang Z (2021) Fermentation transforms the phenolic profiles and bioactivities of plant-based foods. Biotechnology Advances 49: 107763. https://doi.org/10.1016/j.biotechadv.2021.107763

Liu TT, Liu XT, Chen QX, Shi Y (2020) Lipase inhibitors for obesity: A review. Biomedicine and Pharmacotherapy 128: 1–7. https://doi.org/10.1016/j.biopha.2020.110314

Massoud R, Jafari-Dastjerdeh R, Naghavi N, Khosravi-Darani K (2022) All aspects of antioxidant properties of kombucha drink. Biointerface Research in Applied Chemistry 12(3): 4018–4027. https://doi.org/10.33263/BRIAC123.40184027

McDougall GJ, Kulkarni NN, Stewart D (2009) Berry polyphenols inhibit pancreatic lipase activity *in vitro*. Food Chemistry 115(1): 193–199. https://doi.org/10.1016/j.foodchem.2008.11.093

Miller K, Feucht W, Schmid M (2019) Bioactive compounds of strawberry and blueberry and their potential health effects based on human intervention studies: A brief overview. Nutrients 11(7): 1–12. https://doi.org/10.3390/nu11071510

Muvhulawa N, Dludla PV, Ziqubu K, Mthembu SXH, Mthiyane F, Nkambule BB, Mazibuko-Mbeje SE (2022) Rutin ameliorates inflammation and improves metabolic function: A comprehensive analysis of scientific literature. Pharmacological Research 178: 106163. https://doi.org/10.1016/j.phrs.2022.106163

Permatasari HK, Nurkolis F, Gunawan WB, Yusuf VM, Yusuf M, Kusuma RJ, Sabrina N, Muharram FR, Taslim NA, Mayulu N, Batubara SC, Samtiya M, Hardinsyah H, Tsopmo A (2022) Modulation of gut microbiota and markers of metabolic syndrome in mice on cholesterol and fat enriched diet by butterfly pea flower kombucha. Current Research in Food Science 5: 1251–1265. https://doi.org/10.1016/j.crfs.2022.08.005

Permatasari HK, Nurkolis F, Augusta PS, Mayulu N, Kuswari M, Taslim NA, Wewengkang DS, Batubara SC, Ben Gunawan W (2021) Kombucha tea from seagrapes (*Caulerpa racemosa*) potential as a functional anti-ageing food: *in vitro* and in vivo study. Heliyon 7(9): e07944. https://doi.org/10.1016/j.heliyon.2021.e07944

Ruiz-Ojeda FJ, Olza J, Gil Á, Aguilera CM (2018) Chapter 1 – Oxidative Stress and Inflammation in Obesity and Metabolic Syndrome. In: del Moral AM, Aguilera García CMBTO (Eds) Oxidative Stress and Dietary Antioxidants. Academic Press, 15 pp. https://doi.org/10.1016/B978-0-12-812504-5.00001-5

Sancho LEG-G, Yahia EM, González-Aguilar GA (2013) Contribution of major hydrophilic and lipophilic antioxidants from papaya fruit to total antioxidant capacity. Food and Nutrition Sciences 04(08): 93–100. https://doi.org/10.4236/fns.2013.48A012

Selvaraj S, Gurumurthy K (2023) An overview of probiotic health booster-kombucha tea. Chinese Herbal Medicines 15(1): 27–32. https://doi.org/10.1016/j.chmed.2022.06.010

Simos YV, Verginadis II, Toliopoulos IK, Velalopoulou AP, Karagounis IV, Karkabounas SC, Evangelou AM (2012) Effects of catechin and epicatechin on superoxide dismutase and glutathione peroxidase activity, in vivo. Redox Report 17(5): 181–186. https://doi.org/10.1179/1351000212Y.0000000020

Smith Jr DL, Orlandella RM, Allison DB, Norian LA (2021) Diabetes medications as potential calorie restriction mimetics – a focus on the alpha-glucosidase inhibitor acarbose. GeroScience 43(3): 1123–1133. https://doi.org/10.1007/s11357-020-00278-x

Son JW, Kim S (2020) Comprehensive review of current and upcoming anti-obesity drugs. Diabetes and Metabolism Journal 44(6): 802–818. https://doi.org/10.4093/dmj.2020.0258

Villarreal-Soto SA, Beaufort S, Bouajila J, Souchard JP, Taillandier P (2018) Understanding kombucha tea fermentation: A review. Journal of Food Science 83(3): 580–588. https://doi.org/10.1111/1750-3841.14068

Wu R, Yao Y, Jiang Q, Cai M, Liu Q, Wang Y, Wang X (2018) Epigallocatechin gallate targets FTO and inhibits adipogenesis in an mRNA m6A-YTHDF2-dependent manner. International Journal of Obesity 42(7): 1378–1388. https://doi.org/10.1038/s41366-018-0082-5

Yang X, Zhou J, Fan L, Qin Z, Chen Q, Zhao L (2018) Antioxidant properties of a vegetable-fruit beverage fermented with two *Lactobacillus plantarum* strains. Food Science and Biotechnology 27(6): 1719–1726. https://doi.org/10.1007/s10068-018-0411-4

Zhang J, Wang X, Yu O, Tang J, Gu X, Wan X, Fang C (2011) Metabolic profiling of strawberry (*Fragaria* × *ananassa* Duch.) during fruit development and maturation. Journal of Experimental Botany 62(3): 1103–1118. https://doi.org/10.1093/jxb/erq343

Zofia NŁ, Aleksandra Z, Tomasz B, Martyna ZD, Magdalena Z, Zofia HB, Tomasz W (2020) Effect of fermentation time on antioxidant and anti-ageing properties of green coffee kombucha ferments. Molecules 25(22): 1–23. https://doi.org/10.3390/molecules25225394

Zunino SJ, Parelman MA, Freytag TL, Stephensen CB, Kelley DS, Mackey BE, Woodhouse LR, Bonnel EL (2012) Effects of dietary strawberry powder on blood lipids and inflammatory markers in obese human subjects. The British Journal of Nutrition 108(5): 900–909. https://doi.org/10.1017/S0007114511006027

Supplementary material 1

The manufacturer specification

Authors: Adriyan Pramono, William Ben Gunawan, Fahrul Nurkolis, Darmawan Alisaputra, Gilbert Ansell Limen, Muhammad Subhan Alfaqih, Martha Ardiaria

Data type: docx

Link: https://doi.org/10.3897/pharmacia.71.e116794.suppl1