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JURNAL INTERNASIONAL BEREPUTASI

Judul artikel	:	In Vitro and in Silico Analysis of Antilipase, Antioxidant, and Optimization of Granule Effervescent from *Peronema canescens* Jack
Jurnal	:	Tropical Journal of Natural Product Research (TJNPR)
Edisi	:	Tahun 2025, Vol 9 No 2, hal 545 – 553
Penerbit	:	Faculty of Pharmacy, University of Benin, Benin City, Nigeria
Penulis	:	Muhammad R. R. Rahardhian, Nurchasanah, Yasmiwar Susilawati, Sri A. Sumiwi, Dewi Ramonah, Chintiana N. Putri, Ririn Suharsanti

Tabel Tahapan Publikasi Artikel

No	Tahapan Publikasi	Tanggal
1	Submission of manuscript	10 November 2024
2	Editorial Decision “Accepts with major corrections”	17 November 2024
3	Follow-Up on manuscript revision request	1 Desember 2024
4	APC Payment request from TJNPR editorial	1 Desember 2024
5	APC payment report	2 Desember 2024
6	Editorial and Reviewers comments	8 Desember 2024
7	Submit first revised manuscript	19 Desember 2024
8	Editor's comments on first revised manuscripts	20 Desember 2024
9	Submit second revised manuscript	28 Desember 2024
10	Check Galley Proof	2 Februari 2025
11	Article Published Vol 9 issue 2 (28 Februari 2025) on Website www.tjnpr.org	28 Februari 2025
12	TJNPR Published Information on Vol 9 issue 2 (28 Februari 2025)	17 Maret 2025

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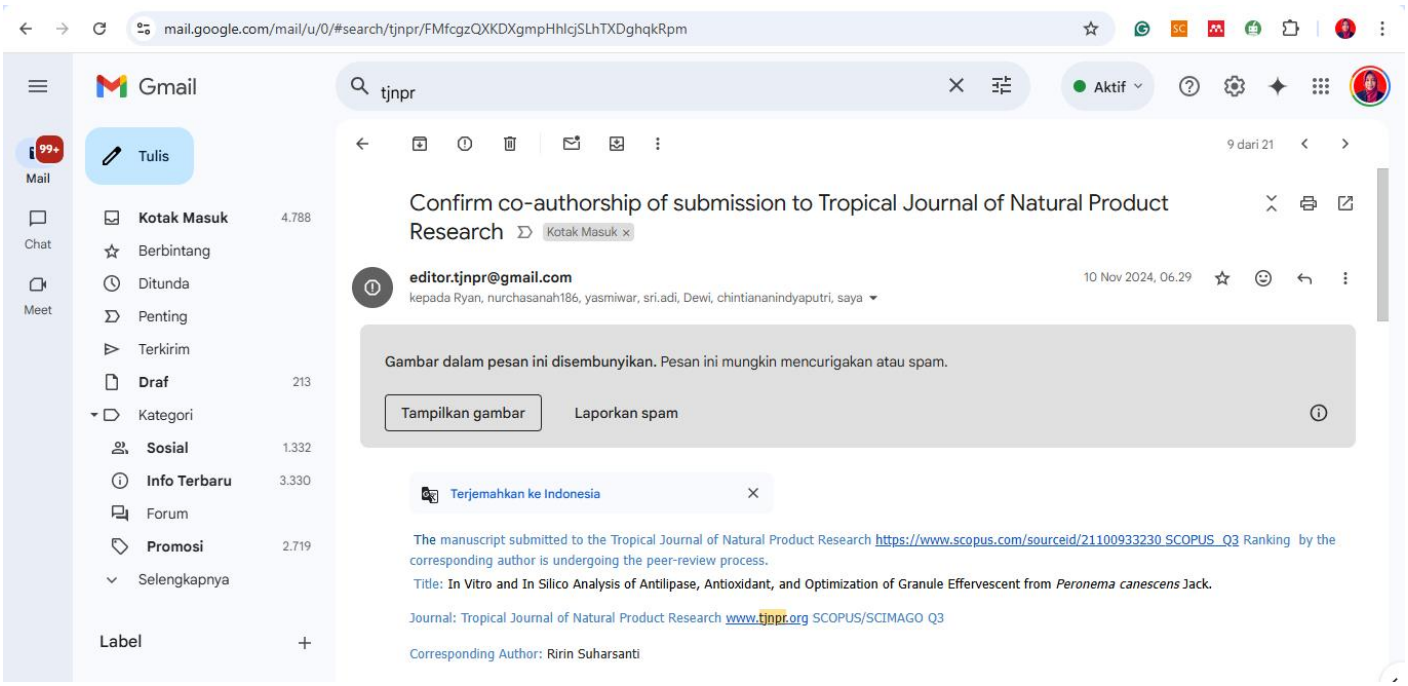

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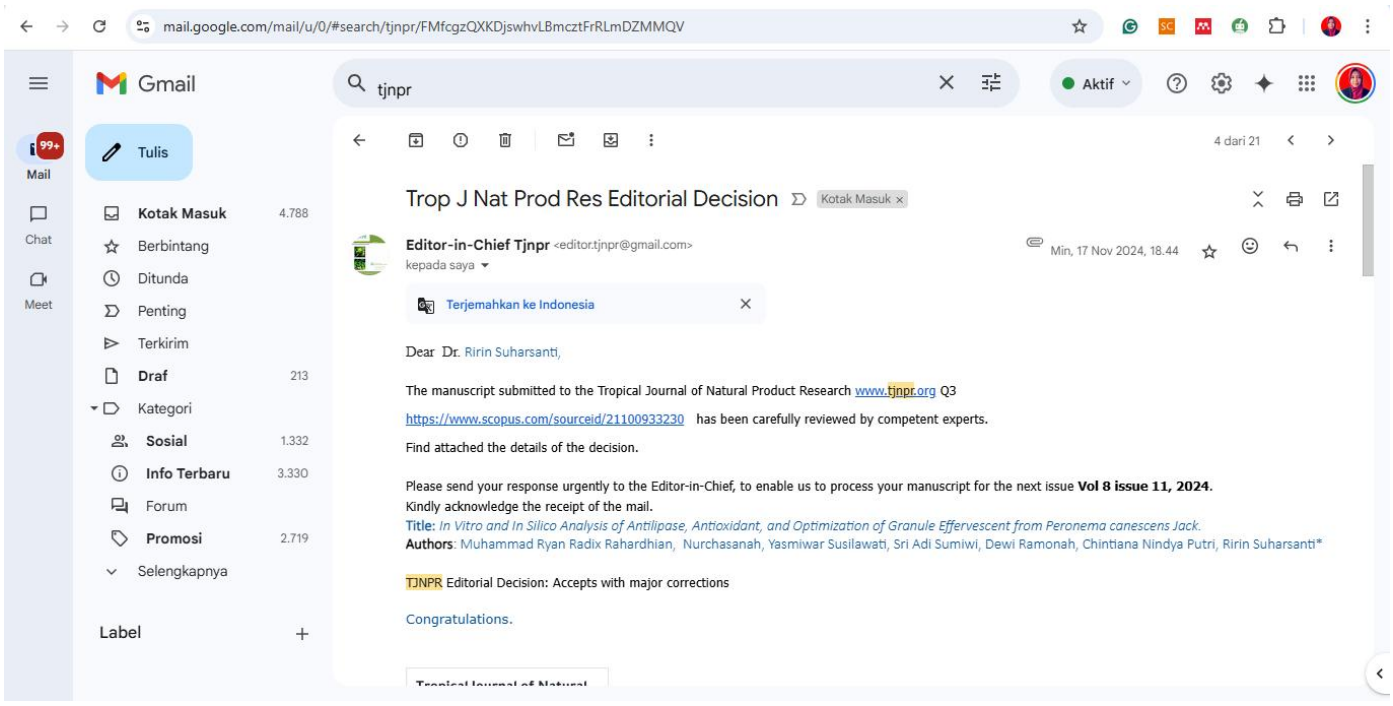

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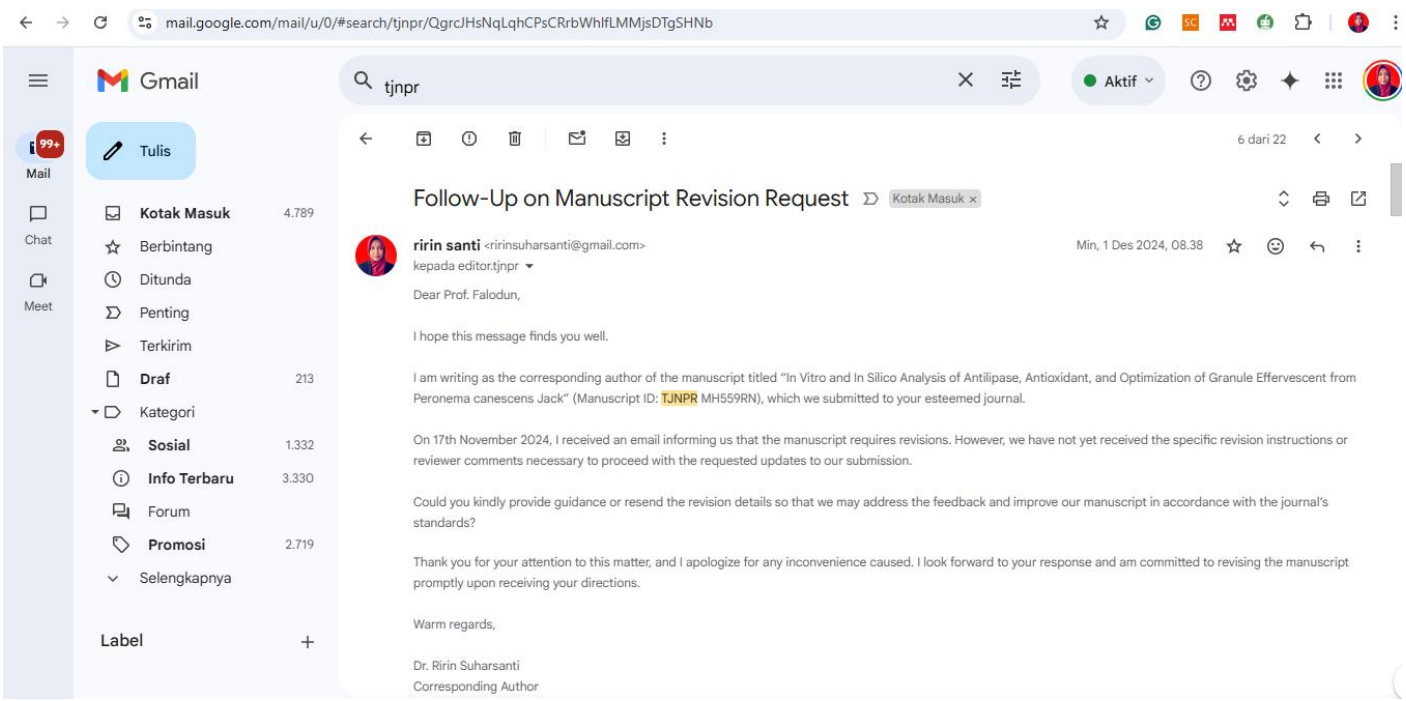

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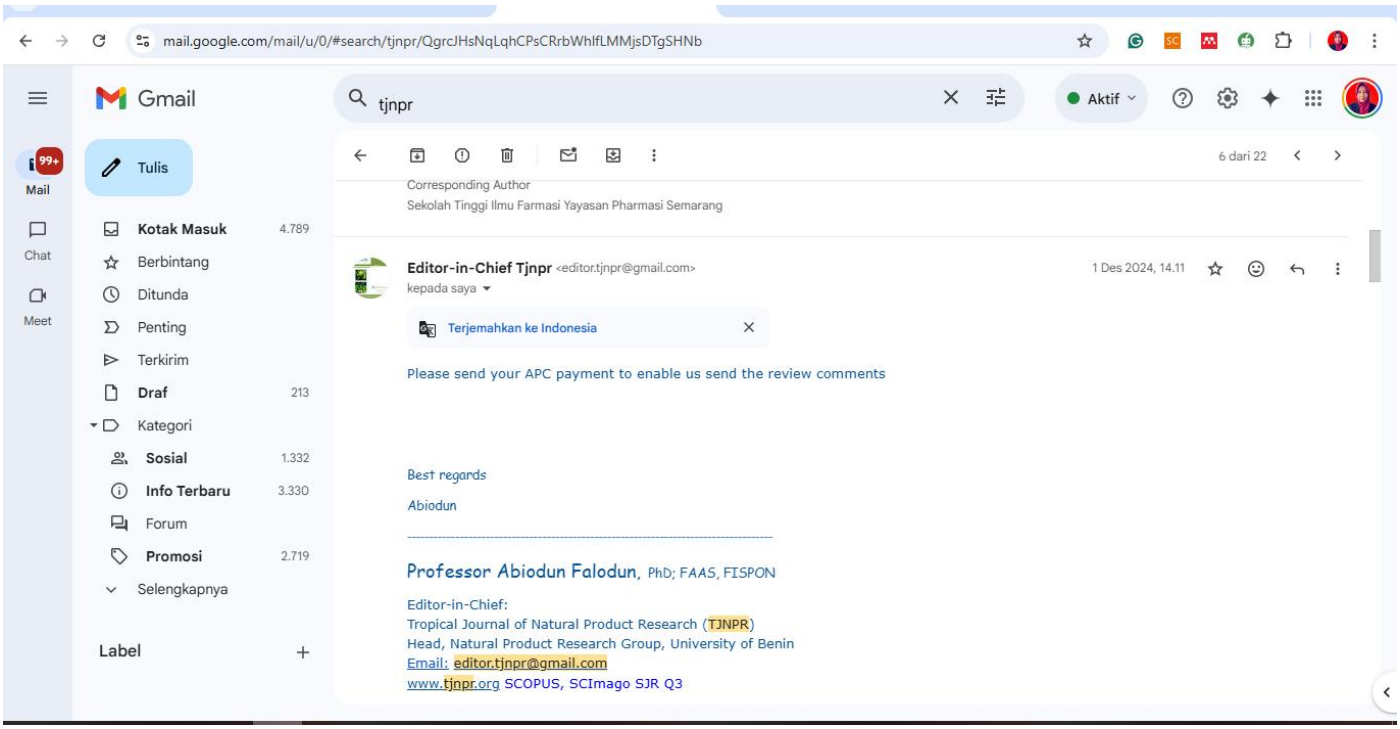

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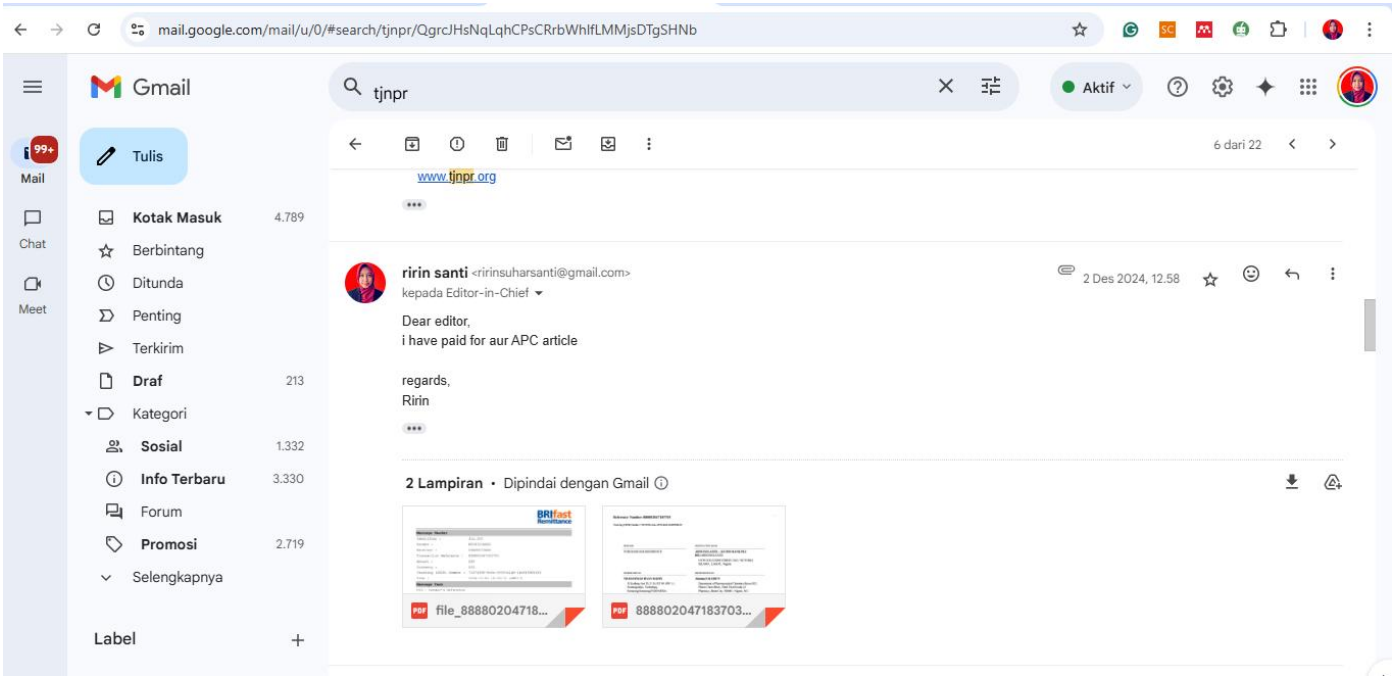

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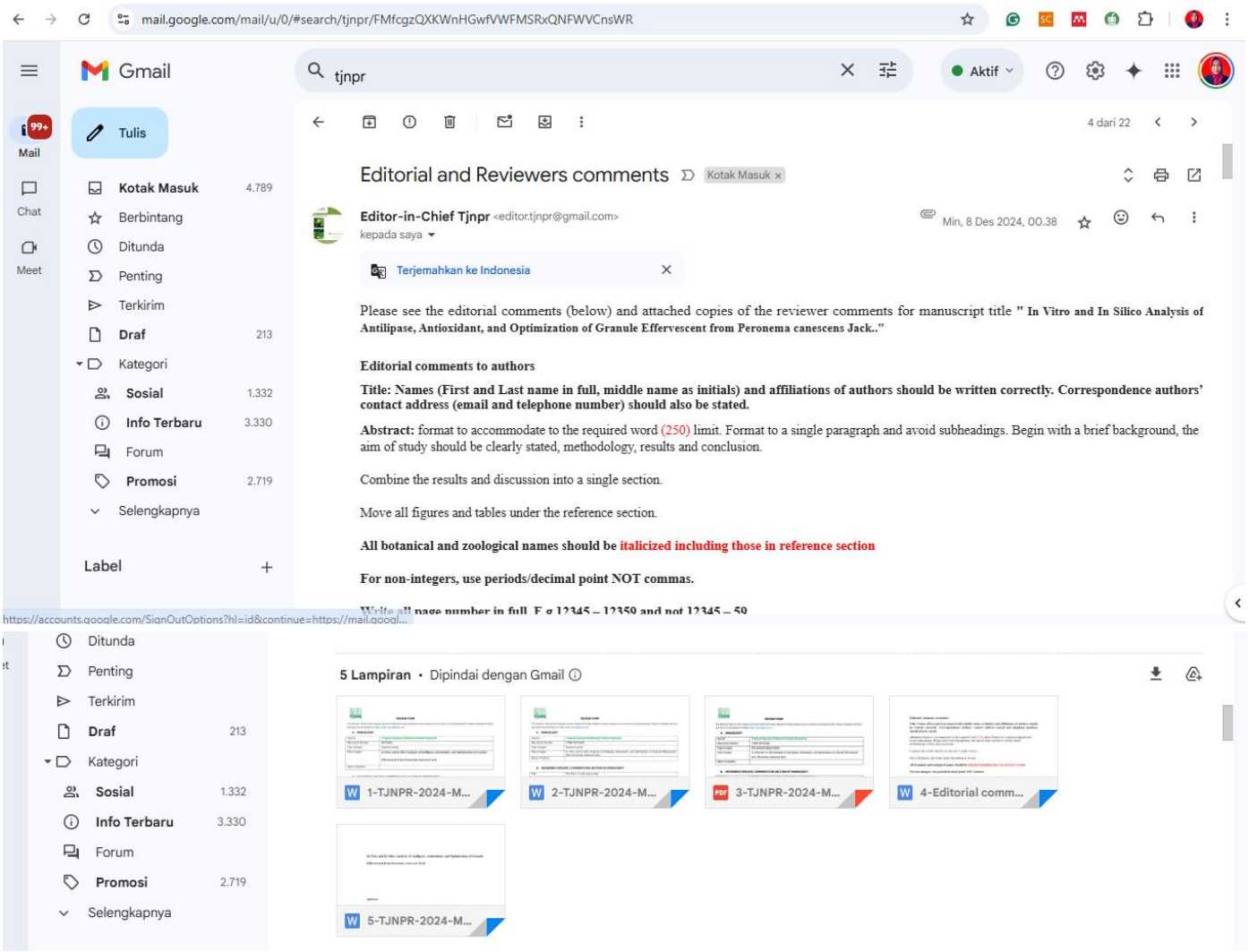

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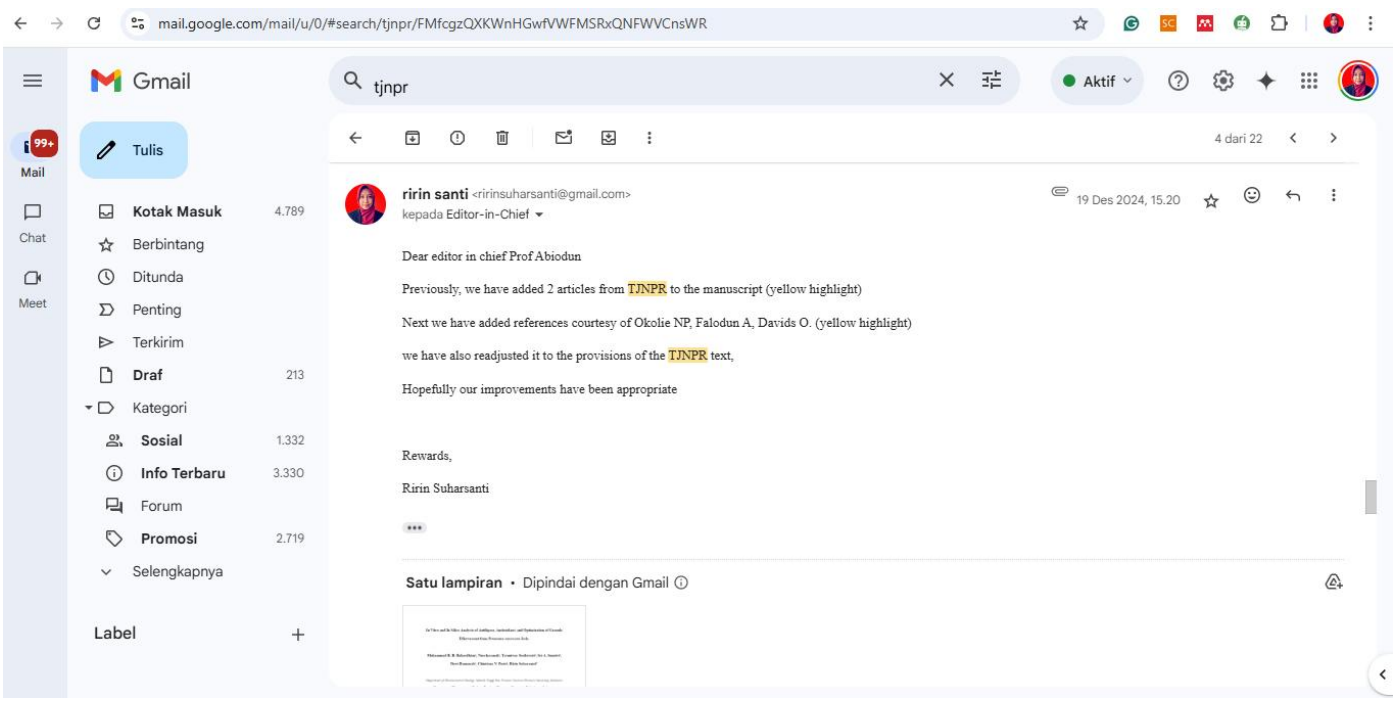

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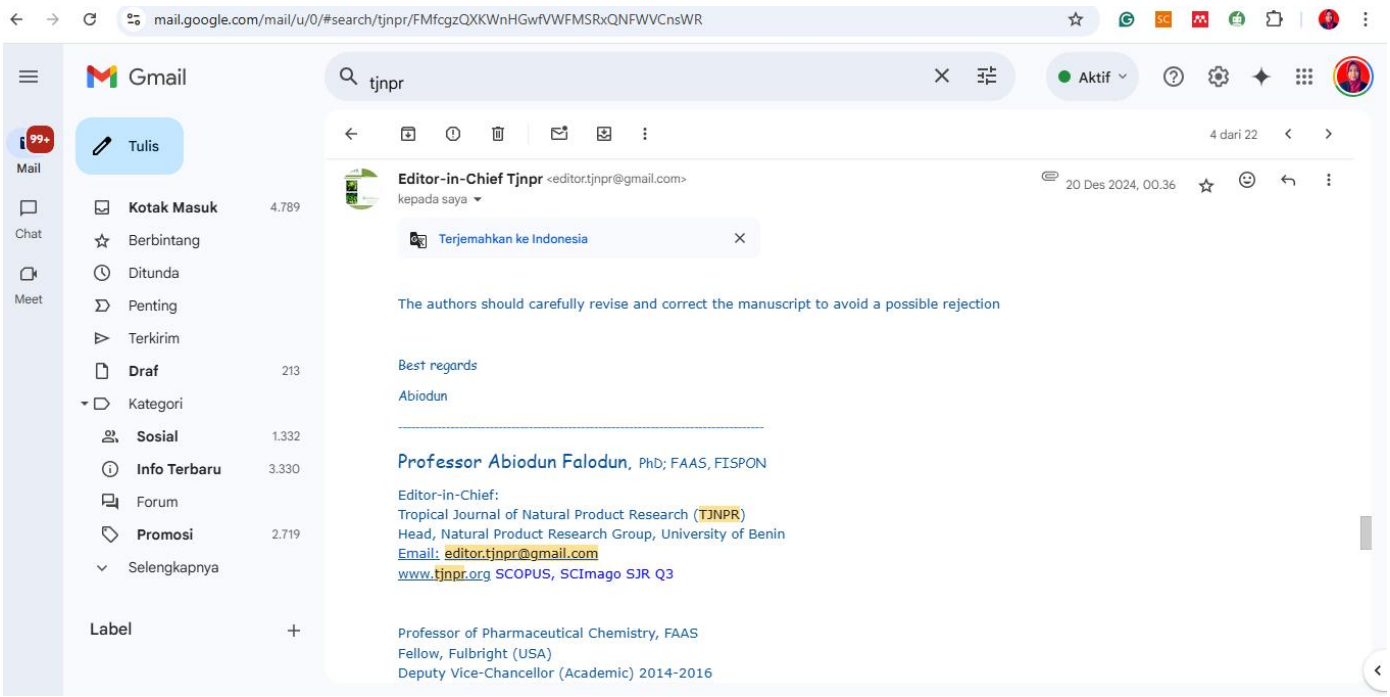

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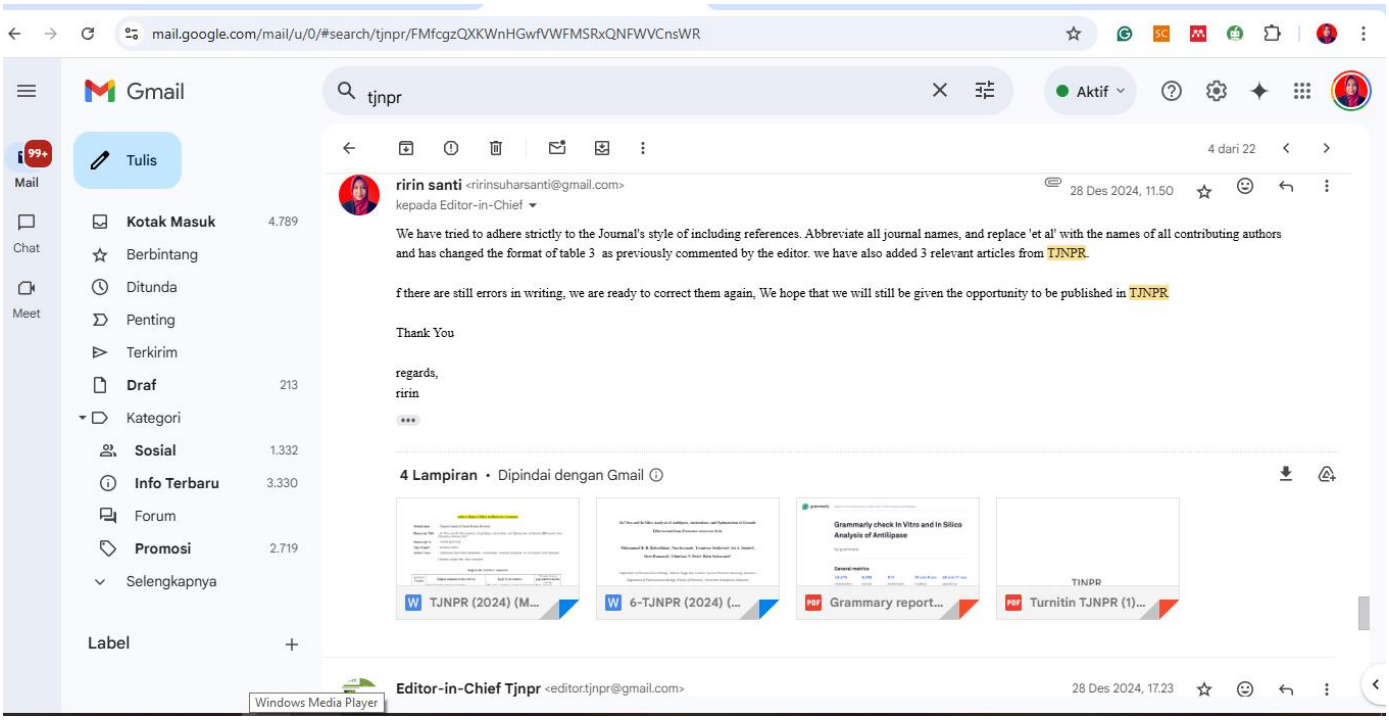

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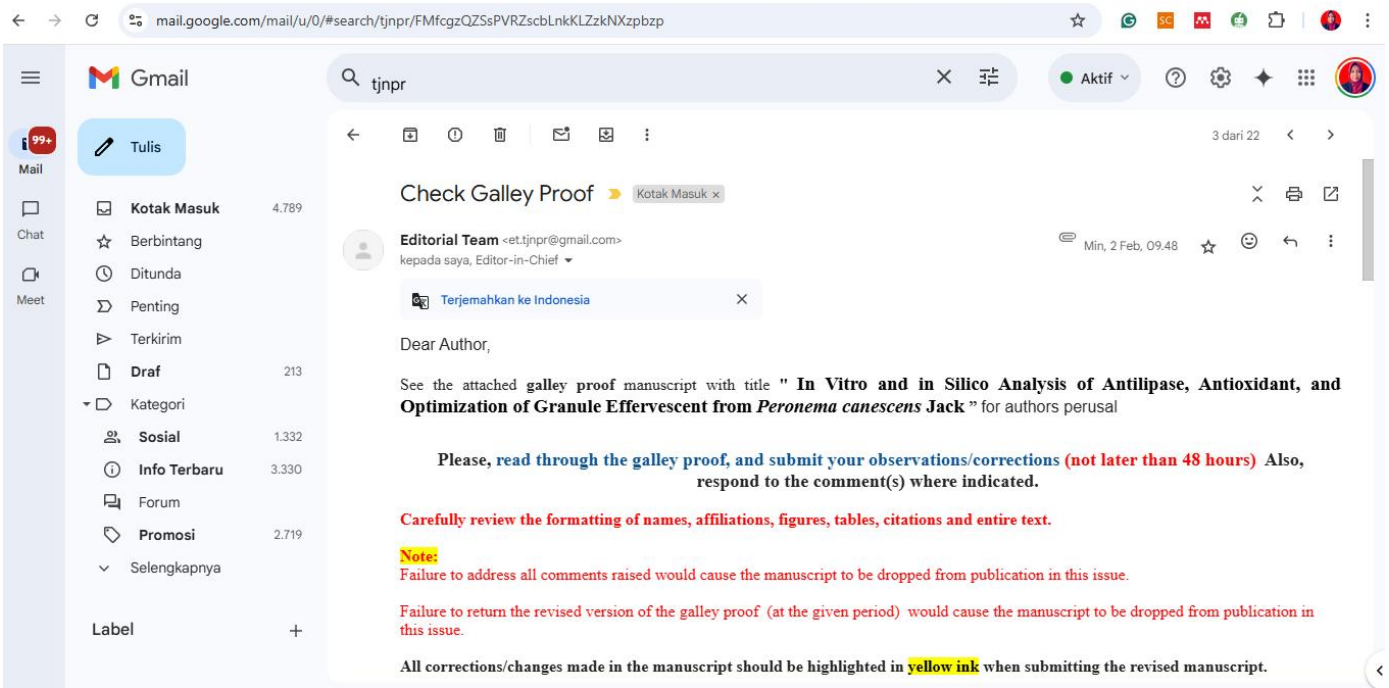

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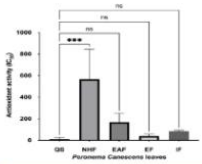

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17th November 2024

Dear Dr Ririn Suharsanti,

Provisional Acceptance letter for Article Manuscript Number TJNPR MH559ARN

Title: *In Vitro and In Silico Analysis of Antilipase, Antioxidant, and Optimization of Granule Effervescent from Peronema canescens Jack.*

Authors: Muhammad Ryan Radix Rahardhian, Nurchasanah, Yasmiwar Susilawati, Sri Adi Sumiwi, Dewi Ramonah, Chintiana Nindya Putri, Ririn Suharsanti*

I am pleased to inform you that your manuscript sent to the Tropical Journal of Natural Product Research has been reviewed and recommended for publication as a research article. However, before the issues raised by the Reviewers are forwarded, to enable you correct your manuscript, accordingly, please pay a publication charge of **$ USD280**. The actual publication of the paper will be in the upcoming issue (**Vol 8 issue 11, 2024**). **Authors are responsible for all bank charges.** Please, the manuscript number (**TJNPR MH559RN**) should be included in the bank transfer.

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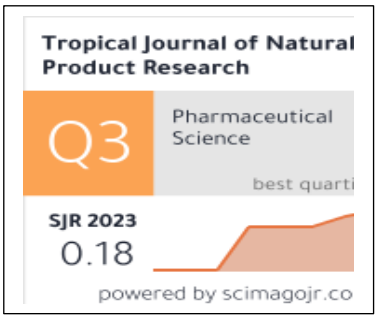

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In Vitro and In Silico Analysis of Antilipase, Antioxidant, and Optimization of Granule Effervescent from *Peronema canescens* Jack.

Abstract

Obesity results from prolonged energy imbalance, with antiobesity treatment targeting pancreatic lipase inhibition. *Peronema canescens* Jack. (PC) known as sungkai, has traditionally been used to treat various ailments. This study aimed to assess PC's antioxidant and antilipase activities and optimize effervescent granule formulations. Phytochemical screening and TLC were performed, followed by antioxidant analysis via the DPPH method and pancreatic antilipase activity using the p-NPB substrate. The ethanol fraction of PC demonstrated potent antioxidant activity (IC_{50} = 47.27 µg/mL), while the insoluble fraction showed the highest pancreatic antilipase activity (67.65%). GC-MS identified active compounds, including dimethyl tetracycline, 2-methoxy-5H-indolo[2,3-b]quinoxaline, and trilaurin, with molecular docking indicating dimethyl tetracycline as the best antilipase candidate via binding to the pancreatic receptor (PDB ID: 1LPB). This compound also met Lipinski's Rule of Five and ADMET criteria, indicating favorable pharmacokinetics and safety. Evaluation of effervescent granules included angle of repose, bulk density, and tapped density, with tartaric and citric acid concentration optimization through Design Expert 13 yielding two optimal formulas: Formula 1 with 13.16% tartaric acid and 0.84% citric acid, and Formula 2 with 13.21% tartaric acid and 0.80% citric acid.

Keywords: *Peronema Canescens* Jack., antioxidant, effervescent granules, molecular docking, pancreatic antilipase.

Introduction

The increasing prevalence of degenerative diseases in Indonesia, alongside infectious diseases, indicates changing health challenges, with obesity emerging as a major concern. RISKESDAS data reveal a rise in obesity rates from 14.8% in 2013 to 21% in 2018[1]. Factors that contribute to obesity encompass environmental factors, urban living, and eating patterns. Diets high in fats and sugars but low in fiber cause an energy imbalance, and when combined with triglyceride buildup, this imbalance triggers oxidative stress and inflammatory responses within the body [2] . This ongoing inflammation, fat accumulation, and suppression of fat breakdown causes adipocyte apoptosis, producing Reactive Oxygen Species (ROS) that harm cells and tissues, thereby raising the risk of degenerative diseases[3]. Antioxidants are essential for neutralizing ROS, helping to reduce the risk of degenerative diseases linked to oxidative stress [4].

In the context of medical treatments, FDA-approved drugs for obesity aim to either decrease calorie absorption or control appetite. Central nervous system (CNS) suppressants, including lorcaserin, liraglutide, phentermine-topiramate, and naltrexone/bupropion, work by targeting appetite-regulating receptors such as 5HT2c, GLP-1, and TAAR-1. On the other hand, Orlistat acts as a lipase inhibitor, reducing the absorption of dietary fats by approximately 30%[5].

People in Indonesia prefer using herbal medicine due to its natural properties, which are perceived as safer and less likely to cause unwanted side effects. In general, herbal medicines are more affordable than synthetic drugs. They also contain a variety of plant-based ingredients. Herbal medicine is considered effective for targeting multiple health issues. Conversely, Orlistat is a therapeutic agent for obesity that works by reducing calorie absorption in the intestinal tract[5]. Nevertheless, the effectiveness of Orlistat is constrained by

52 side effects such as gastrointestinal problems, including oily stools, flatulence, and rectal
53 discharge [6] . These limitations highlight the importance of seeking complementary or
54 alternative treatments, especially natural ones with fewer side effects and potential long-term
55 benefits.

56 Herbal medicine presents a promising alternative to synthetic drugs for managing
57 obesity, thanks to its safety, availability, and ability to target multiple mechanisms. *Peronema*
58 *canescens* Jack. (PC), locally known as sungkai, has attracted attention for its potential
59 therapeutic benefits. Traditionally utilized in Indonesian medicine, the leaves of PC contain
60 secondary metabolites like phenols, triterpenoids, flavonoids, tannins, alkaloids, steroids, and
61 saponins, which have been reported to exhibit anti-inflammatory, antioxidant, antidiabetic,
62 and immune-boosting properties[7]. The bioactive compounds in PC position it as a promising
63 candidate for antiobesity treatments, primarily by inhibiting pancreatic lipase, which helps
64 reduce lipid absorption.

65 Combining in vitro and in silico approaches provides a strong strategy for validating
66 the antiobesity potential of PC. For example, in silico modeling allows for structural
67 predictions and identifying binding sites, enhancing target interaction in drug development[8].
68 Moreover, effervescent granules offer a convenient dosage form by combining acidic and
69 alkaline compounds that release CO_2 upon dissolution. These granules provide high solubility,
70 ease of use, and rapid absorption, making them an ideal delivery system for antioxidants and
71 antilipase agents [9]. Given the therapeutic potential of PC, developing a granule formulation
72 can enhance the accessibility and effectiveness of its bioactive components.

73 While traditional treatments like GLP-1 receptor agonists have proven effective in
74 managing obesity, they are especially beneficial for patients with comorbidities such as type
75 2 diabetes. Other plant-based studies indicate that appetite suppression may occur by
76 activating the 5-HT2C receptor [10] . Additionally, TAAR1 agonists present the potential to
77 address maladaptive eating behaviors associated with metabolic disorders [11] . Inhibitors

78 targeting the lipase enzyme, such as those aimed at PDB proteins 1LPB and 5ZUN, further
79 reinforce the potential of lipase inhibition as a therapeutic target for antiobesity drugs[12].
80 This study is the first comprehensive PC analysis as an antiobesity agent through
81 pancreatic lipase inhibition, integrating in vitro antilipase and antioxidant assays with in silico
82 molecular docking, pharmacokinetic, and toxicity predictions. By focusing on pancreatic
83 lipase inhibition as a mechanism for combating obesity, the study provides insights into the
84 bioactive compounds of PC identified via GC-MS. It evaluates the most promising candidates
85 based on Lipinski's Rule of Five for oral bioavailability. This holistic approach highlights the
86 potential of PC as a safe, accessible, and effective therapy for obesity.

87

88 ## Materials

89 Rotary evaporator (Heidolph-G3), Silica Gel F254 plates, UV lamps (254 nm and 366
90 nm, Evaco GL 220V 50Hz T8 15W), micropipettes (Socorex & Dragon Lab), vortex mixers,
91 UV-Vis spectrophotometer (Shimadzu UV-1780, Serial no. A119161), ELISA reader
92 (Synergy-HTX multi-mode, 96-well plates), GC-MS (QP 2010). *Peronema canescens* Jack
93 (PC), ethanol, n-hexane, ethyl acetate, $FeCl_3$, $MgSO_4$, hydrochloric-ethanolic acid mixture
94 (1:1), hydrochloric acid, Liebermann-Burchard reagent, DPPH (Sigma), quercetin, p.a.
95 methanol, crude porcine pancreatic lipase (PPL), p-nitrophenyl butyrate (p-NPB), phosphate
96 buffer (pH 7.2), DMSO, and orlistat standard.

97 ## Hardware and Software

98 Some of the software used, including the receptors for the test, can be downloaded
99 from the RCSB PDB website (https://www.rcsb.org/). The ligands used in the test are
100 available for download from the PubChem website (https://pubchem.ncbi.nlm.nih.gov/). Test
101 ligands and receptors were created using ChemDraw Professional 15.0, Chem3D 15.0, Biovia
102 Discovery Studio 2021, Command Prompt, and AutoDock Tools 1.5.6. Docking

visualizations were performed using Biovia Discovery Studio 2021. Lipinski's Rule of Five testing was conducted using the Lipinski rules available at (http://www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp). Pharmacokinetics and toxicology testing were performed using the pk-CSM website (https://biosig.lab.uq.edu.au/pkcsm/). Molecular docking simulations were conducted on a laptop (Acer Aspire A314-35, LAPTOP-ML0EUN2).

Methods

The general research methods for the in vitro and in silico analysis of antilipase, antioxidants, and optimization of effervescent granules from PC are outlined in Figure 1.

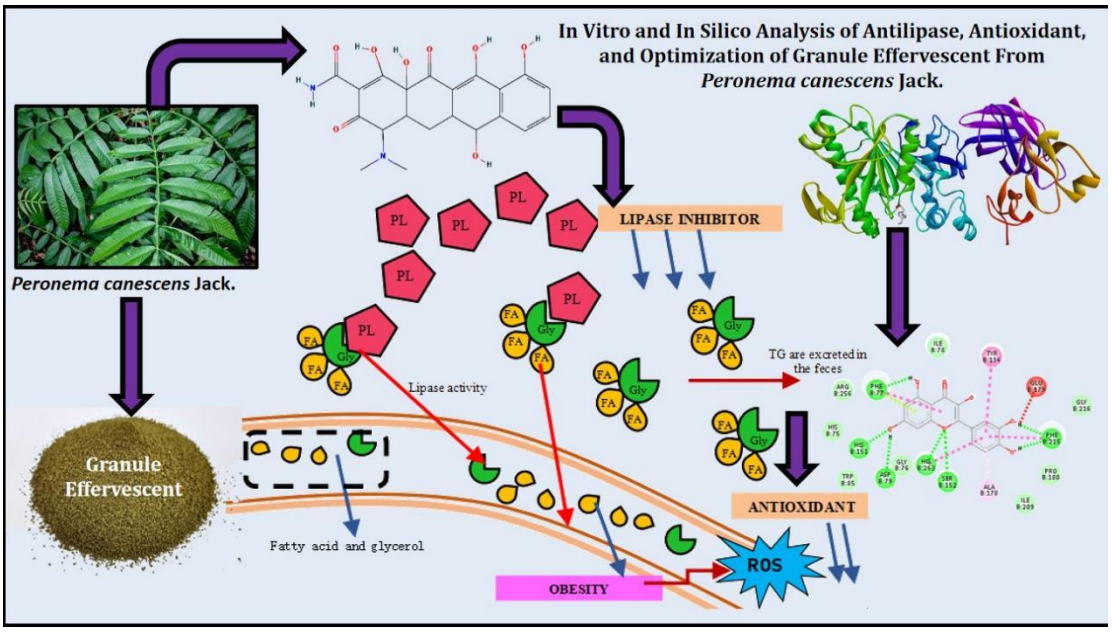


Figure 1. Research methods for in vitro and in silico analysis of antilipase, antioxidants, and optimization of effervescent granules from PC.

Sample preparation, extraction, and fractionation

The sample used in this study was *Peronema canescens*, Jack (PC), sourced from Kayutanam in Padang Pariaman District, West Sumatra, harvested between May and July 2021 from trees measuring 6-7 meters in height. The maceration process was conducted for 3 days (3x24 hours), with occasional stirring and repeated solvent changes using 96% ethanol. The resulting macerate was then filtered and concentrated using a rotary vacuum evaporator, followed by thickening in a water bath at approximately 40°C [13]

Twenty grams of the PC ethanol extract were placed in a beaker with a stir bar and magnetic stirrer. The fractionation process began by adding 100 mL of n-hexane, followed by stirring to separate the liquid from the insoluble extract. This step was repeated 5-6 times, adding 100 mL of n-hexane each time until a clear n-hexane fraction was obtained. Next, 100 mL of ethyl acetate was added to the insoluble n-hexane extract, and the fractionation was repeated until a distinct ethyl acetate fraction was obtained. Subsequently, 100 mL of ethanol solution was used to fractionate the insoluble ethyl acetate extract, repeating the process 5-6 times until a precise ethanol fraction was obtained. The remaining insoluble fraction, treated with ethanol, was designated as the insoluble fraction. The fractions were concentrated using a rotary vacuum evaporator, and the final thickening was performed in a water bath at approximately 50°C to yield a viscous fraction[13].

Antioxidant Activity

The PC fraction was dissolved in methanol and prepared at different concentrations of 10, 20, 30, 40, 50, and 60 µg/mL [14]. The antioxidant activity was determined by adding 1.0 mL of the PC fraction solution to a test tube containing 4.0 mL of 0.1 mM DPPH for each concentration. The mixture was homogenized using a vortex for 1 minute and allowed to stand for the designated time for each test solution. The absorbance of the solution was then

measured at the maximum wavelength. The same procedure was followed to measure the absorbance of the quercetin standard series.

Pancreatic Antilipase Activity

The pancreatic antilipase inhibition activity of the n-hexane, ethyl acetate, ethanol, and insoluble fractions was assessed using 96-well plates and an ELISA reader. The enzyme stock concentration was approximately 0.1 µg/mL, prepared by dissolving 1 mg of solid porcine pancreatic lipase (PPL) powder in 1 mL of buffer solution (a). The fraction was prepared at a concentration of 500 µg/mL (b), and p-NPB was dissolved in 1% DMSO (c) and subsequently diluted with a 50 mM phosphate buffer (pH 7.2, 0.5%) to a final concentration of 2.5 mM in 100 µL (d). Solutions (a), (b), and (d) were mixed and incubated at 37°C for 10 minutes. Each sample was tested in triplicate. Orlistat was used as a positive control, and 1% DMSO was the negative control without inhibitors. One unit of activity is defined as the reaction rate that generates 1 µmol of p-nitrophenyl butyrate at 37°C. Lipase activity inhibition was expressed as the percentage reduction in activity when PPL was incubated with the test compound[15].

Identification of compounds in the active fraction of PC using GC-MS.

GC-MS analysis was conducted at the integrated laboratory of Universitas Islam Indonesia. The active fraction, prepared at a concentration of 500 µg/mL, was injected in a volume of 1.0 µL for analysis using Gas Chromatography coupled with a Flame Ionization Detector (FID) and Mass Spectrometry (MS). The mobile phase consisted of chloroform: ethanol mixture (1:1), and the analysis was performed using an Rtx-5 MS column (5% diphenyl / 95% dimethyl polysiloxane) with specifications of 0.25 µm thickness, 30.0 m length, and 0.25 mm inner diameter. The instrument settings included an initial temperature of 80ºC, an injection temperature of 300ºC, and an ion source temperature of 250ºC. The

oven temperature was gradually increased to 330ºC at 6ºC per minute. The column flow rate was set to 0.74 mL/min with a pressure of 42.3 kPa[16].

Molecular Docking

The receptors used in this study were obtained from the Protein Data Bank in 3D structure format or were drawn using ChemDraw software. These receptors, which are protein macromolecules, were isolated from any irrelevant molecules along with the ligands. The isolation process was performed using Discovery Studio 2021, and the files were saved in pdb format. Optimization involved adding hydrogen atoms, merging nonpolar hydrogens, and calculating Gasteiger charges using AutodockTools 1.5.6. The resulting file was saved in pdbqt format. For ligand preparation, 2D and 3D structures of the selected ligands were created to determine their molecular structure. This was done using ChemDraw Pro 12.0 software. The ligands were then prepared using AutoDockTools 1.5.6, where the compound structures were corrected, and Gasteiger charges were added. The prepared ligands were saved in *pdbqt format [8,12].

Evaluation of Drug Likeliness and ADMET

Assessing the drug-likeness of compounds is based on Lipinski's Rule of Five, which utilizes both experimental and computational approaches to evaluate solubility and permeability in drug discovery and development [17] . The Rule of Five suggests that poor absorption and permeability are likely when the molecular weight exceeds 500, the number of hydrogen bond acceptors is greater than 10, the number of hydrogen bond donors exceeds 5, and the calculated log P (ClogP) is higher than 5 (or MlogP > 4.15). ADMET predictions encompass absorption (CaCO2 permeability), distribution (BBB permeability), metabolism (CYP2D6 substrate), excretion (total clearance), and toxicity (AMES toxicity) [8].

Effervescent formulation

The effervescent formula consists of five different formulations. Each ingredient is weighed and sifted through mesh 30. After sifting, the ingredients are added, extracted, and homogenized. The homogeneous mixture is gradually combined with 95% ethanol until granules are formed. The granules are then sifted through mesh 20/30 and dried. The effervescent formula containing PC extract is presented in Table 1.

Table 1. Formulation of Effervescent Granules from PC Extract.

Ingredient	**Formula**				
	A	**B**	**C**	**D**	**E**
PC Extract	10%	10%	10%	10%	10%
Tartaric Acid	12,72%	12,30%	13,58%	14%	13,15%
Citric Acid	1,58%	2%	0,72%	0,30%	1,15%
Na. Bicarbonate	14,30%	14,30%	14,30%	14,30%	14,30%
Sucrose	60,40%	60,40%	60,40%	60,40%	60,40%
PVP	1%	1%	1%	1%	1%

Results And Discussion

Antioxidant Activity (DPPH)

The antioxidant activity was determined using the DPPH method, with the results expressed as the Inhibition Concentration 50 (IC_{50}). According to [18], a compound is classified as a powerful antioxidant if its IC_{50} is less than 50 µg/mL, strong if IC50 is less than 100 µg/mL, medium if IC_{50} is less than 150 µg/mL, weak if IC_{50} is less than 200 µg/mL, and very weak if IC_{50} is greater than 200 µg/mL. The IC_{50} values obtained in this study for the PC fractions are shown in Table 2. As indicated in Table 2, the ethanol fraction exhibits stronger

antioxidant activity than the other samples, with the order being Ethanol fraction > ethanol extract > insoluble fraction > ethyl acetate fraction > n-hexane fraction. Polar molecules such as flavonoids, phenolics, and glycosides are known for their antioxidant properties. The Ethanol fraction, having the lowest IC_{50} value, shows a significant difference, as denoted by four stars, when compared to the ethyl acetate and n-hexane fractions.

Polar fractions, such as the ethanol and insoluble fractions, contain a higher number of substances capable of donating hydrogen atoms, leading to the formation of a reduced (nonradical) form, which is indicated by the loss of the purple color, as described in reference [18] , This process converts DPPH into a stable hydrazine form, DPP. The DPPH antioxidant activity of the PC fractions is presented in Table 2.

Table 2. Antioxidant activity of PC fractions measured by DPPH assay.

Sample	**IC_{50} ± SD**	**Types of Antioxidants**
Quercetin Standard	23.77 μg/mL	Very strong
N-hexane Fraction	685.70 ±32.15 μg/mL	Very weak
Ethyl Acetate Fraction	201.89 ±20.08 μg/mL	Very weak
Ethanol Fraction	47.27 ±1.90 μg/mL	Very strong
Insoluble Fraction	86.09 ±7.94 μg/mL	Strong

In vitro Pancreatic Antilipase Activity

The inhibition of pancreatic lipase involves the interaction between lipase enzymes and their substrates. This test uses PNPB (P-nitrophenyl butyrate) as the substrate and Porcine Pancreatic Lipase (PPL) as the enzyme. The inhibitory effect is assessed by measuring the hydrolysis of P-nitrophenyl butyrate to P-nitrophenol at a wavelength of 405 nm using an ELISA reader. Pancreatic lipase inhibition by PC was tested at a concentration of 200 μg/mL, with PPL solution in phosphate buffer (pH 7.2) and PNPB solution. One unit of activity is

223 defined as the reaction rate that produces 1 μmol of p-nitrophenol in 10 minutes at 37°C. The
224 inhibition of lipase activity is expressed as the percentage reduction in activity when PPL is
225 incubated with the test compound. PPL was chosen as the enzyme model due to its
226 similarities with human pancreatic lipase (HPL), exhibiting comparable kinetics and enzyme
227 characteristics [19]. According to [15], antilipase activity is considered robust when the inhibition
228 percentage exceeds 50%. The results of the PC fraction at a concentration of 200 μg/mL are
229 shown in Table 3.

230 **Table 3.** Pancreatic antilipase activity of the PC fraction.

Sample	% inhibition of ±SD	Types of Antilipase
Orlistat Standard	61.64% ±9.11%.	Strong
N-hexane fraction	18.66% ±5.21%	Weak
Ethyl Acetate Fraction	67.65% ±8.04%	Strong
Ethanol Fraction	14.22% ±4.69%	Weak
Insoluble fraction	6.45% ±1.13%	Weak

231

232 ## In silico Pancreatic Antilipase Activity

233 Molecular docking validation is performed by redocking, where the native ligand is
234 removed from the protein's active site and then re-docked. A good RMSD score is considered
235 to be <2. The redocking results of the native ligands are shown in Figure 2. The most active
236 fraction is identified as the ethyl acetate fraction. Identification is done using the GC-MS
237 instrument, which reveals three peaks in the ethyl acetate fraction of PC. These peaks
238 correspond to three compounds, which are further tested in silico to determine which ones
239 have the potential to act as inhibitors of the pancreatic lipase enzyme.

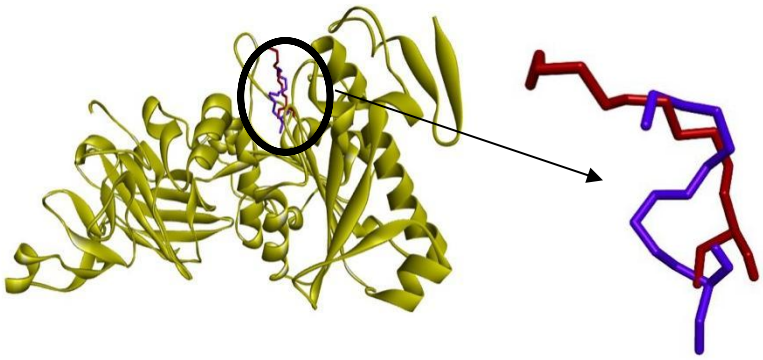
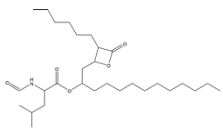
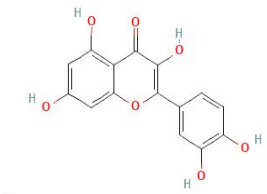
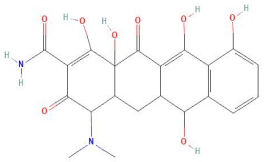
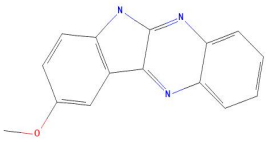
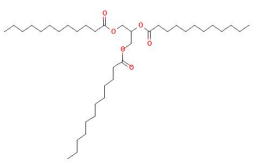


Figure 2. 3D structure of the pancreatic lipase enzyme (PDB ID 1LPB) showing an overlay of the blue (before) and red (after) molecular docking of the native ligand.

The GC-MS identification revealed that the primary compound in the active fraction was Trilaurin, accounting for 54.83% of the area and a similarity index of 59%. The three compounds identified by GC-MS were then prepared for further in silico molecular docking tests. Molecular docking of the quercetin standard, Orlistat, and the three GC-MS compounds was performed to compare the compounds obtained from pancreatic antilipase testing with the standards known to exhibit pancreatic antilipase activity, as reported in previous studies. The results of the molecular docking are presented in Table 4.

Table 4. Binding energy and amino acid residues of Orlistat, quercetin, and PC's ethyl acetate fraction compound.

No	Structure Name	Structure Drawings	Binding Energy	Amino Acid Bonds
1	Orlistat		-6,62	Gly 76, Phe 77, Ile 78, Asp 79, Tyr 114, His 151, **Ser 152,** Leu 153, Ala 178, Glu 179, Pro 180, Ile 209, Phe 215, Arg 256, Ala 259, His 263, Leu 264

2	Quercetin		-8.28 (run 83)	His B:75, Gly B:76, Phe B:77, Ile B:78, Asp B:79, Trp B:85, Tyr B:114, His B:151, **Ser B:152,** Ala B:178, Glu B:179, Pro B:180, Ile B:209, Phe B:215, Gly B:216, Arg B:256, His B:263.
3	Dimethyl Tetracycline		-7.78 (run 14)	Gly B:76, Phe B:77, Ile B:78, Asp B:79, Tyr B:114, His B:151, **Ser B:152,** Leu B:153, Ala B:178, Pro B:180, Phe B:215, Arg B:256, Asp B:257, Ala B:259, Ala B:260, His B:263, Leu B:264.
4	2-methoxy-5H-indole[2,3-b]quinoxaline		-7.25 (run 77)	His B:75, Gly B:76, Phe B:77, Ile B:78, Asp B:79, Trp B:85, Tyr B:114, His B:151, **Ser B:152,** Leu B:153, Ala B:178, Pro B:180, Ile B:209, Phe B:215, His B:263, Leu B:264.
5	Trilaurin		-3.52 (Run of 7)	Ile B:78, Tyr B:114, His B:151, **Ser B:152,** Leu B:153, Ala B:178, Pro B:180, Ile B: 209, Leu B:213, Phe B:215, Trp B:252, Thr B:255, Arg B:256, Ala B:259, Ala B:260, His

B:263, Leu B:264.

The most promising compound is Dimethyl Tetracycline, which exhibits the lowest binding energy and inhibition coefficient values compared to the Orlistat standard. This suggests that Dimethyl Tetracycline has antilipase activity similar to Orlistat. In addition to the binding energy and inhibition coefficient, pancreatic antilipase activity is evaluated based on its interaction with the amino acid serine 152. After analyzing the GC-MS-identified compounds through in silico tests, any compounds that bind to amino acid residues can potentially serve as alternative ligands to replace Orlistat. The next step is to assess whether these compounds can be used as oral drugs by evaluating them according to Lipinski's Rule of Five, as shown in Table 5.

Table 5. Predicted Lipinski's Rule of Five for the Ligands.

No	Molecular Name	Molecular Weight	Log P	Hydrogen Bond Donor (HBD)	Hydrogen Bond Acceptor (HBA)	Polar Voltage Activity (PSA)
1	Quercetin	302,238	1,988	5	7	122,108
2	Dimethyl Tetracycline	430,413	-0,5451	6	9	176,064
3	2-methoxy-5H-indolo[2,3-b]quinoxaline	249,273	3,2729	1	3	108,603
4	Trilaurin	639,015	11,7473	0	6	278,432

The indicates that the natural ligand candidates suitable for use are Quercetin, Dimethyl Tetracycline, and 2-methoxy-5H-indolo[2,3-b]quinoxaline. These compounds meet Lipinski's Rule of Five, with molecular weights under 500 Da, hydrogen bond donors not exceeding 5, hydrogen bond acceptors not exceeding 10, partition coefficients (log P) under 5,

269 and polar surface areas (PSA) under 1025 Å², making them suitable for oral administration.
270 In addition to adhering to Lipinski's Rule, candidate compounds must also pass
271 pharmacokinetic and toxicity assessments conducted using pkCSM software. The results of
272 drug-likeness analysis, along with absorption, distribution, metabolism, excretion, and
273 toxicity (ADMET) predictions, are presented in Table 6.

274 **Table 6.** DMET Prediction for Compounds from the Ethyl Acetate Fraction of PC

No	Molecular Name	Absorption (CaCO2 Permeability) (log Papp in 10-6 cm/sec)	Distribution (VDss (human)) (log L/kg)	Metabolism (CYP2D6) (YES/NO)	Excretion (Total clearance) (log ml/min/kg)	AMES Toxicity (YES/NO)	Hepatotoxicity	Skin Sensitization
1	Orlistat	0,396	-1,017	No	1,679	No	Yes	No
2	Quercetin	-0,277	0,057	No	0,457	No	No	No
3	Dimethyl tetracycline	-0,01	0,605	No	0,354	No	No	No
4	2-methoxy-5H-indolo[2,3-b]quinoxaline	1,301	-0,011	No	0,773	Yes	Yes	No
5	Trilaurin	0,141	-0,821	No	2,232	No	No	No

275

A compound is considered to have blood-brain barrier (BBB) permeability if its log BB value in the distribution phase is greater than 0.3. Molecules with a log BB value below 0.1 are not effectively distributed in the brain. CYP2D6 metabolic parameters predict whether cytochrome P450 will likely metabolize a given molecule. The total clearance (CLtot) parameter indicates excretion rates in log (ml/min/kg). Drug clearance primarily occurs through renal and hepatic clearance (kidney excretion) (liver metabolism and bile excretion). Ames toxicity testing is a commonly used method to evaluate the mutagenic potential of compounds through bacterial assays. Among the candidates, Dimethyl Tetracycline meets both Lipinski's rule of five and ADMET prediction criteria.

The Evaluation of granule preparations includes tests for flow rate, angle of repose, bulk density, tapped density, Carr's compressibility index, and Hausner ratios[20]. In this study, optimization using design experts focuses on flow rate, angle of repose, and Carr's index. Good flow characteristics are defined by the ability of particles to flow independently without clumping, influenced by gravitational force [21] . The flow rate test indicates that all the effervescent granules produced exhibit excellent flow, with a suitable flow time greater than 10 grams per second.

Table 7. Flow rate, angle of repose, and bulk density of the effervescent granules from PC.

Formula	Flow rate	Angle of repose	Bulk density (g/ml)	Tapped density (g/ml)	Hausner ratios	Carr's compressibility index (%)
A	18,66	25,05	0,5205	0,5552	1,0667	6,25
B	20,43	25,85	0,5263	0,5497	1,0445	4,26
C	20,79	27,16	0,5278	0,5638	1,0682	6,38
D	20,63	24,09	0,4957	0,5632	1,1362	11,98
E	18,74	26,03	0,4942	0,5257	1,0637	5,99

The flow rate results of the effervescent granules for each formula are presented in Table 7. Based on the observations for Formula 3 and Formula 4 in Table 7, these formulas exhibit a faster flow time due to a higher tartaric acid content than Formula 1 and Formula 2. Tartaric acid has a higher density than citric acid, which allows granules with a greater tartaric acid content to flow more rapidly because of the increased gravitational force[21]. The angle of repose is the stable angle formed between a pile of cone-shaped particles and a horizontal plane. If the angle is less than 30°, the material is considered to flow easily.

Conversely, if the angle is 40° or greater, the material will likely be difficult to flow. The shape of the granules can influence the value of the angle of repose[22]. Table 7 presents the results of the stationary angle test for formulas 1-5, all of which are below 30°. A stationary angle of no more than 30° indicates excellent flow properties, meaning all the formulas demonstrate good flow behavior. The granules flow more quickly and easily with less friction and tensile force between them. Furthermore, smaller granule sizes tend to increase cohesiveness, reducing the flow velocity and resulting in a higher stationary angle [23].

Determining bulk density includes measuring the actual weight, compressive weight, Hausner factor, and percent compressibility. The Hausner factor is used to compare the actual and compressive weights, helping to assess the flow or free-flowing properties of the powder. All seven formulas meet the qualification of having a Hausner factor of less than 1.25, indicating good flow characteristics. Granule compressibility refers to the ability of the granules to maintain compactness under pressure. Factors such as porosity, type density, particle shape, and moisture content can affect the flow properties of the granules. Good flow properties ensure easier molding of the granules and help maintain uniform weight. The results for the Hausner factor and compressibility are shown in Table 7. The percent compressibility results indicated that Carr's index ranged from 4.26% to 14.59%, which aligns with the literature stating that granules with a Carr's index value below 15% demonstrate good flowability.

The optimal formula using Design Expert is intended to generate the most efficient formula based on the response data from the prepared parameters. The response data, analyzed through ANOVA in Design Expert, is processed to identify the optimal formula [9]. The ideal formula is the one with a desirability value closest to 1. Using the simplex lattice design method in the Design Expert software, the optimal formula was determined to have 65.8126 mg of tartaric acid and 4.1874 mg of citric acid, with a desirability value of 0.862. Before finalizing, the optimal formula requires verification. The results of the formula optimization are shown in Figure 1, with the formula test results from the design expert optimization provided in Table 8.

Table 8. Results of the formula test from Design Expert Optimization.

Formula	Flow rate	Angle of repose	Bulk density (g/ml)	Tapped density (g/ml)	Hausner ratios	Carr's compressibility index (%)
1	20,5	27,61	0,5156	0,6037	1,1709	14,59
2	17,96	24,1	0,5386	0,5898	1,0951	8,68

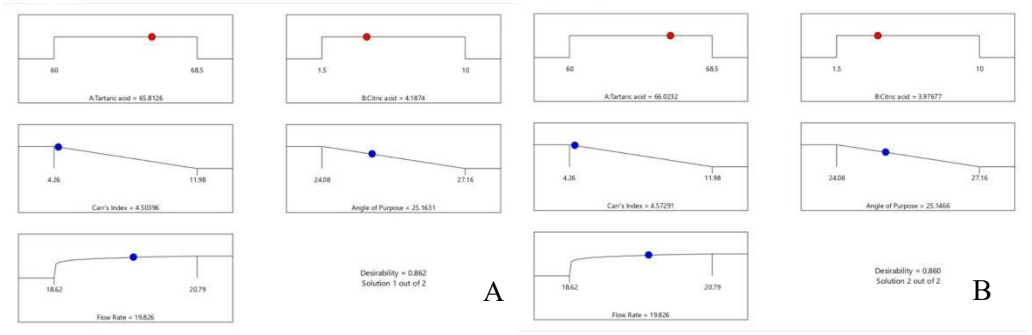
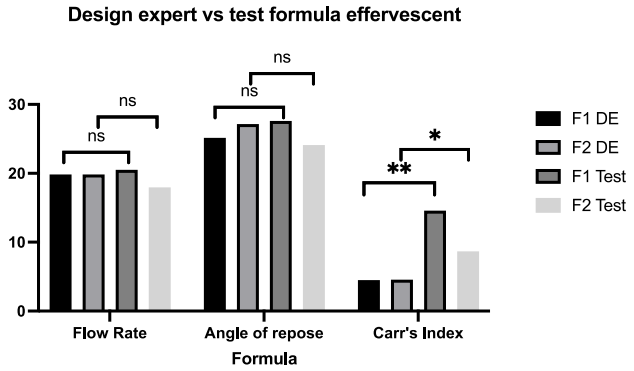


Figure 3. Formula Solution (A) 1 from Design Expert optimization (2) 2 from Design Expert optimization



Note: ns = not Significant (p>0,05), *(p<0,05), F1 DE (Formula 1 Design Expert), F2 DE (Formula 2 Design Expert), F1 Test (Formula 1 test), F2 Test (Formula 2 test)

Figure 4. Formula optimization using Design expert vs test

Based on the GraphPad statistical analysis, the flow rate and angle of repose values from Formula 1 and Formula 2 in both the Design Expert optimization and the actual test results showed no significant difference, indicating that the optimization and laboratory test produced similar outcomes. However, Carr's index test revealed a discrepancy between the Design Expert optimization and the test results, as effervescent granules are highly sensitive to room temperature, which may have influenced the test outcomes.

Additional research is needed to isolate compounds from PC based on the results of the in silico data. An integrated study of network pharmacology and component analysis should be conducted to explore the molecular mechanisms of PC extract in treating obesity [24] . In

348 silico antiobesity activity should be explored using additional receptor targets, as the
349 antiobesity mechanism extends beyond pancreatic lipase. Central nervous system
350 mechanisms can be investigated, targeting receptors such as GLP-1 (liraglutide), 5-HT2c
351 (lorcaserin), and TAAR-1 (phentermine). Further research is needed to explore other
352 antiobesity strategies beyond the pancreatic lipase inhibition pathway or in vivo methods.
353

354 ## Conclusion

355 The antioxidant activity of the PC fraction, evaluated using the DPPH method,
356 revealed the ethanol fraction as the most potent, with an IC50 of 47.2712 µg/mL. The ethyl
357 acetate fraction showed the highest pancreatic antilipase activity, with a 67.65% inhibition
358 rate. GC-MS analysis identified three active compounds in the PC fraction:
359 Demethyltetracycline, 2-methoxy-5H-indolo[2,3-b]quinoxaline, and Trilaurin, all of which
360 demonstrated pancreatic antilipase activity in silico, with Dimethyl Tetracycline showing the
361 most potential. Formula optimization using the Design Expert software resulted in two
362 formulas. The flow rate and angle of repose values from the design expert and the laboratory
363 tests did not show significant differences, indicating that the optimization and experimental
364 results aligned. However, differences were observed in the Carr's Index test between the
365 design expert optimization and the lab results.
366

367 **Conflict of Interest**

368 The authors declare no conflict of interest.
369

370 **Authors' Declaration**

371 The authors affirm that the work presented in this article is original, and they accept full
372 responsibility for any claims related to the content of the article.

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In Vitro and In Silico Analysis of Antilipase, Antioxidant, and Optimization of Granule Effervescent from *Peronema canescens* Jack.

Abstract

Obesity results from prolonged energy imbalance, with antiobesity treatment targeting pancreatic lipase inhibition. *Peronema canescens* Jack. (PC) known as sungkai, has traditionally been used to treat various ailments. This study aimed to assess PC's antioxidant and antilipase activities and optimize effervescent granule formulations. Phytochemical screening and TLC were performed, followed by antioxidant analysis via the DPPH method and pancreatic antilipase activity using the p-NPB substrate. The ethanol fraction of PC demonstrated potent antioxidant activity (IC_{50} = 47.27 µg/mL), while the insoluble fraction showed the highest pancreatic antilipase activity (67.65%). GC-MS identified active compounds, including dimethyl tetracycline, 2-methoxy-5H-indolo[2,3-b]quinoxaline, and trilaurin, with molecular docking indicating dimethyl tetracycline as the best antilipase candidate via binding to the pancreatic receptor (PDB ID: 1LPB). This compound also met Lipinski's Rule of Five and ADMET criteria, indicating favorable pharmacokinetics and safety. Evaluation of effervescent granules included angle of repose, bulk density, and tapped density, with tartaric and citric acid concentration optimization through Design Expert 13 yielding two optimal formulas: Formula 1 with 13.16% tartaric acid and 0.84% citric acid, and Formula 2 with 13.21% tartaric acid and 0.80% citric acid.

Keywords: *Peronema Canescens* Jack., antioxidant, effervescent granules, molecular docking, pancreatic antilipase.

Introduction

The increasing prevalence of degenerative diseases in Indonesia, alongside infectious diseases, indicates changing health challenges, with obesity emerging as a major concern. RISKESDAS data reveal a rise in obesity rates from 14.8% in 2013 to 21% in 2018[1]. Factors that contribute to obesity encompass environmental factors, urban living, and eating patterns. Diets high in fats and sugars but low in fiber cause an energy imbalance, and when combined with triglyceride buildup, this imbalance triggers oxidative stress and inflammatory responses within the body [2] . This ongoing inflammation, fat accumulation, and suppression of fat breakdown causes adipocyte apoptosis, producing Reactive Oxygen Species (ROS) that harm cells and tissues, thereby raising the risk of degenerative diseases[3]. Antioxidants are essential for neutralizing ROS, helping to reduce the risk of degenerative diseases linked to oxidative stress [4].

In the context of medical treatments, FDA-approved drugs for obesity aim to either decrease calorie absorption or control appetite. Central nervous system (CNS) suppressants, including lorcaserin, liraglutide, phentermine-topiramate, and naltrexone/bupropion, work by targeting appetite-regulating receptors such as 5HT2c, GLP-1, and TAAR-1. On the other hand, Orlistat acts as a lipase inhibitor, reducing the absorption of dietary fats by approximately 30%[5].

People in Indonesia prefer using herbal medicine due to its natural properties, which are perceived as safer and less likely to cause unwanted side effects. In general, herbal medicines are more affordable than synthetic drugs. They also contain a variety of plant-

based ingredients. Herbal medicine is considered effective for targeting multiple health issues. Conversely, Orlistat is a therapeutic agent for obesity that works by reducing calorie absorption in the intestinal tract[5]. Nevertheless, the effectiveness of Orlistat is constrained by side effects such as gastrointestinal problems, including oily stools, flatulence, and rectal discharge [6] . These limitations highlight the importance of seeking complementary or alternative treatments, especially natural ones with fewer side effects and potential long-term benefits.

Herbal medicine presents a promising alternative to synthetic drugs for managing obesity, thanks to its safety, availability, and ability to target multiple mechanisms. *Peronema canescens* Jack. (PC), locally known as sungkai, has attracted attention for its potential therapeutic benefits. Traditionally utilized in Indonesian medicine, the leaves of PC contain secondary metabolites like phenols, triterpenoids, flavonoids, tannins, alkaloids, steroids, and saponins, which have been reported to exhibit anti-inflammatory, antioxidant, antidiabetic, and immune-boosting properties[7]. The bioactive compounds in PC position it as a promising candidate for antiobesity treatments, primarily by inhibiting pancreatic lipase, which helps reduce lipid absorption.

Combining in vitro and in silico approaches provides a strong strategy for validating the antiobesity potential of PC. For example, in silico modeling allows for structural predictions and identifying binding sites, enhancing target interaction in drug development[8]. Moreover, effervescent granules offer a convenient dosage form by combining acidic and alkaline compounds that release CO_2 upon dissolution. These granules provide high solubility, ease of use, and rapid absorption, making them an ideal delivery system for antioxidants and antilipase agents [9]. Given the therapeutic potential of PC, developing a granule formulation can enhance the accessibility and effectiveness of its bioactive components.

While traditional treatments like GLP-1 receptor agonists have proven effective in managing obesity, they are especially beneficial for patients with comorbidities such as type

2 diabetes. Other plant-based studies indicate that appetite suppression may occur by activating the 5-HT2C receptor [10] . Additionally, TAAR1 agonists present the potential to address maladaptive eating behaviors associated with metabolic disorders [11] . Inhibitors targeting the lipase enzyme, such as those aimed at PDB proteins 1LPB and 5ZUN, further reinforce the potential of lipase inhibition as a therapeutic target for antiobesity drugs[12].

This study is the first comprehensive PC analysis as an antiobesity agent through pancreatic lipase inhibition, integrating in vitro antilipase and antioxidant assays with in silico molecular docking, pharmacokinetic, and toxicity predictions. By focusing on pancreatic lipase inhibition as a mechanism for combating obesity, the study provides insights into the bioactive compounds of PC identified via GC-MS. It evaluates the most promising candidates based on Lipinski's Rule of Five for oral bioavailability. This holistic approach highlights the potential of PC as a safe, accessible, and effective therapy for obesity.

Materials

Rotary evaporator (Heidolph-G3), Silica Gel F254 plates, UV lamps (254 nm and 366 nm, Evaco GL 220V 50Hz T8 15W), micropipettes (Socorex & Dragon Lab), vortex mixers, UV-Vis spectrophotometer (Shimadzu UV-1780, Serial no. A119161), ELISA reader (Synergy-HTX multi-mode, 96-well plates), GC-MS (QP 2010). *Peronema canescens* Jack (PC), ethanol, n-hexane, ethyl acetate, $FeCl_3$, $MgSO_4$, hydrochloric-ethanolic acid mixture (1:1), hydrochloric acid, Liebermann-Burchard reagent, DPPH (Sigma), quercetin, p.a. methanol, crude porcine pancreatic lipase (PPL), p-nitrophenyl butyrate (p-NPB), phosphate buffer (pH 7.2), DMSO, and orlistat standard.

Hardware and Software

Some of the software used, including the receptors for the test, can be downloaded from the RCSB PDB website (https://www.rcsb.org/). The ligands used in the test are

available for download from the PubChem website (https://pubchem.ncbi.nlm.nih.gov/). Test ligands and receptors were created using ChemDraw Professional 15.0, Chem3D 15.0, Biovia Discovery Studio 2021, Command Prompt, and AutoDock Tools 1.5.6. Docking visualizations were performed using Biovia Discovery Studio 2021. Lipinski's Rule of Five testing was conducted using the Lipinski rules available at (http://www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp). Pharmacokinetics and toxicology testing were performed using the pk-CSM website (https://biosig.lab.uq.edu.au/pkcsm/). Molecular docking simulations were conducted on a laptop (Acer Aspire A314-35, LAPTOP-ML0EUN2).

Methods

The general research methods for the in vitro and in silico analysis of antilipase, antioxidants, and optimization of effervescent granules from PC are outlined in Figure 1.

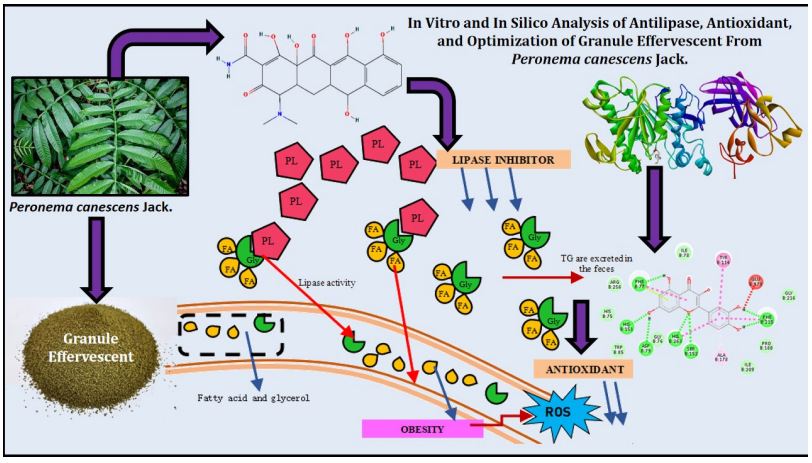

Figure 1. Research methods for in vitro and in silico analysis of antilipase, antioxidants, and optimization of effervescent granules from PC.

Sample preparation, extraction, and fractionation

The sample used in this study was *Peronema canescens*, Jack (PC), sourced from Kayutanam in Padang Pariaman District, West Sumatra, harvested between May and July 2021 from trees measuring 6-7 meters in height. The maceration process was conducted for 3 days (3x24 hours), with occasional stirring and repeated solvent changes using 96% ethanol. The resulting macerate was then filtered and concentrated using a rotary vacuum evaporator, followed by thickening in a water bath at approximately 40°C [13]

Comment[II1]: Include GPS location

Twenty grams of the PC ethanol extract were placed in a beaker with a stir bar and magnetic stirrer. The fractionation process began by adding 100 mL of n-hexane, followed by stirring to separate the liquid from the insoluble extract. This step was repeated 5-6 times, adding 100 mL of n-hexane each time until a clear n-hexane fraction was obtained. Next, 100 mL of ethyl acetate was added to the insoluble n-hexane extract, and the fractionation was repeated until a distinct ethyl acetate fraction was obtained. Subsequently, 100 mL of ethanol solution was used to fractionate the insoluble ethyl acetate extract, repeating the process 5-6 times until a precise ethanol fraction was obtained. The remaining insoluble fraction, treated with ethanol, was designated as the insoluble fraction. The fractions were concentrated using a rotary vacuum evaporator, and the final thickening was performed in a water bath at approximately 50°C to yield a viscous fraction[13].

Antioxidant Activity

The PC fraction was dissolved in methanol and prepared at different concentrations of 10, 20, 30, 40, 50, and 60 µg/mL [14]. The antioxidant activity was determined by adding 1.0 mL of the PC fraction solution to a test tube containing 4.0 mL of 0.1 mM DPPH for each concentration. The mixture was homogenized using a vortex for 1 minute and allowed to stand for the designated time for each test solution. The absorbance of the solution was then

measured at the maximum wavelength. The same procedure was followed to measure the absorbance of the quercetin standard series.

Comment[II2]: Specify the wavelength

Reply[II3]: Mention instrument used alongside the model, manufacturer and country

Pancreatic Antilipase Activity

The pancreatic antilipase inhibition activity of the n-hexane, ethyl acetate, ethanol, and insoluble fractions was assessed using 96-well plates and an ELISA reader. The enzyme stock concentration was approximately 0.1 µg/mL, prepared by dissolving 1 mg of solid porcine pancreatic lipase (PPL) powder in 1 mL of buffer solution (a). The fraction was prepared at a concentration of 500 µg/mL (b), and p-NPB was dissolved in 1% DMSO (c) and subsequently diluted with a 50 mM phosphate buffer (pH 7.2, 0.5%) to a final concentration of 2.5 mM in 100 µL (d). Solutions (a), (b), and (d) were mixed and incubated at 37°C for 10 minutes. Each sample was tested in triplicate. Orlistat was used as a positive control, and 1% DMSO was the negative control without inhibitors. One unit of activity is defined as the reaction rate that generates 1 µmol of p-nitrophenyl butyrate at 37°C. Lipase activity inhibition was expressed as the percentage reduction in activity when PPL was incubated with the test compound[15].

Comment[II4]: Mention instrument used alongside the model, manufacturer and country

Identification of compounds in the active fraction of PC using GC-MS.

GC-MS analysis was conducted at the integrated laboratory of Universitas Islam Indonesia. The active fraction, prepared at a concentration of 500 µg/mL, was injected in a volume of 1.0 µL for analysis using Gas Chromatography coupled with a Flame Ionization Detector (FID) and Mass Spectrometry (MS). The mobile phase consisted of chloroform: ethanol mixture (1:1), and the analysis was performed using an Rtx-5 MS column (5% diphenyl / 95% dimethyl polysiloxane) with specifications of 0.25 µm thickness, 30.0 m length, and 0.25 mm inner diameter. The instrument settings included an initial temperature of 80ºC, an injection temperature of 300ºC, and an ion source temperature of 250ºC. The

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oven temperature was gradually increased to 330ºC at 6ºC per minute. The column flow rate was set to 0.74 mL/min with a pressure of 42.3 kPa[16].

Molecular Docking

The receptors used in this study were obtained from the Protein Data Bank in 3D structure format or were drawn using ChemDraw software. These receptors, which are protein macromolecules, were isolated from any irrelevant molecules along with the ligands. The isolation process was performed using Discovery Studio 2021, and the files were saved in pdb format. Optimization involved adding hydrogen atoms, merging nonpolar hydrogens, and calculating Gasteiger charges using AutodockTools 1.5.6. The resulting file was saved in pdbqt format. For ligand preparation, 2D and 3D structures of the selected ligands were created to determine their molecular structure. This was done using ChemDraw Pro 12.0 software. The ligands were then prepared using AutoDockTools 1.5.6, where the compound structures were corrected, and Gasteiger charges were added. The prepared ligands were saved in *pdbqt format [8,12].

Comment[II6]: remove

Evaluation of Drug Likeliness and ADMET

Assessing the drug-likeness of compounds is based on Lipinski's Rule of Five, which utilizes both experimental and computational approaches to evaluate solubility and permeability in drug discovery and development [17] . The Rule of Five suggests that poor absorption and permeability are likely when the molecular weight exceeds 500, the number of hydrogen bond acceptors is greater than 10, the number of hydrogen bond donors exceeds 5, and the calculated log P (ClogP) is higher than 5 (or MlogP > 4.15). ADMET predictions encompass absorption (CaCO2 permeability), distribution (BBB permeability), metabolism (CYP2D6 substrate), excretion (total clearance), and toxicity (AMES toxicity) [8].

Effervescent formulation

The effervescent formula consists of five different formulations. Each ingredient is weighed and sifted through mesh 30. After sifting, the ingredients are added, extracted, and homogenized. The homogeneous mixture is gradually combined with 95% ethanol until granules are formed. The granules are then sifted through mesh 20/30 and dried. The effervescent formula containing PC extract is presented in Table 1.

Table 1. Formulation of Effervescent Granules from PC Extract.

Ingredient	**Formula**				
	A	**B**	**C**	**D**	**E**
PC Extract	10%	10%	10%	10%	10%
Tartaric Acid	12,72%	12,30%	13,58%	14%	13,15%
Citric Acid	1,58%	2%	0,72%	0,30%	1,15%
Na. Bicarbonate	14,30%	14,30%	14,30%	14,30%	14,30%
Sucrose	60,40%	60,40%	60,40%	60,40%	60,40%
PVP	1%	1%	1%	1%	1%

Results And Discussion

Antioxidant Activity (DPPH)

The antioxidant activity was determined using the DPPH method, with the results expressed as the Inhibition Concentration 50 (IC_{50}). According to [18], a compound is classified as a powerful antioxidant if its IC_{50} is less than 50 µg/mL, strong if IC50 is less than 100 µg/mL, medium if IC_{50} is less than 150 µg/mL, weak if IC_{50} is less than 200 µg/mL, and very weak if IC_{50} is greater than 200 µg/mL. The IC_{50} values obtained in this study for the PC fractions are shown in Table 2. As indicated in Table 2, the ethanol fraction exhibits stronger

antioxidant activity than the other samples, with the order being Ethanol fraction > ethanol extract > insoluble fraction > ethyl acetate fraction > n-hexane fraction. Polar molecules such as flavonoids, phenolics, and glycosides are known for their antioxidant properties. The Ethanol fraction, having the lowest IC_{50} value, shows a significant difference, as denoted by four stars, when compared to the ethyl acetate and n-hexane fractions.

Polar fractions, such as the ethanol and insoluble fractions, contain a higher number of substances capable of donating hydrogen atoms, leading to the formation of a reduced (nonradical) form, which is indicated by the loss of the purple color, as described in reference [18] , This process converts DPPH into a stable hydrazine form, DPP. The DPPH antioxidant activity of the PC fractions is presented in Table 2.

Comment[II7]: in full prior to abbreviations

Table 2. Antioxidant activity of PC fractions measured by DPPH assay.

Sample	IC_{50} ± SD	Types of Antioxidants
Quercetin Standard	23.77 µg/mL	Very strong
N-hexane Fraction	685.70 ±32.15 µg/mL	Very weak
Ethyl Acetate Fraction	201.89 ±20.08 µg/mL	Very weak
Ethanol Fraction	47.27 ±1.90 µg/mL	Very strong
Insoluble Fraction	86.09 ±7.94 µg/mL	Strong

Comment[II8]: include reference – citation needed

Comment[II9]: this result should be presented as graph used in the estimation of the IC50 with their corresponding error bars

In vitro Pancreatic Antilipase Activity

The inhibition of pancreatic lipase involves the interaction between lipase enzymes and their substrates. This test uses PNPB (P-nitrophenyl butyrate) as the substrate and Porcine Pancreatic Lipase (PPL) as the enzyme. The inhibitory effect is assessed by measuring the hydrolysis of P-nitrophenyl butyrate to P-nitrophenol at a wavelength of 405 nm using an ELISA reader. Pancreatic lipase inhibition by PC was tested at a concentration of 200 µg/mL, with PPL solution in phosphate buffer (pH 7.2) and PNPB solution. One unit of activity is

223 defined as the reaction rate that produces 1 µmol of p-nitrophenol in 10 minutes at 37°C. The
224 inhibition of lipase activity is expressed as the percentage reduction in activity when PPL is
225 incubated with the test compound. PPL was chosen as the enzyme model due to its
226 similarities with human pancreatic lipase (HPL), exhibiting comparable kinetics and enzyme
227 characteristics [19]. According to [15], antilipase activity is considered robust when the inhibition
228 percentage exceeds 50%. The results of the PC fraction at a concentration of 200 µg/mL are
229 shown in Table 3.

230 **Table 3.** Pancreatic antilipase activity of the PC fraction.

Sample	% inhibition of ±SD	Types of Antilipase
Orlistat Standard	61.64% ±9.11%.	Strong
N-hexane fraction	18.66% ±5.21%	Weak
Ethyl Acetate Fraction	67.65% ±8.04%	Strong
Ethanol Fraction	14.22% ±4.69%	Weak
Insoluble fraction	6.45% ±1.13%	Weak

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232 ## In silico Pancreatic Antilipase Activity

233 Molecular docking validation is performed by redocking, where the native ligand is
234 removed from the protein's active site and then re-docked. A good RMSD score is considered
235 to be <2. The redocking results of the native ligands are shown in Figure 2. The most active
236 fraction is identified as the ethyl acetate fraction. Identification is done using the GC-MS
237 instrument, which reveals three peaks in the ethyl acetate fraction of PC. These peaks
238 correspond to three compounds, which are further tested in silico to determine which ones
239 have the potential to act as inhibitors of the pancreatic lipase enzyme.

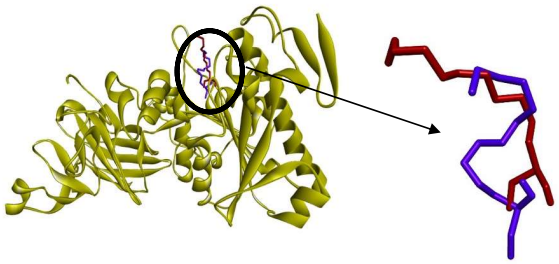
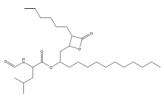
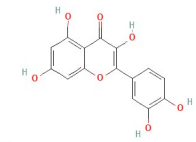
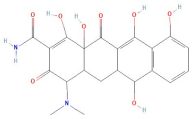
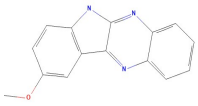
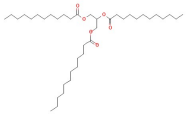


Figure 2. 3D structure of the pancreatic lipase enzyme (PDB ID 1LPB) showing an overlay of the blue (before) and red (after) molecular docking of the native ligand.

The GC-MS identification revealed that the primary compound in the active fraction was Trilaurin, accounting for 54.83% of the area and a similarity index of 59%. The three compounds identified by GC-MS were then prepared for further in silico molecular docking tests. Molecular docking of the quercetin standard, Orlistat, and the three GC-MS compounds was performed to compare the compounds obtained from pancreatic antilipase testing with the standards known to exhibit pancreatic antilipase activity, as reported in previous studies. The results of the molecular docking are presented in Table 4.

Table 4. Binding energy and amino acid residues of Orlistat, quercetin, and PC's ethyl acetate fraction compound.

No	Structure Name	Structure Drawings	Binding Energy	Amino Acid Bonds
1	Orlistat		-6,62	Gly 76, Phe 77, Ile 78, Asp 79, Tyr 114, His 151, **Ser 152,** Leu 153, Ala 178, Glu 179, Pro 180, Ile 209, Phe 215, Arg 256, Ala 259, His 263, Leu 264

2	Quercetin		-8.28 (run of 83)	His B:75, Gly B:76, Phe B:77, Ile B:78, Asp B:79, Trp B:85, Tyr B:114, His B:151, **Ser B:152,** Ala B:178, Glu B:179, Pro B:180, Ile B:209, Phe B:215, Gly B:216, Arg B:256, His B:263.
3	Dimethyl Tetracycline		-7.78 (run of 14)	Gly B:76, Phe B:77, Ile B:78, Asp B:79, Tyr B:114, His B:151, **Ser B:152,** Leu B:153, Ala B:178, Pro B:180, Phe B:215, Arg B:256, Asp B:257, Ala B:259, Ala B:260, His B:263, Leu B:264.
4	2-methoxy-5H-indole[2,3-b]quinoxaline		-7.25 (run of 77)	His B:75, Gly B:76, Phe B:77, Ile B:78, Asp B:79, Trp B:85, Tyr B:114, His B:151, **Ser B:152,** Leu B:153, Ala B:178, Pro B:180, Ile B:209, Phe B:215, His B:263, Leu B:264.
5	Trilaurin		-3.52 (Run of 7)	Ile B:78, Tyr B:114, His B:151, **Ser B:152,** Leu B:153, Ala B:178, Pro B:180, Ile B: 209, Leu B:213, Phe B:215, Trp B:252, Thr B:255, Arg B:256, Ala B:259, Ala B:260, His

B:263, Leu B:264.

The most promising compound is Dimethyl Tetracycline, which exhibits the lowest binding energy and inhibition coefficient values compared to the Orlistat standard. This suggests that Dimethyl Tetracycline has antilipase activity similar to Orlistat. In addition to the binding energy and inhibition coefficient, pancreatic antilipase activity is evaluated based on its interaction with the amino acid serine 152. After analyzing the GC-MS-identified compounds through in silico tests, any compounds that bind to amino acid residues can potentially serve as alternative ligands to replace Orlistat. The next step is to assess whether these compounds can be used as oral drugs by evaluating them according to Lipinski's Rule of Five, as shown in Table 5.

Table 5. Predicted Lipinski's Rule of Five for the Ligands.

No	Molecular Name	Molecular Weight	Log P	Hydrogen Bond Donor (HBD)	Hydrogen Bond Acceptor (HBA)	Polar Voltage Activity (PSA)
1	Quercetin	302,238	1,988	5	7	122,108
2	Dimethyl Tetracycline	430,413	-0,5451	6	9	176,064
3	2-methoxy-5H-indolo[2,3-b]quinoxaline	249,273	3,2729	1	3	108,603
4	Trilaurin	639,015	11,7473	0	6	278,432

Comment[II11]: do not use commas as a decimal point rather use periods

The ~~Lipinski table~~ indicates that the natural ligand candidates suitable for use are Quercetin, Dimethyl Tetracycline, and 2-methoxy-5H-indolo[2,3-b]quinoxaline. These compounds meet Lipinski's Rule of Five, with molecular weights under 500 Da, hydrogen bond donors not exceeding 5, hydrogen bond acceptors not exceeding 10, partition

Inserted[II]: t Table 5

coefficients (log P) under 5, and polar surface areas (PSA) under 1025 Å², making them suitable for oral administration. In addition to adhering to Lipinski's Rule, candidate compounds must also pass pharmacokinetic and toxicity assessments conducted using pkCSM software. The results of drug-likeness analysis, along with absorption, distribution, metabolism, excretion, and toxicity (ADMET) predictions, are presented in Table 6.

Table 6. DMET Prediction for Compounds from the Ethyl Acetate Fraction of PC

No	Molecular Name	Absorption (CaCO2 Permeability) (log Papp in 10-6 cm/sec)	Distribution (VDss (human)) (log L/kg)	Metabolism (CYP2D6) (YES/NO)	Excretion (Total clearance) (log ml/min/kg)	AMES Toxicity (YES/NO)	Hepatotoxicity	Skin Sensitization
1	Orlistat	0,396	-1,017	No	1,679	No	Yes	No
2	Quercetin	-0,277	0,057	No	0,457	No	No	No
3	Dimethyl tetracycline	-0,01	0,605	No	0,354	No	No	No
4	2-methoxy-5H-indolo[2,3-b]quinoxaline	1,301	-0,011	No	0,773	Yes	Yes	No
5	Trilaurin	0,141	-0,821	No	2,232	No	No	No

Comment[II12]: do not use commas as a decimal point rather use periods

A compound is considered to have blood-brain barrier (BBB) permeability if its log BB value in the distribution phase is greater than 0.3. Molecules with a log BB value below 0.1 are not effectively distributed in the brain. CYP2D6 metabolic parameters predict whether cytochrome P450 will likely metabolize a given molecule. The total clearance (CLtot) parameter indicates excretion rates in log (ml/min/kg). Drug clearance primarily occurs through renal and hepatic clearance (kidney excretion) (liver metabolism and bile excretion). Ames toxicity testing is a commonly used method to evaluate the mutagenic potential of compounds through bacterial assays. Among the candidates, Dimethyl Tetracycline meets both Lipinski's rule of five and ADMET prediction criteria.

The Evaluation of granule preparations includes tests for flow rate, angle of repose, bulk density, tapped density, Carr's compressibility index, and Hausner ratios[20]. In this study, optimization using design experts focuses on flow rate, angle of repose, and Carr's index. Good flow characteristics are defined by the ability of particles to flow independently without clumping, influenced by gravitational force [21] . The flow rate test indicates that all the effervescent granules produced exhibit excellent flow, with a suitable flow time greater than 10 grams per second.

Table 7. Flow rate, angle of repose, and bulk density of the effervescent granules from PC.

Formula	Flow rate	Angle of repose	Bulk density (g/ml)	Tapped density (g/ml)	Hausner ratios	Carr's compressibility index (%)
A	18,66	25,05	0,5205	0,5552	1,0667	6,25
B	20,43	25,85	0,5263	0,5497	1,0445	4,26
C	20,79	27,16	0,5278	0,5638	1,0682	6,38
D	20,63	24,09	0,4957	0,5632	1,1362	11,98
E	18,74	26,03	0,4942	0,5257	1,0637	5,99

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The flow rate results of the effervescent granules for each formula are presented in Table 7. Based on the observations for Formula 3 and Formula 4 in Table 7, these formulas exhibit a faster flow time due to a higher tartaric acid content than Formula 1 and Formula 2. Tartaric acid has a higher density than citric acid, which allows granules with a greater tartaric acid content to flow more rapidly because of the increased gravitational force[21]. The angle of repose is the stable angle formed between a pile of cone-shaped particles and a horizontal plane. If the angle is less than 30°, the material is considered to flow easily.

Conversely, if the angle is 40° or greater, the material will likely be difficult to flow. The shape of the granules can influence the value of the angle of repose[22]. Table 7 presents the results of the stationary angle test for formulas 1-5, all of which are below 30°. A stationary angle of no more than 30° indicates excellent flow properties, meaning all the formulas demonstrate good flow behavior. The granules flow more quickly and easily with less friction and tensile force between them. Furthermore, smaller granule sizes tend to increase cohesiveness, reducing the flow velocity and resulting in a higher stationary angle [23].

Determining bulk density includes measuring the actual weight, compressive weight, Hausner factor, and percent compressibility. The Hausner factor is used to compare the actual and compressive weights, helping to assess the flow or free-flowing properties of the powder. All seven formulas meet the qualification of having a Hausner factor of less than 1.25, indicating good flow characteristics. Granule compressibility refers to the ability of the granules to maintain compactness under pressure. Factors such as porosity, type density, particle shape, and moisture content can affect the flow properties of the granules. Good flow properties ensure easier molding of the granules and help maintain uniform weight. The results for the Hausner factor and compressibility are shown in Table 7. The percent compressibility results indicated that Carr's index ranged from 4.26% to 14.59%, which aligns with the literature stating that granules with a Carr's index value below 15% demonstrate good flowability.

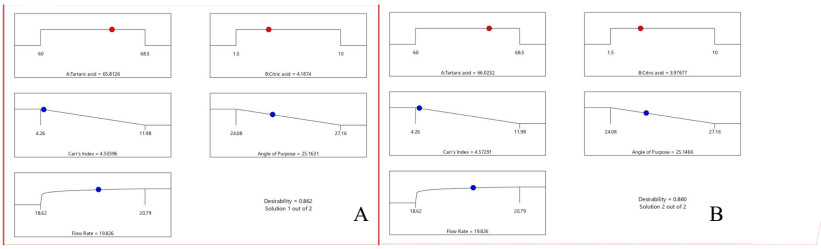
The optimal formula using Design Expert is intended to generate the most efficient formula based on the response data from the prepared parameters. The response data, analyzed through ANOVA in Design Expert, is processed to identify the optimal formula [9]. The ideal formula is the one with a desirability value closest to 1. Using the simplex lattice design method in the Design Expert software, the optimal formula was determined to have 65.8126 mg of tartaric acid and 4.1874 mg of citric acid, with a desirability value of 0.862. Before finalizing, the optimal formula requires verification. The results of the formula optimization are shown in Figure 1, with the formula test results from the design expert optimization provided in Table 8.

Comment[II14]: ?

Table 8. Results of the formula test from Design Expert Optimization.

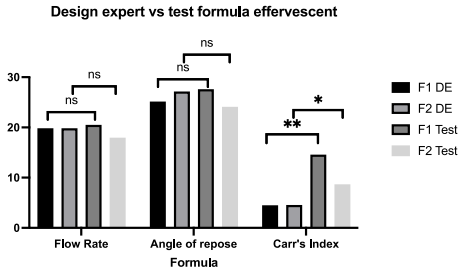
Formula	Flow rate	Angle of repose	Bulk density (g/ml)	Tapped density (g/ml)	Hausner ratios	Carr's compressibility index (%)
1	20,5	27,61	0,5156	0,6037	1,1709	14,59
2	17,96	24,1	0,5386	0,5898	1,0951	8,68

Comment[II15]: do not use commas as a decimal point rather use periods



Comment[II16]: all images should be clear and readable – at least 300dpi

Figure 3. Formula Solution (A) 1 from Design Expert optimization (2) 2 from Design Expert optimization



Note: ns = not Significant (p>0,05), *(p<0,05), F1 DE (Formula 1 Design Expert), F2 DE (Formula 2 Design Expert), F1 Test (Formula 1 test), F2 Test (Formula 2 test)

Figure 4. Formula optimization using Design expert vs test

Based on the GraphPad statistical analysis, the flow rate and angle of repose values from Formula 1 and Formula 2 in both the Design Expert optimization and the actual test results showed no significant difference, indicating that the optimization and laboratory test produced similar outcomes. However, Carr's index test revealed a discrepancy between the Design Expert optimization and the test results, as effervescent granules are highly sensitive to room temperature, which may have influenced the test outcomes.

Additional research is needed to isolate compounds from PC based on the results of the in silico data. An integrated study of network pharmacology and component analysis should be conducted to explore the molecular mechanisms of PC extract in treating obesity [24]. In

silico antiobesity activity should be explored using additional receptor targets, as the antiobesity mechanism extends beyond pancreatic lipase. Central nervous system mechanisms can be investigated, targeting receptors such as GLP-1 (liraglutide), 5-HT2c (lorcaserin), and TAAR-1 (phentermine). Further research is needed to explore other antiobesity strategies beyond the pancreatic lipase inhibition pathway or in vivo methods.

Conclusion

The antioxidant activity of the PC fraction, evaluated using the DPPH method, revealed the ethanol fraction as the most potent, with an IC50 of 47.2712 µg/mL. The ethyl acetate fraction showed the highest pancreatic antilipase activity, with a 67.65% inhibition rate. GC-MS analysis identified three active compounds in the PC fraction: Demethyltetracycline, 2-methoxy-5H-indolo[2,3-b]quinoxaline, and Trilaurin, all of which demonstrated pancreatic antilipase activity in silico, with Dimethyl Tetracycline showing the most potential. Formula optimization using the Design Expert software resulted in two formulas. The flow rate and angle of repose values from the design expert and the laboratory tests did not show significant differences, indicating that the optimization and experimental results aligned. However, differences were observed in the Carr's Index test between the design expert optimization and the lab results.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors affirm that the work presented in this article is original, and they accept full responsibility for any claims related to the content of the article.

Acknowledgement

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Comment[EF17]: Cite two related published articles from TJNPR

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A. MANUSCRIPT

Journal	**Tropical Journal of Natural Product Research**
Manuscript Number	TJNPR MH559AR
Type of paper	Research article
Title of paper	In Vitro and In Silico Analysis of Antilipase, Antioxidant, and Optimization of Granule Effervescent from *Peronema canescens* Jack.
Name of Authors	

B. REVIEWER'S SPECIFIC COMMENTS PER SECTION OF MANUSCRIPT

Title	The title is mostly appropriate
Abstract	The abstract word count is < 250. The authors should correct all grammatical, spellings and punctuation errors. • The abstract should be revised as recommended
Keywords	Keywords are okay
Introduction	The aim and objectives of the research are obvious. The authors should include a statement of research novelty. Authors should briefly discuss the relevance of the research methods to the research. All grammatical, spellings and punctuation errors should be corrected. The literature review should mostly be contextual. Use recent citations. Abbreviated words should be mentioned in full prior to abbreviations. • The introduction should be improved as recommended
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Results	All results should be presented based on the journal guideline. Images should be of a very high quality/resolution (300 dpi) – some images were not clear and readable. Authors should forward all supplementary data (input and output files) associated with the research to the journal management for validations and authentications. Use footnotes were necessary to define terms. Crosscheck the methodologies to ensure that results obtained tally with methods used. The results of the IC50 (antioxidant assay) should be presented as graphs used in the estimation of the IC50. Authors should include the GCMS spectra obtained from the analysis. Authors shouldn't use commas as decimal points. • The results obtained are mostly appropriate for the methods used.

Discussion	The discussions should be mostly statistically comparative. Authors should discuss mainly the significant findings in details. Authors should use recent citations in discussing their findings. All grammatical, spellings and punctuation errors should be corrected. • The discussion should be improved
Conclusion	Authors should capture the future prospects of the research.
References	Authors should ensure that all the references follow the journal guideline. The number of cited references must tally with those at the references sections
Figures	All figures should be presented based on the journal guideline. All figures should be of a very high quality/resolution. Figures 3 and 4 captured were not discussed in the manuscript.
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Note: Further corrections are contained in the reviewed manuscript

C. REVIEWER'S GENERAL COMMENTS AND REMARKS

The authors studied "In Vitro and In Silico Analysis of Antilipase, Antioxidant, and Optimization of Granule Effervescent from *Peronema canescens* Jack.". The abstract should be revised as recommended. The introduction should be improved as recommended. Methods used are mostly appropriate for the research. The results obtained are mostly appropriate for the methods used. The discussion should be improved. Authors should capture the future prospects of the research. Authors should ensure that all the references follow the journal guideline. The number of cited references must tally with those at the references sections.

Authors should Include methods for the statistical analysis.

Mention the software used in the statistical analysis alongside the manufacturer, version and release year.

Authors should Include the number of replicate measurements.

The results of the IC50 (antioxidant assay) should be presented as graphs used in the estimation of the IC50.

Authors should include the GCMS spectra obtained from the analysis.

Some images were not clear and readable

The manuscript should be formatted based on the journal guideline

The manuscript should be grammar edited by a professional English language editor – very important

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Authors should forward all supplementary data (input and output files) associated with the research to the journal management for validations and authentications.

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A. MANUSCRIPT

Journal	**Tropical Journal of Natural Product Research**
Manuscript Number	TJNPR MH559AR
Type of paper	Full article/Original Article
Title of paper	*In Vitro* and *In Silico* Analysis of Anti-lipase, Antioxidant, and Optimization of Granule Effervescent from *Peronema canescens* Jack.
Name of Authors	-

B. REVIEWER'S SPECIFIC COMMENTS PER SECTION OF MANUSCRIPT

Abstract	A brief conclusion should be added at the end of abstract.
Introduction	Many grammatical errors and typos appear in the text such as anti-lipase, anti-obesity, *in vitro*, *in silico*, etc. It is suggested that the authors revisit and improve the English quality of the manuscript.
Methodology	In Figure 4, statistical analysis was applied to examine the granule characteristics. Which statistical method was used here? The statistical analysis should be mentioned in the Methods section in detail.
Results	-
Discussion	1. The IC50 of ethanol fraction is higher than that of quercetin standard showing the better antioxidant activity of the standard as compared to the PC fraction. How did the authors explain this result? 2. What is the correlation of antioxidant activity and anti-lipase potency of the PC towards the anti-obesity as the primary claim of this study? 3. Ethanol fraction was revealed as the most potent anti-oxidant agent, while ethyl acetate showed the highest pancreatic anti-lipase activity. Why do these two assays demonstrate different findings? Which type of fraction was finally used in the effervescent formula? And why? Authors are suggested to discuss it more profoundly in the Results and Discussion Section. 4. Why is the optimized effervescent granule formula not continued with the tablet formulation? 5. Authors need to enrich the Discussion with more relevant references
Conclusion	-
References	All references should have a consistent style and format according to the journal guidelines.
Figures, Tables	Figure legends in Figure 3 are too small.

C. REVIEWER'S GENERAL COMMENTS AND REMARKS

Comments may be continued onto another sheet if necessary.

In this manuscript (TJNPR MH559AR), *in vitro* and *in silico* approaches were applied to assess the antioxidant and anti-lipase activities of *Peronema canescens* Jack. and further optimize effervescent granule formulations. In general, this work is well-organized and of current interest for the obesity phytotherapy community. However, some issues need to be addressed before being accepted in the TJNPR journal.

1. The title is not precise and can be changed to "*In Vitro* and *In Silico* Evaluation of Phytochemical Compounds from *Peronema canescens* Jack. and Optimization towards Effervescent Granules"
2. Many grammatical errors and typos appear in the text such as anti-lipase, anti-obesity, *in vitro*, *in silico*, etc. It is suggested that the authors revisit and improve the English quality of the manuscript.
3. Figure legends in Figure 3 are too small.
4. The IC50 of ethanol fraction is higher than that of quercetin standard showing the better antioxidant activity of the standard as compared to the PC fraction. How did the authors explain this result?
5. In Figure 4, statistical analysis was applied to examine the granule characteristics. Which statistical method was used here? The statistical analysis should be mentioned in the Methods section in detail.
6. What is the correlation of antioxidant activity and anti-lipase potency of the PC towards the anti-obesity as the primary claim of this study?
7. Ethanol fraction was revealed as the most potent anti-oxidant agent, while ethyl acetate showed the highest pancreatic anti-lipase activity. Why do these two assays demonstrate different findings? Which type of fraction was finally used in the effervescent formula? And why? Authors are suggested to discuss it more profoundly in the Results and Discussion Section.
8. Why is the optimized effervescent granule formula not continued with the tablet formulation?
9. Authors need to enrich the Discussion with more relevant references.
10. All references should have a consistent style and format according to the journal guidelines.

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A. MANUSCRIPT

Journal	**Tropical Journal of Natural Product Research**
Manuscript Number	MH559AR
Type of paper	Research article
Title of paper	**In Vitro and In Silico Analysis of Antilipase, Antioxidant, and Optimization of Granule Effervescent from *Peronema canescens* Jack.**
Name of Authors	

B. REVIEWER'S SPECIFIC COMMENTS PER SECTION OF MANUSCRIPT

Abstract	Line 9 sungkai, change to Sungkai Line 12 DPPH, describe DPPH Line 19 ADMET, describe admet
Introduction	Line 31 RISKESDAS, describe RISKESDAS Line 55-56 add reference Line 63 add reference
Methodology	Line 140 add reference
Results	Every data change comma to dot and consistently 2 digits behind the dot
Discussion	
Conclusion	Add a closing statement that emphasizes the contribution of the research to scientific development or its applications.
References	IC50 change comma to dot and consistently 2 digits behind the dot
Figures, Tables	Every table change comma to dot and consistently 2 digits behind the dot

C. REVIEWER'S GENERAL COMMENTS AND REMARKS

Comments may be continued onto another sheet if necessary.

The manuscript demonstrates a solid research approach and valuable findings, but several areas could benefit from further clarification and consistency to improve its clarity and readability. Specifically, terminology and acronyms should be fully described upon their first mention, such as DPPH, ADMET, and RISKESDAS, to ensure the audience is clear on their meaning. Additionally, referencing should be more consistent, with key statements backed by appropriate citations where needed

BUKTI KORESPONDENSI

REVISED MANUSCRIPT

In Vitro and In Silico Analysis of Antilipase, Antioxidant, and Optimization of Granule Effervescent from *Peronema canescens* Jack.

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Abstract : Obesity results from prolonged energy imbalance, with anti-obesity treatment targeting pancreatic lipase inhibition. *Peronema canescens* Jack. (PC) known as Sungkai, has traditionally been used to treat various ailments. This study aimed to assess PC antioxidant and antilipase activities and optimize effervescent granule formulations. Phytochemical screening and thin-layer chromatography (TLC) were performed, followed by antioxidant analysis using 2,2-diphenyl-1-picrylhydazyl (DPPH) and pancreatic antilipase activity, using the p-NPB substrate, were employed. The ethanol fraction of PC demonstrated potent antioxidant activity (IC_{50} = 47.27 µg/mL), while the insoluble fraction showed the highest pancreatic antilipase activity (67.65%). Gas chromatography-mass spectrometry (GC-MS) identified active compounds, including dimethyl tetracycline, 2-methoxy-5H-indolo[2,3-b] quinoxaline, and trilaurin, with molecular docking study indicating dimethyl tetracycline was the most effective antilipase candidate, binding to the pancreatic receptor (PDB ID: 1LPB). This compound also met Lipinski's Rule of Five and ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity), suggesting favorable pharmacokinetics and safety. Evaluation of effervescent granules included angle of repose, bulk density, and tapped density. Optimization of tartaric and citric acid concentration using Design Expert 13 yielding two optimal formulas: Formula 1 with 13.16% tartaric acid and 0.84% citric acid, and Formula 2 with 13.21% tartaric acid and 0.80% citric acid. PC leaves have the potential to be an antioxidant and anti-obesity and can be developed into effervescent formula.

Keywords: *Peronema Canescens* Jack., antioxidant, effervescent granules, molecular docking, pancreatic antilipase.

Introduction

The increasing prevalence of degenerative diseases in Indonesia, alongside infectious diseases, indicates changing health challenges, with obesity emerging as a major concern. RISKESDAS (Indonesia's basic health research) data reveal a rise in obesity rates from 14.8% in 2013 to 21% in 2018.[1] Factors that contribute to obesity encompass environmental factors, urban living, and eating patterns. Diets high in fats and sugars but low in fiber cause an energy imbalance, which, when combined with triglyceride buildup, this imbalance triggers oxidative stress and inflammatory responses within the body.[2] This ongoing inflammation, fat accumulation, and suppression of fat breakdown causes adipocyte apoptosis, producing Reactive Oxygen Species (ROS) that harm cells and tissues, raising the risk of degenerative diseases.[3] Antioxidants are essential for neutralizing ROS, helping to reduce the risk of degenerative diseases linked to oxidative stress.[4]

In the context of medical treatments, FDA-approved drugs for obesity aim to either decrease calorie absorption or control appetite. Central nervous system (CNS) suppressants, including lorcaserin, liraglutide, phentermine-topiramate, and naltrexone/bupropion, work by targeting appetite-regulating receptors such as 5HT2c, GLP-1, and TAAR-1. On the other hand, Orlistat acts as a lipase inhibitor, reducing the absorption of dietary fats by approximately 30%.[5]

People in Indonesia prefer using herbal medicine due to its natural properties, which are perceived as safer and less likely to cause unwanted side effects. In general, herbal medicines are more affordable than synthetic drugs. They also contain a variety of plant-based ingredients. Herbal medicine is considered effective for targeting multiple health issues. Conversely, Orlistat is a therapeutic agent for obesity that reduces calorie absorption in the intestinal tract.[5] Nevertheless, the effectiveness of Orlistat is constrained by side effects such

as gastrointestinal problems, including oily stools, flatulence, and rectal discharge.[6] These limitations highlight the importance of seeking complementary or alternative treatments, especially natural ones with fewer side effects and potential long-term benefits.

Herbal medicine presents a promising alternative to synthetic drugs for managing obesity, thanks to its safety, availability, and ability to target multiple mechanisms. *Peronema canescens* Jack. (PC), locally known as Sungkai, has attracted attention for its potential therapeutic benefits. Traditionally utilized in Indonesian medicine, the leaves of PC contain secondary metabolites like phenols, triterpenoids, flavonoids, tannins, alkaloids, steroids, and saponins, which have been reported to exhibit anti-inflammatory, antioxidant, antidiabetic, and immune-boosting properties.[7] The bioactive compounds in PC position it as a promising candidate for anti-obesity treatments, primarily by inhibiting pancreatic lipase, which helps reduce lipid absorption.

Recent studies have highlighted the potential of plant-based compounds for pancreatic lipase inhibition, particularly in treating obesity.[8] For example, in silico modeling allows for structural predictions and identifying binding sites, enhancing target interaction in drug development.[9] Moreover, effervescent granules offer a convenient dosage form by combining acidic and alkaline compounds that release CO_2 upon dissolution. These granules provide high solubility, ease of use, and rapid absorption, making them an ideal delivery system for antioxidants and antilipase agents.[10] Given the therapeutic potential of PC, developing a granule formulation can enhance the accessibility and effectiveness of its bioactive components.

While traditional treatments like GLP-1 receptor agonists have proven effective in managing obesity, they are especially beneficial for patients with comorbidities such as type 2 diabetes. Other plant-based studies indicate that appetite suppression may occur by activating the 5-HT2C receptor.[11] Additionally, TAAR1 agonists present the potential to

address maladaptive eating behaviors associated with metabolic disorders.[12] Inhibitors targeting the lipase enzyme, such as those aimed at PDB proteins 1LPB and 5ZUN, further reinforce the potential of lipase inhibition as a therapeutic target for anti-obesity drugs.[13]

The methods employed in this study, including phytochemical screening, DPPH antioxidant assay, pancreatic antilipase activity testing, and molecular docking, are specifically chosen to assess the bioactive compounds in PC and their potential for obesity treatment. These approaches are highly relevant as they combine experimental and computational techniques to identify promising antilipase candidates. This is the first study to comprehensively evaluate PC antioxidant and antilipase activities while optimizing effervescent granule formulations. The integration of in vitro and in silico approaches in this study provides a novel insight into the potential therapeutic uses of PC in combating obesity. This holistic approach highlights the potential of PC as a safe, accessible, and effective therapy for obesity.

Materials and Methods

Materials

Rotary evaporator (Heidolph-G3), Silica Gel F254 plates, UV lamps (254 nm and 366 nm, Evaco GL 220V 50Hz T8 15W), micropipettes (Socorex & Dragon Lab), vortex mixers, UV-Vis Spectrophotometer (Shimadzu UV-1780, Shimadzu Corporation, Japan), ELISA reader (Synergy HTX, Agilent, USA), GC-MS (Shimadzu QP 2010 SE, Shimadzu Corporation, Japan). *Peronema canescens* Jack (PC), ethanol, n-hexane, ethyl acetate, $FeCl_3$, $MgSO_4$, hydrochloric-ethanolic acid mixture (1:1), hydrochloric acid, Liebermann-Burchard reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH, Sigma-Aldrich, USA, 97% purity, analytical grade) was used for antioxidant assays, quercetin, p.a. methanol, crude porcine pancreatic lipase (PPL), P-nitrophenyl butyrate (p-NPB, Sigma-Aldrich, USA, 98% purity, analytical

grade) was used for pancreatic antilipase activity, phosphate buffer (pH 7.2), DMSO, and orlistat standard.

Hardware and Software

Some of the software used, including the receptors for the test, can be downloaded from the RCSB PDB website (https://www.rcsb.org/). The ligands used in the test are available for download from the PubChem website (https://pubchem.ncbi.nlm.nih.gov/). Test ligands and receptors were created using ChemDraw Professional 15.0, Chem3D 15.0, Biovia Discovery Studio 2021, Command Prompt, and AutoDock Tools 1.5.6. Docking visualizations were performed using Biovia Discovery Studio 2021. Lipinski's Rule of Five testing was conducted using the Lipinski rules available at (http://www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp). Pharmacokinetics and toxicology testing were performed using the pk-CSM website (https://biosig.lab.uq.edu.au/pkcsm/). Molecular docking simulations were conducted on a laptop (Acer Aspire A314-35, Laptop-ML0EUN2).

Methods

The general research methods for the in vitro and in silico analysis of antilipase, antioxidants, and optimization of effervescent granules from PC are outlined in Figure 1.

Sample preparation, extraction, and fractionation

The sample used in this study was *Peronema canescens*, Jack (PC), sourced from Kayutanam in Padang Pariaman District, West Sumatra, Latitude : 0°29'46.8312", Longitude : 100°20'7.638", Altitude: Located at an altitude of between 100 to 1000 meters above sea level. Harvested between May and July 2021 from trees measuring 6-7 meters in height. The maceration process was conducted for 3 days (3x24 hours), with occasional stirring and repeated solvent changes using 96% ethanol. The resulting macerate was then filtered and concentrated using a rotary vacuum evaporator, followed by thickening in a water bath at approximately 40°C.[14–16]

Twenty grams of the PC ethanol extract were placed in a beaker with a stir bar and magnetic stirrer. The fractionation process began by adding 100 mL of n-hexane, followed by stirring to separate the liquid from the insoluble extract. This step was repeated 5-6 times, adding 100 mL of n-hexane each time until a clear n-hexane fraction was obtained. Next, 100 mL of ethyl acetate was added to the insoluble n-hexane extract, and the fractionation was repeated until a distinct ethyl acetate fraction was obtained. Subsequently, 100 mL of ethanol solution was used to fractionate the insoluble ethyl acetate extract, repeating the process 5-6 times until a precise ethanol fraction was obtained. The remaining insoluble fraction, treated with ethanol, was designated as the insoluble fraction. The fractions were concentrated using a rotary vacuum evaporator, and the final thickening was performed in a water bath at approximately 50°C to yield a viscous fraction.[14]

Antioxidant Activity

The PC fraction was dissolved in methanol and prepared at 10, 20, 30, 40, 50, and 60 µg/mL.[17] The antioxidant activity was determined by adding 1.0 mL of the PC fraction solution to a test tube containing 4.0 mL of 0.1 mM DPPH for each concentration. The mixture was homogenized using a vortex for 1 minute and allowed to stand for the designated time for each test solution. The absorbance of the solution was then measured wavelength at 516.0 nm using a UV-Vis spectrophotometer (Shimadzu UV-1780, Shimadzu Corporation, Japan). The same procedure was followed to measure the absorbance of the quercetin standard series.

Pancreatic Antilipase Activity

The pancreatic antilipase inhibition activity of the n-hexane, ethyl acetate, ethanol, and insoluble fractions was assessed using 96-well plates and an ELISA reader (Synergy HTX, Agilent, USA). The enzyme stock concentration was approximately 0.1 µg/mL, prepared by dissolving 1 mg of solid porcine pancreatic lipase (PPL) powder in 1 mL of buffer solution

(a). The fraction was prepared at a concentration of 500 µg/mL (b), and p-NPB was dissolved in 1% DMSO (c) and subsequently diluted with a 50 mM phosphate buffer (pH 7.2, 0.5%) to a final concentration of 2.5 mM in 100 µL (d). Solutions (a), (b), and (d) were mixed and incubated at 37°C for 10 minutes. Each sample was tested in triplicate. Orlistat was used as a positive control, and 1% DMSO was the negative control without inhibitors. One unit of activity is defined as the reaction rate that generates 1 µmol of p-nitrophenyl butyrate at 37°C. Lipase activity inhibition was expressed as the percentage reduction in activity when PPL was incubated with the test compound.[18]

Identification of compounds in the active fraction of PC using GC-MS.

GC-MS analysis was conducted at the integrated laboratory of Universitas Islam Indonesia. The active fraction, prepared at a concentration of 500 µg/mL, was injected in a volume of 1.0 µL for analysis using Gas Chromatography coupled with a Flame Ionization Detector (FID) and Mass Spectrometry (MS) (Shimadzu QP 2010 SE, Shimadzu Corporation, Japan). The mobile phase consisted of chloroform: ethanol mixture (1:1), and the analysis was performed using an Rtx-5 MS column (5% diphenyl / 95% dimethyl polysiloxane) with specifications of 0.25 µm thickness, 30.0 m length, and 0.25 mm inner diameter. The instrument settings included an initial temperature of 80ºC, an injection temperature of 300ºC, and an ion source temperature of 250ºC. The oven temperature was gradually increased to 330ºC at 6ºC per minute. The column flow rate was set to 0.74 mL/min with a pressure of 42.3 kPa.[19]

Molecular Docking

The receptors used in this study were obtained from the Protein Data Bank in 3D structure format or were drawn using ChemDraw software. These receptors, which are protein macromolecules, were isolated from any irrelevant molecules along with the ligands. The isolation process was performed using Discovery Studio 2021, and the files were saved

in pdb format. Optimization involved adding hydrogen atoms, merging nonpolar hydrogens, and calculating Gasteiger charges using AutodockTools 1.5.6. The resulting file was saved in pdbqt format. For ligand preparation, 2D and 3D structures of the selected ligands were created to determine their molecular structure using ChemDraw Pro 12.0 software. The ligands were then prepared using AutoDockTools 1.5.6, where the compound structures were corrected, and Gasteiger charges were added. The prepared ligands were saved and ready for docking.[9,13]

Evaluation of Drug Likeliness and ADMET

Assessing the drug-likeness of compounds is based on Lipinski's Rule of Five, which utilizes both experimental and computational approaches to evaluate solubility and permeability in drug discovery and *development*.[20] The Rule of Five suggests that poor absorption and permeability are likely when the molecular weight exceeds 500, the number of hydrogen bond acceptors is greater than 10, the number of hydrogen bond donors exceeds 5, and the calculated log P (ClogP) is higher than 5 (or MlogP > 4.15). ADMET predictions encompass absorption (CaCO2 permeability), distribution (BBB permeability), metabolism (CYP2D6 substrate), excretion (total clearance), and toxicity (AMES toxicity).[9]

Effervescent formulation

The effervescent formula consists of five different formulations. Each ingredient is weighed and sifted through mesh 30. After sifting, the ingredients are added, extracted, and homogenized. The homogeneous mixture is gradually combined with 95% ethanol until granules are formed. The granules are then sifted through mesh 20/30 and dried. The effervescent formula containing PC extract is presented in Table 1.

Data Analysis

The data were expressed as the mean ± standard deviation (SD) of experiments in triplicate. This statistical analysis in this study was carried out with one-way anova using a

GraphPad Prism (Version 9.5.1 (528), 2023. Graph Pad Inc. software San Diego, CA, USA). IC_{50} value represented the concentration of the test sample causing 50% inhibition, in which the value <0.05 was considered significant.

Results And Discussion

Antioxidant Activity (DPPH)

The antioxidant activity was determined using the DPPH method, with the results expressed as the Inhibition Concentration 50 (IC_{50}). According to,[21] a compound is classified as a powerful antioxidant if its IC_{50} is less than 50 µg/mL, strong if IC_{50} is less than 100 µg/mL, medium if IC_{50} is less than 150 µg/mL, weak if IC_{50} is less than 200 µg/mL, and very weak if IC_{50} is greater than 200 µg/mL. The IC_{50} values obtained in this study for the PC fractions are shown in Figure 2. Quercetin as a positive control had the highest antioxidant activity with an IC_{50} value of 23.77 µg/mL, which is classified as very strong according to the criteria established by,[21] and the ethanol fraction with C50 of 47.27 ± 1.90 µg/mL, which is categorized as very strong exhibits stronger antioxidant activity than the other fraction. In contrast, the ethyl acetate fraction exhibited a much weaker antioxidant potential with an IC_{50} of 201.89 ± 20.08 µg/mL, classified as very weak. The N-hexane fraction showed the highest IC_{50} value at 685.70 ± 32.15 µg/mL, indicating very weak antioxidant activity. The insoluble fraction had an IC_{50} value of 86.09 ± 7.94 µg/mL, falling under the strong category for antioxidant activity. with the order being Ethanol fraction > ethanol extract > insoluble fraction > ethyl acetate fraction > n-hexane fraction. The OH group on quercetin can function as a hydrogen donor. Quercetin can donate hydrogen atoms to neutralize free radicals, reducing the potential for cell oxidative damage.[21] Polar molecules such as flavonoids, phenolics, and glycosides are known for their antioxidant properties. The Ethanol fraction,

having the lowest IC_{50} value, shows a significant difference, as denoted by four stars, when compared to the ethyl acetate and n-hexane fractions.

Polar fractions, such as the ethanol and insoluble fractions, contain a higher number of substances capable of donating hydrogen atoms, leading to the formation of a reduced (nonradical) form, which is indicated by the loss of the purple color, as described in reference,[21] This process reduces 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals into a stable nonradical hydrazine derivative, resulting in a color change. The DPPH antioxidant activity of the PC fractions is presented in Figure 2.

In vitro Pancreatic Antilipase Activity

The inhibition of pancreatic lipase involves the interaction between lipase enzymes and their substrates. This test uses PNPB (P-nitrophenyl butyrate) as the substrate and Porcine Pancreatic Lipase (PPL) as the enzyme. The inhibitory effect is assessed by measuring the hydrolysis of P-nitrophenyl butyrate to P-nitrophenol at a wavelength of 405 nm using an ELISA reader. Pancreatic lipase inhibition by PC was tested at a concentration of 200 µg/mL, with PPL solution in phosphate buffer (pH 7.2) and PNPB solution. One unit of activity is defined as the reaction rate that produces 1 µmol of p-nitrophenol in 10 minutes at 37°C. The inhibition of lipase activity is expressed as the percentage reduction in activity when PPL is incubated with the test compound. PPL was chosen as the enzyme model due to its similarities with human pancreatic lipase (HPL), exhibiting comparable kinetics and enzyme characteristics.[22] According to,[18] antilipase activity is robust when the inhibition percentage exceeds 50%. The results of the PC fraction at a concentration of 200 µg/mL are shown in Table 2. Similar to our findings, a recent study demonstrated that flavonoid-rich plant extracts exhibit strong antioxidant and antilipase activities, making them potential candidates for anti-obesity therapy.[5]

Obesity triggers inflammatory processes in excess lipogenesis, inhibits lipolysis, and increases adipocyte apoptosis. This matter increases the release of Reactive Oxygen Species (ROS) and will cause oxidative stress. Oxidative stress caused by obesity can result in damage to cells and tissues and trigger the emergence of degenerative diseases.[23] Antioxidants help neutralize radicals and reduce the risk of complications from degenerative diseases. Therefore, supplementation with antioxidants will reduce the risk of obesity-related complications and oxidative stress.[24]

The ethanol fraction proved the most potent antioxidant agent, while ethyl acetate showed pancreatic antilipase activity. Differences in the compounds that guide these two activities. These results follow research on other materials that show that the ethyl acetate fraction has higher anti-obesity activity than the ethanol fraction. The ethanol fraction has stronger antioxidant activity than the ethyl acetate fraction. Regarding compound content, the ethanol fraction has higher total phenolic content and total flavonoid content than the ethyl acetate fraction.[25] It is necessary to prove the levels of PC leaf extracts and fractions regarding the levels of compounds, not only phenolics and flavonoids but also other groups of compounds. So, to support both activities, an effervescent preparation will be made from PC leaves extract. Effervescent granules are preferred because they are easy to use, dissolve easily in water, and taste better. Compared to tablet preparations, effervescent granules reduce stomach irritation, which sometimes occurs when tablet preparations are swallowed directly, and reduce the risk of blockage in the esophagus because they are completely dissolved in liquid before consumption.

In silico Pancreatic Antilipase Activity

Molecular docking validation is performed by redocking. The redocking results of the native ligands are shown in Figure 3. The blue structure represents the initial conformation of the enzyme-ligand complex before molecular docking. In contrast, the red structure shows the

optimized docking pose of the native ligand after computational refinement. The close alignment between the pre-and post-docking structures, indicated by a root mean square deviation (RMSD) value below 2 Å, confirms the reliability and accuracy of the docking method used in this study.

The GC-MS identification revealed that the primary compound in the active fraction was Trilaurin, accounting for 54.83% of the area and a similarity index of 59%. The three compounds identified by GC-MS were then prepared for further in silico molecular docking tests. Molecular docking of the quercetin standard, Orlistat, and the three GC-MS compounds was performed to compare the compounds obtained from pancreatic antilipase testing with the standards known to exhibit pancreatic antilipase activity, as reported in previous studies. The results of the molecular docking are presented in Table 3.

The most promising compound is Dimethyl Tetracycline, which exhibits the lowest binding energy and inhibition coefficient values compared to the Orlistat standard. The Dimethyl Tetracycline has antilipase activity similar to Orlistat. In addition to the binding energy and inhibition coefficient, pancreatic antilipase activity is evaluated based on its interaction with the amino acid serine 152. After analyzing the GC-MS-identified compounds through in silico tests, any compounds that bind to amino acid residues can potentially serve as alternative ligands to replace Orlistat. The next step is to assess whether these compounds can be used as oral drugs by evaluating them according to Lipinski's Rule of Five, as shown in Table 4.

Table 4 indicates that the natural ligand candidates suitable for use are Quercetin, Dimethyl Tetracycline, and 2-methoxy-5H-indolo[2,3-b]quinoxaline. These compounds meet Lipinski's Rule of Five, with molecular weights under 500 Da, hydrogen bond donors not exceeding 5, hydrogen bond acceptors not exceeding 10, partition coefficients (log P) under 5, and polar surface areas (PSA) under 1025 $Å^2$, making them suitable for oral administration.

In addition to adhering to Lipinski's Rule, candidate compounds must also pass pharmacokinetic and toxicity assessments conducted using pkCSM software. The results of drug-likeness analysis, along with absorption, distribution, metabolism, excretion, and toxicity (ADMET) predictions, are presented in Table 5.

A compound is considered to have blood-brain barrier (BBB) permeability if its log BB value in the distribution phase is greater than 0.3. Molecules with a log BB value below 0.1 are not effectively distributed in the brain. CYP2D6 metabolic parameters predict whether cytochrome P450 will likely metabolize a given molecule. The total clearance (CLtot) parameter indicates excretion rates in log (ml/min/kg). Drug clearance primarily occurs through renal and hepatic clearance (kidney excretion) (liver metabolism and bile excretion). Ames toxicity testing is a commonly used method to evaluate the mutagenic potential of compounds through bacterial assays. Among the candidates, Dimethyl Tetracycline meets both Lipinski's rule of five and ADMET prediction criteria.

The Evaluation of granule preparations includes tests for flow rate, angle of repose, bulk density, tapped density, Carr's compressibility index, and Hausner ratios.[26] In this study, optimization using design experts focuses on flow rate, angle of repose, and Carr's index. Good flow characteristics are defined by the ability of particles to flow independently without clumping, influenced by gravitational force.[27] The flow rate test indicates that all the effervescent granules produced exhibit excellent flow, with a suitable flow time greater than 10 grams per second.

The flow rate results of the effervescent granules for each formula are presented in Table 6. Based on the observations for Formula 3 and Formula 4 in Table 6, these formulas exhibit a faster flow time due to a higher tartaric acid content than Formula 1 and Formula 2. Tartaric acid has a higher density than citric acid, which allows granules with a greater tartaric acid content to flow more rapidly because of the increased gravitational force.[27] The

angle of repose is the stable angle formed between a pile of cone-shaped particles and a horizontal plane. If the angle is less than 30°, the material is considered to flow easily.

Conversely, if the angle is 40° or greater, the material will likely be difficult to flow. The shape of the granules can influence the value of the angle of repose.[28] Table 6 presents the results of the stationary angle test for formulas 1-5, all of which are below 30°. A stationary angle of no more than 30° indicates excellent flow properties, meaning all the formulas demonstrate good flow behavior. The granules flow more quickly and easily with less friction and tensile force between them. Furthermore, smaller granule sizes increase cohesiveness, reducing the flow velocity and resulting in a higher stationary angle.[29]

Determining bulk density includes measuring the actual weight, compressive weight, Hausner factor, and percent compressibility. The Hausner factor is used to compare the actual and compressive weights, helping to assess the flow or free-flowing properties of the powder. All seven formulas meet the qualification of having a Hausner factor of less than 1.25, indicating good flow characteristics. Granule compressibility refers to the ability of the granules to maintain compactness under pressure. Factors such as porosity, type density, particle shape, and moisture content can affect the flow properties of the granules. Good flow properties ensure easier molding of the granules and help maintain uniform weight. The results for the Hausner factor and compressibility are shown in Table 6. The percent compressibility results indicated that Carr's index ranged from 4.26% to 14.59%, which aligns with the literature stating that granules with a Carr's index value below 15% demonstrate good flowability.

The optimal formula using Design Expert is intended to generate the most efficient formula based on the response data from the prepared parameters. The response data, analyzed through ANOVA in Design Expert, is processed to identify the optimal formula.[10] The ideal formula is the one with a desirability value closest to 1. Using the simplex lattice design

method in the Design Expert software, the optimal formula was determined to have 65.81 mg of tartaric acid and 4.19 mg of citric acid, with a desirability value of 0.86. Before finalizing, the optimal formula requires verification. The results of design expert optimization formula 1 solution and formula 2 solution are shown in Figure 4, with the formula test results from the design expert optimization provided in Table 7.

This study utilized a Design Expert to optimize the effervescent granule formulation containing PC extract Figure 4. This software allows integrated analysis to evaluate interactions between formulation variables and determine the optimal combination of ingredients used. The optimization of the effervescent granule formulation resulted in two optimal formulas. Formula 1: 13.16% tartaric acid and 0.84% citric acid. Formula 2: 13.21% tartaric acid and 0.80% citric acid. The desirability score for both formulas was 0.862, indicating a high optimization level. The flow rate and angle of repose parameters from the optimized formulas showed no significant differences compared to laboratory experimental results ($p > 0.05$), suggesting the predictive model's accuracy in Figure 5.

Based on the GraphPad Version 9.5.1 (528), 2023 statistical analysis Figure 5, the flow rate and angle of repose values from Formula 1 and Formula 2 in both the Design Expert optimization and the actual test results showed no significant difference, indicating that the optimization and laboratory test produced similar outcomes. However, Carr's index test revealed a discrepancy between the Design Expert optimization and the test results, as effervescent granules are highly sensitive to room temperature, which may have influenced the test outcomes.

Additional research is needed to isolate compounds from PC based on the results of the in silico data. An integrated study of network pharmacology and component analysis should be conducted to explore the molecular mechanisms of PC extract in treating obesity.[30] In silico anti-obesity activity should be explored using additional receptor targets, as the anti-

obesity mechanism extends beyond pancreatic lipase. Central nervous system mechanisms can be investigated, targeting receptors such as GLP-1 (liraglutide), 5-HT2c (lorcaserin), and TAAR-1 (phentermine). In vivo, testing is also recommended to validate the efficacy and safety of these compounds in animal models and clinical settings. Furthermore, advanced formulations, such as nanocarrier systems, could be developed to enhance PC-based products' bioavailability and therapeutic potential. Future studies should focus on the antioxidant properties of the sample using comprehensive methods.[31] These include determination of hydrogen peroxide scavenging capacity, determination of ferric reducing power, determination of nitric oxide (NO) scavenging activity, determination of ascorbic acid, determination of vitamin e, and assessment of lipid peroxidation inhibition. These assays will provide a deeper understanding of the antioxidant potential and the mechanisms by which the sample mitigates oxidative stress.

Conclusion

The antioxidant activity of the PC fraction, evaluated using the DPPH method, revealed that the ethanol fraction exhibited significantly stronger antioxidant activity (IC_{50} = 47.27 ± 1.90 µg/mL) compared to the ethyl acetate fraction (IC_{50} = 201.89 ± 20.08 µg/mL, $p < 0.05$). This difference highlights the greater presence of polar compounds, such as flavonoids and phenolics, in the ethanol fraction. Among the identified compounds, dimethyl tetracycline showed the lowest binding energy (-7.78 kcal/mol) in molecular docking studies, suggesting its potential as a strong pancreatic lipase inhibitor. This was further supported by its adherence to Lipinski's Rule of Five, indicating good oral bioavailability. Formula optimization using the Design Expert software resulted in two formulas. The flow rate and angle of repose values from the design expert and the laboratory tests did not show significant differences, indicating that the optimization and experimental results aligned.

However, differences were observed in the Carr's Index test between the design expert optimization and the lab results.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors affirm that the work presented in this article is original, and they accept full responsibility for any claims related to the article's content.

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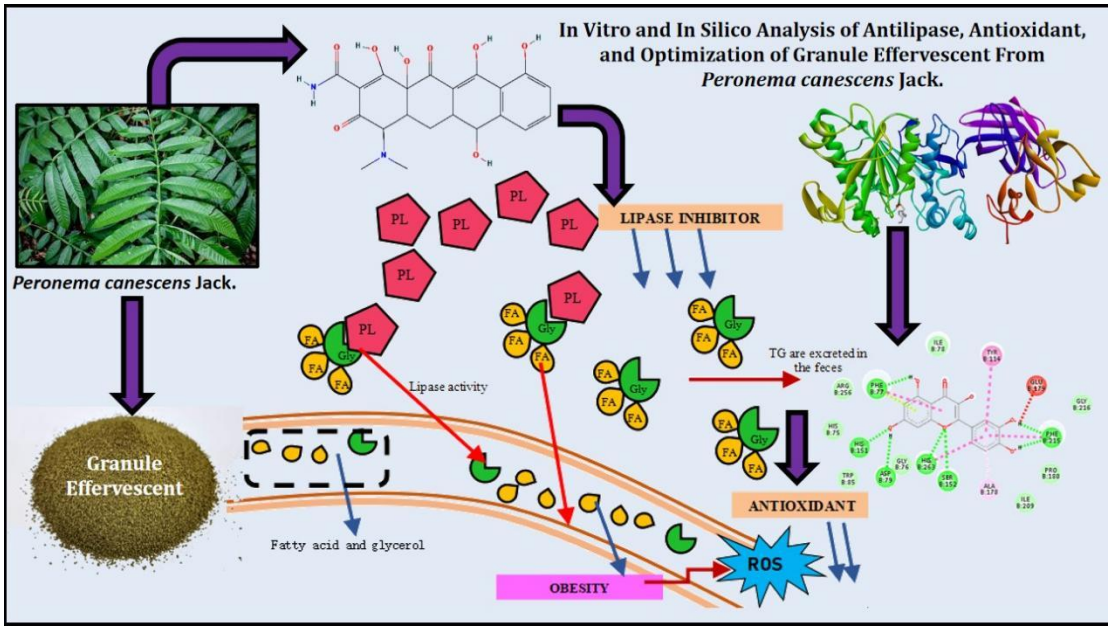

542

543 **Figure 1:** Research methods for *in vitro* and *in silico* analysis of antilipase, antioxidants, and

544 optimization of effervescent granules from PC.

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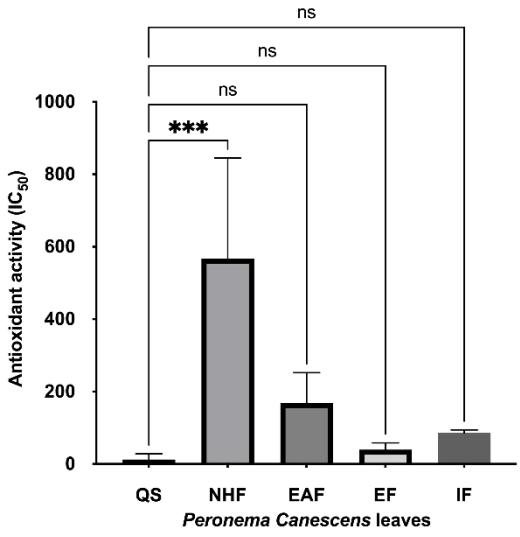

546

547 **Figure 2:** Antioxidant activity of PC fractions measured by DPPH assay, ns = not Significant

548 (p>0,05), ***(p<0,001), QS (Quercetin standard), NHF (n-Hexane fraction, EAF (Ethyl acetate

549 fraction), EF (Ethanol fraction), IF (Insoluble fraction).

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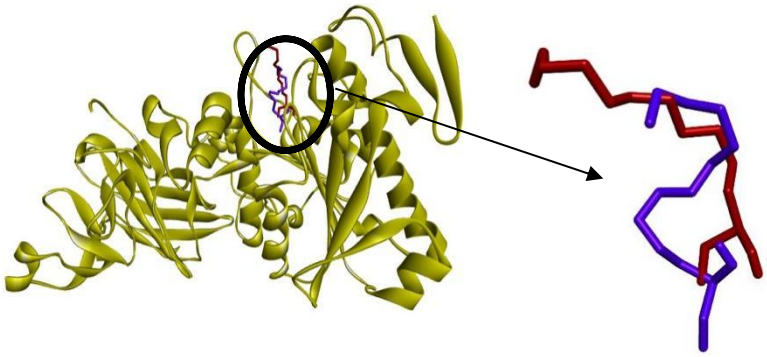

Figure 3: 3D structure of the pancreatic lipase enzyme (PDB ID 1LPB) showing an overlay of the blue (before) and red (after) molecular docking of the native ligand.

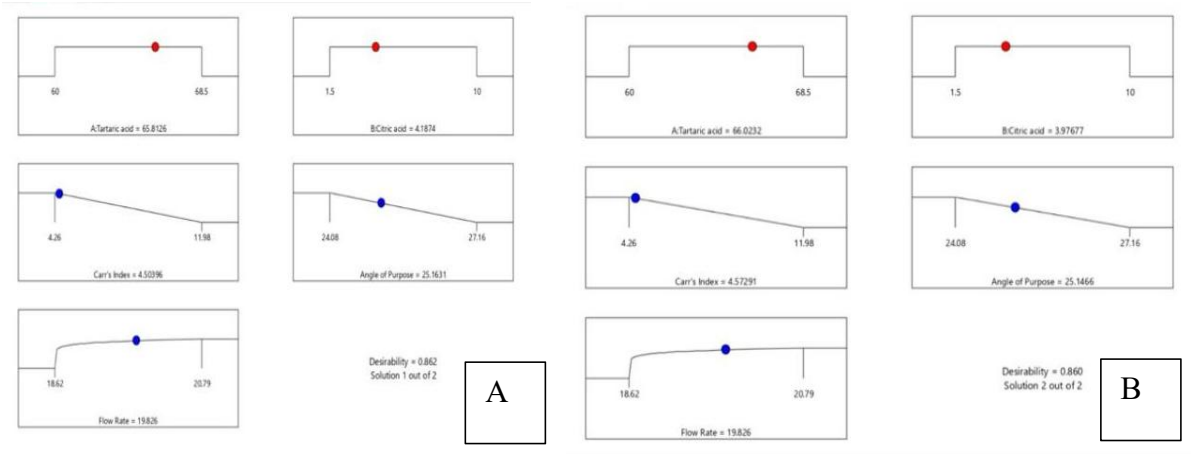

Figure 4: Design Expert optimization (A) Formula 1 Solution (B) Formula 2 Solution

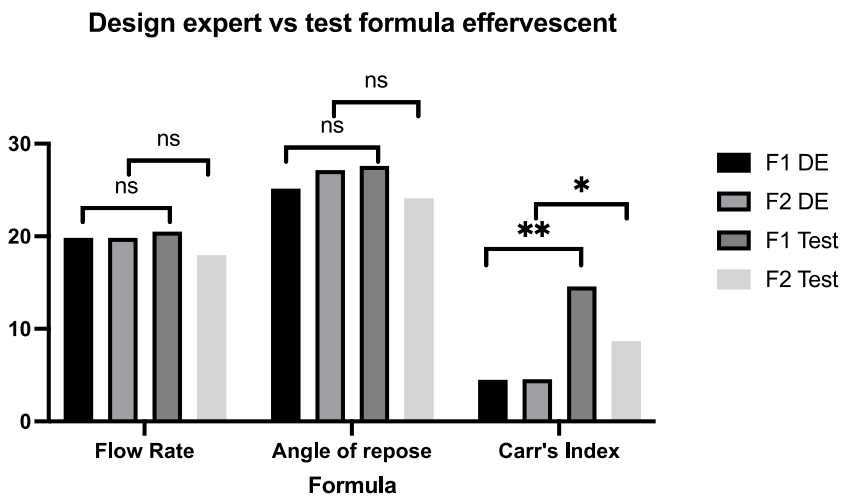

Figure 5: Formula optimization using Design expert vs test, ns = not Significant (p>0,05), *(p<0,05), F1 DE (Formula 1 Design Expert), F2 DE (Formula 2 Design Expert), F1 Test (Formula 1 test), F2 Test (Formula 2 test)

Table 1: Formulation of Effervescent Granules from PC Extract.

Ingredient	**Formula**				
	A	**B**	**C**	**D**	**E**
PC Extract	10%	10%	10%	10%	10%
Tartaric Acid	12.72%	12.30%	13.58%	14%	13.15%
Citric Acid	1.58%	2%	0.72%	0.30%	1.15%
Na. Bicarbonate	14.30%	14.30%	14.30%	14.30%	14.30%
Sucrose	60.40%	60.40%	60.40%	60.40%	60.40%
PVP	1%	1%	1%	1%	1%

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589 **Table 2:** Pancreatic antilipase activity of the PC fraction.

590 **Sample**	**% inhibition of ±SD**	**Types of Antilipase** [2]
591 Orlistat Standard	61.64±9.11	Strong
592 N-hexane fraction	18.66±5.21	Weak
593 Ethyl Acetate Fraction	67.65 ±8.04	Strong
594 Ethanol Fraction	14.22±4.69	Weak
595 Insoluble fraction	6.45±1.13	Weak

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Table 3: Molecular docking scores and interactions of the identified compounds.

No	Structure Name	Binding Energy	Amino Acid Bonds
1	Orlistat	-6.62	Gly 76, Phe 77, Ile 78, Asp 79, Tyr 114, His 151, **Ser 152,** Leu 153, Ala 178, Glu 179, Pro 180, Ile 209, Phe 215, Arg 256, Ala 259, His 263, Leu 264
2	Quercetin	-8.28 (Run of 83)	His B:75, Gly B:76, Phe B:77, Ile B:78, Asp B:79, Trp B:85, Tyr B:114, His B:151, **Ser B:152,** Ala B:178, Glu B:179, Pro B:180, Ile B:209, Phe B:215, Gly B:216, Arg B:256, His B:263.
3	Dimethyl Tetracycline	-7.78 (Run of 14)	Gly B:76, Phe B:77, Ile B:78, Asp B:79, Tyr B:114, His B:151, **Ser B:152,** Leu B:153, Ala B:178, Pro B:180, Phe B:215, Arg B:256, Asp B:257, Ala B:259, Ala B:260, His B:263, Leu B:264.
4	2-methoxy-5H-indole[2,3-b]quinoxaline	-7.25 (Run of 77)	His B:75, Gly B:76, Phe B:77, Ile B:78, Asp B:79, Trp B:85, Tyr B:114, His B:151, **Ser B:152,** Leu B:153, Ala B:178, Pro B:180, Ile B:209, Phe B:215, His B:263, Leu B:264.
5	Trilaurin	-3.52 (Run of 7)	Ile B:78, Tyr B:114, His B:151, **Ser B:152,** Leu B:153, Ala B:178, Pro B:180, Ile B: 209, Leu B:213, Phe B:215, Trp B:252, Thr B:255, Arg B:256, Ala B:259, Ala B:260, His B:263, Leu B:264.

627 **Table 4:** Predicted Lipinski's Rule of Five for the Ligands.

No	Molecular Name	Molecular Weight	Log P	Hydrogen Bond Donor (HBD)	Hydrogen Bond Acceptor (HBA)	Polar Voltage Activity (PSA)
1	Quercetin	302.24	1.99	5	7	122.11
2	Dimethyl Tetracycline	430.41	-0.55	6	9	176.06
3	2-methoxy-5H-indolo[2,3-b]quinoxaline	249.27	3.27	1	3	108.60
4	Trilaurin	639.02	11.75	0	6	278.43

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629 **Table 5:** ADMET Prediction for Compounds from the Ethyl Acetate Fraction of PC

No	Molecular Name	Absorption (CaCO2 Permeability) (log Papp in 10-6 cm/sec)	Distribution (VDss (human)) (log L/kg)	Metabolism (CYP2D6) (YES/NO)	Excretion (Total clearance) (log ml/min/kg)	AMES Toxicity (YES/NO)	Hepato toxicity	Skin Sensitization
1	Orlistat	0.40	-1.02	No	1.68	No	Yes	No
2	Quercetin	-0.28	0.06	No	0.46	No	No	No
3	Dimethyl tetracycline	-0.01	0.61	No	0.35	No	No	No
4	2-methoxy-5H-indolo[2,3-b]quinoxaline	1.30	-0.01	No	0.77	Yes	Yes	No
5	Trilaurin	0.14	-0.82	No	2.23	No	No	No

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631 **Table 6:** Flow rate, angle of repose, and bulk density of the effervescent granules from PC.

Formula	Flow rate	Angle of repose	Bulk density (g/ml)	Tapped density (g/ml)	Hausner ratios	Carr's compressibility index (%)
A	18.66	25.05	0.52	0.56	1.07	6.25
B	20.43	25.85	0.53	0.55	1.04	4.26
C	20.79	27.16	0.53	0.56	1.07	6.38
D	20.63	24.09	0.50	0.56	1.14	11.98
E	18.74	26.03	0.49	0.53	1.06	5.99

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633 **Table 7:** Results of the formula test from Design Expert Optimization.

Formula	Flow rate	Angle of repose	Bulk density (g/ml)	Tapped density (g/ml)	Hausner ratios	Carr's compressibility index (%)
1	20.50	27.61	0.52	0.60	1.17	14.59
2	17.96	24.10	0.54	0.59	1.10	8.68

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BUKTI KORESPONDENSI

Author's Reply to Editor and Reviewers Comments

Journal name : Tropical Journal of Natural Product Research

Manuscript Title : *In Vitro and In Silico Analysis of Antilipase, Antioxidant, and Optimization of Granule Effervescent from Peronema canescens Jack.*

Manuscript No : TJNPR MH559AR

Type of paper : Research Article

Authors Name : Muhammad Ryan Radix Rahardhian, Nurchasanah, Yasmiwar Susilawati, Sri Adi Sumiwi, Dewi Ramonah, Chintiana Nindya Putri, Ririn Suharsanti

Reply to the reviewers' comments

Reviewer Number	Original comments of the reviewer	Reply by the author(s)	Changes done on page number and line number
1	Line 9 sungkai, change to Sungkai	The term "sungkai" on line 9 has been corrected to "Sungkai" as per the suggestion. We have ensured consistency in capitalization throughout the manuscript.	Page 1 line 19
1	Line 21 ADMET, describe admet	We have added a description of ADMET.	Page 2, line 31-32

		ADMET refers to the Absorption, Distribution, Metabolism, Excretion, and Toxicity properties of compounds, which are critical parameters in evaluating the pharmacokinetics and safety profile of potential drug candidates.	
1	Line 31 RISKESDAS, describe RISKESDAS	We have added a description of RISKESDAS. RISKESDAS refers to "Indonesia basic health research".	Page 2 line 45
1	Line 55-56 add reference	We have added an appropriate reference to support the statement on lines 55-56. The updated reference is now included in the revised manuscript and properly cited in the reference list.	Page 3, line 65-66
1	Line 63 add reference	We have added an appropriate reference to support the statement on lines 63. The updated reference is now included in the revised manuscript and properly cited in the reference list.	Page 3, line 75
1	Line 140 add reference	We have added an appropriate reference to support the statement on lines 140. The updated reference is now included in the revised manuscript and properly cited in the reference list.	Page 6, line 151
1	Every data change comma to dot and consistently 2 digits behind the dot	We have revised all numerical data in the manuscript to ensure consistency in formatting. All commas have been replaced with dots as decimal separators, and all numerical values are now	Page 24, table 1 Page 24, table 2 Page 24, table 3 Page 26, table 4 Page 26, table 5 Page 27, table 6

		presented with two digits after the decimal point.	Page 27, table 7
1	Add a closing statement that emphasizes the contribution of the research to scientific development or its applications.	We have added a closing statement in the conclusion about contribution of this research to scientific development and its potential applications. In vivo studies are also recommended to validate the efficacy and safety of these compounds in animal models and clinical settings. Furthermore, advanced formulations, such as nanocarrier systems, could be developed to enhance PC-based products' bioavailability and therapeutic potential.	Page 16, line 392-395
1	In line 359 Demethyltetracycline, 2-methoxy-5H-indolol [2,3-bquinoxaline rewrite to Demethyltetracycline, 2-methoxy-5H-indol [2,3]quinoxaline	We have revised Demethyltetracycline, 2-methoxy-5H-indol [2,3]quinoxaline	Page 27, table 6
2	The abstract word count is < 250. The authors should correct all grammatical, spellings and punctuation errors.	Thank you for the feedback. We have revised the abstract to meet the recommended word count of <250 words, and we have carefully corrected all grammatical, spelling, and punctuation errors. The abstract has been revised as suggested	Page 1 Line 19-37
2	The aim and objectives of the research are obvious. The authors should include a statement of research novelty. Authors should briefly discuss the relevance of the research methods to the research. All grammatical, spellings and punctuation errors should be corrected. The literature review should	Thank you for your valuable feedback. We have revised the manuscript based on your suggestions: 1. We have included a statement of the research novelty.	1. Page 4 line 97-100 2. Page 5 line 106-113 3. Page 1-18 4. Page 3 line 66

	mostly be contextual. Use recent citations. Abbreviated words should be mentioned in full prior to abbreviations.	2. We have clarified the relevance of the research methods to the research. 3. All grammatical, spelling, and punctuation errors have been corrected. 4. The literature review has been updated to include recent and relevant citations, providing better context. 5. Abbreviations are now fully defined before being abbreviated.	5. Page 1, line 12, 13, 16, 20
2	Include methods for the statistical analysis. Mention the software used in the statistical analysis alongside the manufacturer, version and release year. Include the number of replicate measurements. All chemicals and reagents used should be mentioned alongside the manufacturer, % purity, conc., grade and specificities. All equipment and instrument used should be mentioned alongside their model, manufacturer and country. Authors should specify instruments/equipment used for each experiment. All methods used should be validated with relevant citations. All grammatical, spellings and punctuation errors should be corrected. Authors should include the GPS location of the sample collection	Thank you for the constructive feedback. We have revised the manuscript as per your suggestions: 1. We have included the statistical analysis methods, specifying the software used and relevant details. 2. All chemicals and reagents have been listed alongside the manufacturer, purity, concentration, grade, and specificities. 3. The instruments and equipment used for each experiment have been mentioned, along with their model, manufacturer, and country. 4. We have added citations to validate the methods used in the	1. Page 9, line 209-214 2. Page 5, 106-113 3. Page 7 line 154-155 4. Page 10 line 250, page 11 line 268 5. Page 6, line 132

		study. 5. The GPS location of the sample collection site has been included	
2	All results should be presented based on the journal guideline. Images should be of a very high quality/resolution (300 dpi) – some images were not clear and readable. Authors should forward all supplementary data (input and output files) associated with the research to the journal management for validations and authentications. Use footnotes were necessary to define terms. Crosscheck the methodologies to ensure that results obtained tally with methods used. The results of the IC50 (antioxidant assay) should be presented as graphs used in the estimation of the IC50. Authors should include the GCMS spectra obtained from the analysis. Authors shouldn't use commas as decimal points.	Thank you for your detailed feedback. We have addressed the comments as follows: 1. We have revised the results section to align with the journal guidelines. 2. We have ensured that all images meet the required resolution of 300 dpi and are clear and readable. 3. Footnotes have been added where necessary to define terms. 4. The IC50 results from the antioxidant assay are now presented as graphs, as requested. 5. Thank you for your feedback. Due to the extensive nature of the GC-MS spectra data, we have included it as supplementary material, which has been submitted alongside the manuscript for review and validation 6. We have corrected all instances of commas used as decimal points and replaced them with periods	1. Page 9, line 216-page 17 395 2. Page 23, figure 3 3. Page 22, Figure 2. Page 23 figure 5 4. Page 13, Figure 5 5. Page 4 line 96 Page 8 line 167 6. Page 24, table 1 Page 24, table 2 Page 24, table 3 Page 26, table 4 Page 26, table 5 Page 27, table 6 Page 27, tablel 7
2	The discussions should be mostly statistically comparative. Authors should discuss mainly the	Thank you for your valuable feedback.	1. Page 22, Figure 2 Page

	significant findings in details. Authors should use recent citations in discussing their findings. All grammatical, spellings and punctuation errors should be corrected	1. Statistically Comparative Discussion 2. Focus on Significant Findings 3. Recent Citations for Discussion 4. Grammatical, Spelling, and Punctuation Corrections	23 Figure 5. 2. Page 11, Line 398-409 3. Page 11, line 267-269 4. Page 1-18
2	Authors should capture the future prospects of the research.	Thank you for the suggestion. We have added a section discussing the future prospects of the research, highlighting potential applications, further research directions, and broader implications	Page 16, line 392-395
2	Authors should ensure that all the references follow the journal guideline. The number of cited references must tally with those at the references sections	Thank you for highlighting this issue. We have thoroughly reviewed and revised the manuscript to ensure that all references adhere to the journal's formatting guidelines. Additionally, we have cross-checked the in-text citations to ensure they tally with the references listed in the references section.	Page 21, line 622-24 line 721
2	All figures should be presented based on the journal guideline. All figures should be of a very high quality/resolution. Figures 3 and 4 captured were not discussed in the manuscript	Thank you for your constructive feedback. We have revised the manuscript to ensure that all figures comply with the journal guidelines and meet the required resolution of at least 300 dpi. Figures 3 and 4 have been discussed in the manuscript, and their relevance to the study has been clarified.	Page 23, Figure 3 Page 12, line 284 Page 16, line 367 Page 16, line 371
2	All tables should be presented based on the journal	Thank you for your valuable feedback.	Page 24, table 1

	guideline. Table captured was discussed in the manuscript.	We have revised the manuscript to ensure all tables comply with the journal's formatting guidelines. Additionally, all tables have been reviewed to confirm they are appropriately discussed and integrated into the manuscript.	Page 24, table 2 Page 24, table 3 Page 26, table 4 Page 26, table 5 Page 27, table 6 Page 27, tablel 7
2	Remove *	Thank you for the feedback. We have removed the asterisk (*) from the term 'pdbqt' in the manuscript to improve clarity and consistency	Page 8 line 192
2	in full prior to abbreviations	Thank you for your comment. We have revised the manuscript to include the full form of DPPH, TLC, GC-MC, p-NPB, and ADMET before its abbreviation to ensure clarity for the readers	Page 1, line 23-32
2	include reference – citation needed	Thank you for the comment. We have added the appropriate reference to support the statement about the types of antioxidants in the manuscript	Page 9, line 224
2	include reference – citation	Thank you for the suggestion. We have added the necessary reference to support the discussion on the types of anti-lipase in the manuscript	Page 11. Line 258
2	do not use commas as a decimal point rather use periods	Thank you for the feedback. We have revised the manuscript to use periods (.) as the decimal point instead of commas to align with international formatting standards	Page 24, table 1 Page 24, table 2 Page 24, table 3 Page 26, table 4 Page 26, table 5 Page 27, table 6 Page 27, tablel 7
2	Figure 1	Change to Figure 4	Page 20 line 375

2	all images should be clear and readable – at least 300dpi	All images presented in this manuscript have been reviewed and updated to ensure they meet a minimum resolution of 300 dpi for clarity and readability	Page 26, table 4
3	A brief conclusion should be added at the end of abstract.	A conclusion has been added at the end of the abstract : *Peronema canescens* Jack. (PC) leaves has the potential as an antioxidant and antiobesity and can be developed into effervescent formula.	Page 2 Line 36-37
3	Many grammatical errors and typos appear in the text such as anti-lipase, anti-obesity, in vitro, in silico, etc. It is suggested that the authors revisit and improve the English quality of the manuscript.	has been corrected throughout the manuscript	has been corrected throughout the manuscript
3	In Figure 4, statistical analysis was applied to examine the granule characteristics. Which statistical method was used here? The statistical analysis should be mentioned in the Methods section in detail.	Data analysis has been added to the method : The data were expressed as the mean ± standard deviation (SD) of experiments in triplicate. This statistical analysis in this study was carried out with one way anova using a GraphPad Prism (version 9.5.1 (528), 2023. Graph Pad Inc. software San Diego, CA, USA). IC50 value represented the concentration of the test sample causing 50% inhibition in which value <0.05 was considered significant.	Page 16 Line 370-378
3	The IC_{50} of ethanol fraction is higher than that of quercetin standard showing the better antioxidant activity of the standard as compared to the PC fraction. How did the authors explain this result?	There is a writing error regarding the results related to table 2, the author has corrected the sentence in the discussion section and added an explanation to the related results : As indicated in Figure 2, quercetin as a	Page 9 Line 222-233

		positive control had the highest antioxidant activity and the ethanol fraction exhibits stronger antioxidant activity than the other fraction samples, with the order being Ethanol fraction > ethanol extract > insoluble fraction > ethyl acetate fraction > n-hexane fraction. The OH group on quercetin can function as a hydrogen donor. This means that quercetin can donate hydrogen atoms to neutralize free radicals, thereby reducing the potential for oxidative damage to cells**.**	
3	What is the correlation of antioxidant activity and anti-lipase potency of the PC towards the anti-obesity as the primary claim of this study?	has been added to the discussion section of the relationship between antioxidants and anti-obesity : Obesity triggers inflammatory processes, lipogenesis excess, inhibit lipolysis, and increase adipocyte apoptosis. This matter increases the release of Reactive Oxygen Species (ROS) and will cause oxidative stress. Oxidative stress caused by obesity can result damage to cells and tissues and trigger the emergence of diseases degenerative. Antioxidants help neutralize radicals free and reduces the risk of complications from degenerative diseases. Therefore Supplementation with antioxidants will reduce the risk of disease complications associated with obesity and oxidative stress.	Page 12 Line 260-266
3	Ethanol fraction was revealed as the most potent anti-oxidant agent, while ethyl acetate showed the highest pancreatic anti-lipase activity. Why do these two assays	has been added to the discussion section: The ethanol fraction proved to be the most	Page 12 Line 267-275

	demonstrate different findings? Which type of fraction was finally used in the effervescent formula? And why? Authors are suggested to discuss it more profoundly in the Results and Discussion Section.	potent antioxidant agent, while ethyl acetate showed pancreatic anti-lipase activity highest. This is because there are differences in the compounds that guide these two activities. These results are in accordance with research on other materials that the ethyl acetate fraction has higher anti-obesity activity than the ethanol fraction, while the ethanol fraction has stronger antioxidant activity than the ethyl acetate fraction. When related to compound content, the ethanol fraction has higher total phenolic content and total flavonoid content than the ethyl acetate fraction. It is necessary to prove the levels of PC leaf extracts and fractions regarding the levels of compounds not only phenolics and flavonoids, but also other groups of compounds. So to support both activities an effervescent preparation will be made from PC leaves extract.	
3	Why is the optimized effervescent granule formula not continued with the tablet formulation?	has been added to the discussion section: Effervescent granules are preferred because they are easy to use, dissolve easily in water, taste better, and when compared to tablet preparations, effervescent granules reduce stomach irritation which sometimes occurs when tablet preparations are swallowed directly and reduce the risk of blockage in the esophagus because they are completely dissolved in liquid. before consumption.	Page 12 Line 275-280

3	Authors need to enrich the Discussion with more relevant references	References have been added to support the results	Page 9, line 222-Page 17, line 395
3	Figure legends in Figure 3 are too small.	figure 3 has been enlarged and moved to the bottom	Page 23, Figure 3
Editorial comments	Cite two related published articles from TJNPR	Thank you for the suggestion. We have added references to two related published articles from *TJNPR* to strengthen the background and context of our study 1. Chike-Ekwughe A, John-Africa LB, Adebayo AH, Ogunlana OO. Evaluation of the In vitro and In silico Pancreatic Lipase Inhibitory Activity of Ethanol Leaf Extract of Tapinanthus cordifolius and its Effect on Oral Glucose Tolerance in Mice. Trop J Nat Prod Res. 2024;8(8):8168–8175. 2. Syofyan, S, Almahdy, A, Wulandari, A, Alen, Y, Diliarosta, S, Kurniawan, H, Noverial, N, Putra, P.P, Dillasamola, D. Effects of Ethanol Extract of Sungkai (Peronema canescens Jack.) on Fertility of Female Wistar Mice (Mus musculus L.). Trop J Nat	Page 19 line 460 Page 20 line 485 Page 21 line 498

		Prod Res. 2023;7(5):2863–2866. 3. Falodun A, Siraj R, Choudhary MI. GC-MS Insecticidal Leaf essential oil of P. staudtii Hutch and Dalz (Icacinaceae). Trop J. Pharm Res. 2009; 82:139-143.	
	Cite related published article	Okolie NP, Falodun A, Oluseyi D. Evaluation of the Antioxidant Activity of Root Extract of Pepper Fruit (Dennetia Tripetala), and It's Potential for the Inhibition of Lipid Peroxidation. Afr J. of Trad Compl and Altern Med. 2014;11(3):221–227.	Page 22 line 530
	Adhere strictly to the Journal's style for listing references. Abbreviate all journal names, and replace 'et al' with names of all contributing authors		
	All journal titles should be abbreviated ex : Trop J Nat Prod Res	We have abbreviated all journal titles, et al is not allowed in the references	Line 441-532
	The table format should be like table 1 (table 3)	We have adapted table 3 to the format of table 1	Page 27 line 618

Note : Highlighted turquoise for changes to response reviewer.

Trop J Nat Prod Res, February 2025; 9(2): ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

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Original Research Article

In Vitro and in Silico Analysis of Antilipase, Antioxidant, and Optimization of Granule Effervescent from *Peronema canescens* Jack

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ABSTRACT

Obesity results from prolonged energy imbalance, with anti-obesity treatment targeting pancreatic lipase inhibition. *Peronema canescens* Jack. (PC) known as Sungkai, has traditionally been used to treat various ailments. This study aimed to assess PC antioxidant and antilipase activities and optimize effervescent granule formulations. Phytochemical screening and thin-layer chromatography (TLC) were performed, followed by antioxidant analysis using 2,2-diphenyl-1-picrylhydazyl (DPPH) and pancreatic antilipase activity, using the p-NPB substrate, were employed. The ethanol fraction of PC demonstrated potent antioxidant activity (IC_{50} = 47.27 µg/mL), while the insoluble fraction showed the highest pancreatic antilipase activity (67.65%). Gas chromatography-mass spectrometry (GC-MS) identified active compounds, including dimethyl tetracycline, 2-methoxy-5H-indolo[2,3-b] quinoxaline, and trilaurin, with molecular docking study indicating dimethyl tetracycline was the most effective antilipase candidate, binding to the pancreatic receptor (PDB ID: 1LPB). This compound also met Lipinski's Rule of Five and ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity), suggesting favorable pharmacokinetics and safety. Evaluation of effervescent granules included angle of repose, bulk density, and tapped density. Optimization of tartaric and citric acid concentration using Design Expert 13 yielding two optimal formulas: Formula 1 with 13.16% tartaric acid and 0.84% citric acid, and Formula 2 with 13.21% tartaric acid and 0.80% citric acid. PC leaves have the potential to be an antioxidant and anti-obesity and can be developed into effervescent formula.

Keywords: *Peronema Canescens* Jack., Antioxidant, Effervescent granules, Molecular docking, Pancreatic antilipase.

Introduction

The increasing prevalence of degenerative diseases in Indonesia, alongside infectious diseases, indicates changing health challenges, with obesity emerging as a major concern. RISKESDAS (Indonesia's basic health research) data reveal a rise in obesity rates from 14.8% in 2013 to 21% in 2018.[1] Factors that contribute to obesity encompass environmental factors, urban living, and eating patterns. Diets high in fats and sugars but low in fiber cause an energy imbalance, which, when combined with triglyceride buildup, this imbalance triggers oxidative stress and inflammatory responses within the body.[2] This ongoing inflammation, fat accumulation, and suppression of fat breakdown causes adipocyte apoptosis, producing Reactive Oxygen Species (ROS) that harm cells and tissues, raising the risk of degenerative diseases.[3] Antioxidants are essential for neutralizing ROS, helping to reduce the risk of degenerative diseases linked to oxidative stress.[4] In the context of medical treatments, FDA-approved drugs for obesity aim to either decrease calorie absorption or control appetite.

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Citation: Rahardhian MRR, Nurchasanah, Susilawati Y, Sumiwi SA, Ramonah D, Putri CN, Suharsanti R. In Vitro and in Silico Analysis of Antilipase, Antioxidant, and Optimization of Granule Effervescent from *Peronema canescens* Jack. Trop J Nat Prod Res. 2025; 9(2):

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Central nervous system (CNS) suppressants, including lorcaserin, liraglutide, phentermine-topiramate, and naltrexone/bupropion, work by targeting appetite-regulating receptors such as 5HT2c, GLP-1, and TAAR-1. On the other hand, Orlistat acts as a lipase inhibitor, reducing the absorption of dietary fats by approximately 30%.[5] People in Indonesia prefer using herbal medicine due to its natural properties, which are perceived as safer and less likely to cause unwanted side effects. In general, herbal medicines are more affordable than synthetic drugs. They also contain a variety of plant-based ingredients. Herbal medicine is considered effective for targeting multiple health issues. Conversely, Orlistat is a therapeutic agent for obesity that reduces calorie absorption in the intestinal tract.[5] Nevertheless, the effectiveness of Orlistat is constrained by side effects such as gastrointestinal problems, including oily stools, flatulence, and rectal discharge.[6] These limitations highlight the importance of seeking complementary or alternative treatments, especially natural ones with fewer side effects and potential long-term benefits. Herbal medicine presents a promising alternative to synthetic drugs for managing obesity, thanks to its safety, availability, and ability to target multiple mechanisms. *Peronema canescens* Jack. (PC), locally known as Sungkai, has attracted attention for its potential therapeutic benefits. Traditionally utilized in Indonesian medicine, the leaves of PC contain secondary metabolites like phenols, triterpenoids, flavonoids, tannins, alkaloids, steroids, and saponins, which have been reported to exhibit anti-inflammatory, antioxidant, antidiabetic, and immune-boosting properties.[7] The bioactive compounds in PC position it as a promising candidate for anti-obesity treatments, primarily by inhibiting

pancreatic lipase, which helps reduce lipid absorption. Recent studies have highlighted the potential of plant-based compounds for pancreatic lipase inhibition, particularly in treating obesity.[8] For example, in silico modeling allows for structural predictions and identifying binding sites, enhancing target interaction in drug development.[9] Moreover, effervescent granules offer a convenient dosage form by combining acidic and alkaline compounds that release CO_2 upon dissolution. These granules provide high solubility, ease of use, and rapid absorption, making them an ideal delivery system for antioxidants and antilipase agents.[10] Given the therapeutic potential of PC, developing a granule formulation can enhance the accessibility and effectiveness of its bioactive components. While traditional treatments like GLP-1 receptor agonists have proven effective in managing obesity, they are especially beneficial for patients with comorbidities such as type 2 diabetes. Other plant-based studies indicate that appetite suppression may occur by activating the 5-HT2C receptor.[11] Additionally, TAAR1 agonists present the potential to address maladaptive eating behaviors associated with metabolic disorders.[12] Inhibitors targeting the lipase enzyme, such as those aimed at PDB proteins 1LPB and 5ZUN, further reinforce the potential of lipase inhibition as a therapeutic target for anti-obesity drugs.[13]

The methods employed in this study, including phytochemical screening, DPPH antioxidant assay, pancreatic antilipase activity testing, and molecular docking, are specifically chosen to assess the bioactive compounds in PC and their potential for obesity treatment. These approaches are highly relevant as they combine experimental and computational techniques to identify promising antilipase candidates. This is the first study to comprehensively evaluate PC antioxidant and antilipase activities while optimizing effervescent granule formulations. The integration of in vitro and in silico approaches in this study provides a novel insight into the potential therapeutic uses of PC in combating obesity. This holistic approach highlights the potential of PC as a safe, accessible, and effective therapy for obesity.

Materials and Methods

Materials

Rotary evaporator (Heidolph-G3), Silica Gel F254 plates, UV lamps (254 nm and 366 nm, Evaco GL 220V 50Hz T8 15W), micropipettes (Socorex & Dragon Lab), vortex mixers, UV-Vis Spectrophotometer (Shimadzu UV-1780, Shimadzu Corporation, Japan), ELISA reader (Synergy HTX, Agilent, USA), GC-MS (Shimadzu QP 2010 SE, Shimadzu Corporation, Japan). *Peronema canescens* Jack (PC), ethanol, n-hexane, ethyl acetate, $FeCl_3$, $MgSO_4$, hydrochloric-ethanolic acid mixture (1:1), hydrochloric acid, Liebermann-Burchard reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH, Sigma-Aldrich, USA, 97% purity, analytical grade) was used for antioxidant assays, quercetin, p.a. methanol, crude porcine pancreatic lipase (PPL), P-nitrophenyl butyrate (p-NPB, Sigma-Aldrich, USA, 98% purity, analytical grade) was used for pancreatic antilipase activity, phosphate buffer (pH 7.2), DMSO, and orlistat standard.

Hardware and Software

Some of the software used, including the receptors for the test, can be downloaded from the RCSB PDB website (https://www.rcsb.org/). The ligands used in the test are available for download from the PubChem website (https://pubchem.ncbi.nlm.nih.gov/). Test ligands and receptors were created using ChemDraw Professional 15.0, Chem3D 15.0, Biovia Discovery Studio 2021, Command Prompt, and AutoDock Tools 1.5.6. Docking visualizations were performed using Biovia Discovery Studio 2021. Lipinski's Rule of Five testing was conducted using the Lipinski rules available at (http://www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp). Pharmacokinetics and toxicology testing were performed using the pk-CSM website (https://biosig.lab.uq.edu.au/pkcsm/). Molecular docking simulations were conducted on a laptop (Acer Aspire A314-35, Laptop-ML0EUN2).

Methods

The general research methods for the in vitro and in silico analysis of antilipase, antioxidants, and optimization of effervescent granules from PC are outlined in Figure 1.

Sample preparation, extraction, and fractionation

The sample used in this study was *Peronema canescens*, Jack (PC), sourced from Kayutanam in Padang Pariaman District, West Sumatra, Latitude : 0°29'46.8312", Longitude : 100°20'7.638", Altitude: Located at an altitude of between 100 to 1000 meters above sea level. Harvested between May and July 2021 from trees measuring 6-7 meters in height. The maceration process was conducted for 3 days (3x24 hours), with occasional stirring and repeated solvent changes using 96% ethanol. The resulting macerate was then filtered and concentrated using a rotary vacuum evaporator, followed by thickening in a water bath at approximately 40°C.[14–16]

Twenty grams of the PC ethanol extract were placed in a beaker with a stir bar and magnetic stirrer. The fractionation process began by adding 100 mL of n-hexane, followed by stirring to separate the liquid from the insoluble extract. This step was repeated 5-6 times, adding 100 mL of n-hexane each time until a clear n-hexane fraction was obtained. Next, 100 mL of ethyl acetate was added to the insoluble n-hexane extract, and the fractionation was repeated until a distinct ethyl acetate fraction was obtained. Subsequently, 100 mL of ethanol solution was used to fractionate the insoluble ethyl acetate extract, repeating the process 5-6 times until a precise ethanol fraction was obtained. The remaining insoluble fraction, treated with ethanol, was designated as the insoluble fraction. The fractions were concentrated using a rotary vacuum evaporator, and the final thickening was performed in a water bath at approximately 50°C to yield a viscous fraction.[14]

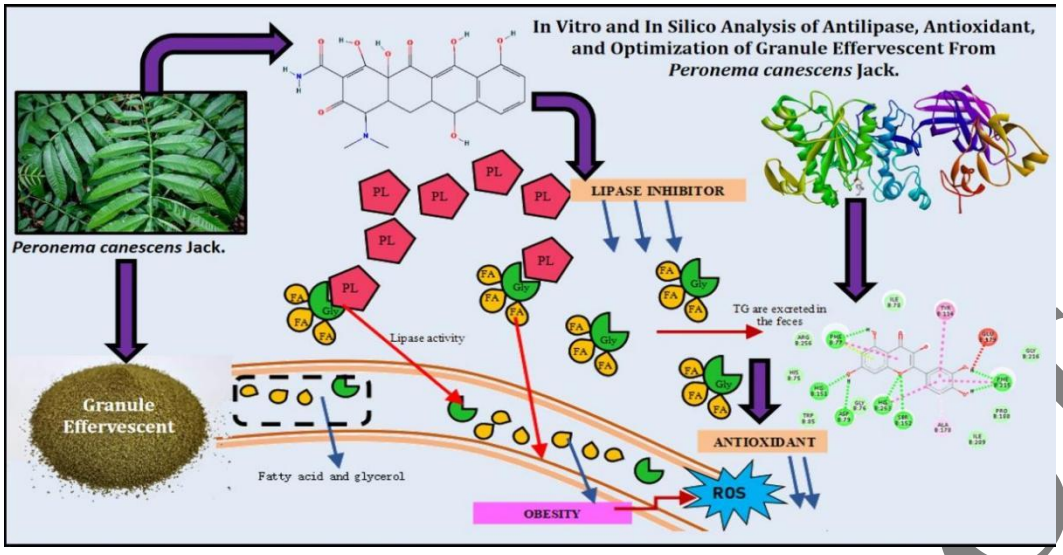

Figure 1: Research methods for *in vitro* and *in silico* analysis of antilipase, antioxidants, and optimization of effervescent granules from PC.

Antioxidant Activity

The PC fraction was dissolved in methanol and prepared at 10, 20, 30, 40, 50, and 60 µg/mL.[17] The antioxidant activity was determined by adding 1.0 mL of the PC fraction solution to a test tube containing 4.0 mL of 0.1 mM DPPH for each concentration. The mixture was homogenized using a vortex for 1 minute and allowed to stand for the designated time for each test solution. The absorbance of the solution was then measured wavelength at 516.0 nm using a UV-Vis spectrophotometer (Shimadzu UV-1780, Shimadzu Corporation, Japan). The same procedure was followed to measure the absorbance of the quercetin standard series.

Pancreatic Antilipase Activity

The pancreatic antilipase inhibition activity of the n-hexane, ethyl acetate, ethanol, and insoluble fractions was assessed using 96-well plates and an ELISA reader (Synergy HTX, Agilent, USA). The enzyme stock concentration was approximately 0.1 µg/mL, prepared by dissolving 1 mg of solid porcine pancreatic lipase (PPL) powder in 1 mL of buffer solution (a). The fraction was prepared at a concentration of 500 µg/mL (b), and p-NPB was dissolved in 1% DMSO (c) and subsequently diluted with a 50 mM phosphate buffer (pH 7.2, 0.5%) to a final concentration of 2.5 mM in 100 µL (d). Solutions (a), (b), and (d) were mixed and incubated at 37°C for 10 minutes. Each sample was tested in triplicate. Orlistat was used as a positive control, and 1% DMSO was the negative control without inhibitors. One unit of activity is defined as the reaction rate that generates 1 µmol of p-nitrophenyl butyrate at 37°C. Lipase activity inhibition was expressed as the percentage reduction in activity when PPL was incubated with the test compound.[18]

Identification of compounds in the active fraction of PC using GC-MS.

GC-MS analysis was conducted at the integrated laboratory of Universitas Islam Indonesia. The active fraction, prepared at a concentration of 500 µg/mL, was injected in a volume of 1.0 µL for analysis using Gas Chromatography coupled with a Flame Ionization Detector (FID) and Mass Spectrometry (MS) (Shimadzu QP 2010 SE, Shimadzu Corporation, Japan). The mobile phase consisted of chloroform: ethanol mixture (1:1), and the analysis was performed using an Rtx-5 MS column (5% diphenyl / 95% dimethyl polysiloxane) with specifications of 0.25 µm thickness, 30.0 m length, and 0.25 mm inner diameter. The instrument settings included an initial temperature of 80ºC, an injection temperature of 300ºC, and an ion source temperature of 250ºC. The oven temperature was gradually increased to 330ºC at 6ºC per minute. The column flow rate was set to 0.74 mL/min with a pressure of 42.3 kPa.[19]

Molecular Docking

The receptors used in this study were obtained from the Protein Data Bank in 3D structure format or were drawn using ChemDraw software. These receptors, which are protein macromolecules, were isolated from any irrelevant molecules along with the ligands. The isolation process was performed using Discovery Studio 2021, and the files were saved in pdb format. Optimization involved adding hydrogen atoms, merging nonpolar hydrogens, and calculating Gasteiger charges using AutodockTools 1.5.6. The resulting file was saved in pdbqt format. For ligand preparation, 2D and 3D structures of the selected ligands were created to determine their molecular structure using ChemDraw Pro 12.0 software. The ligands were then prepared using AutoDockTools 1.5.6, where the compound structures were corrected, and Gasteiger charges were added. The prepared ligands were saved and ready for docking.[9,13]

Evaluation of Drug Likeliness and ADMET

Assessing the drug-likeness of compounds is based on Lipinski's Rule of Five, which utilizes both experimental and computational approaches to evaluate solubility and permeability in drug discovery and *development*.[20] The Rule of Five suggests that poor absorption and permeability are likely when the molecular weight exceeds 500, the number of hydrogen bond acceptors is greater than 10, the number of hydrogen bond donors exceeds 5, and the calculated log P (ClogP) is higher than 5 (or MlogP > 4.15). ADMET predictions encompass absorption (CaCO2 permeability), distribution (BBB permeability), metabolism (CYP2D6 substrate), excretion (total clearance), and toxicity (AMES toxicity).[9]

Effervescent formulation

The effervescent formula consists of five different formulations. Each ingredient is weighed and sifted through mesh 30. After sifting, the ingredients are added, extracted, and homogenized. The homogeneous mixture is gradually combined with 95% ethanol until granules are formed. The granules are then sifted through mesh 20/30 and dried. The effervescent formula containing PC extract is presented in Table 1.

Table 1: Formulation of Effervescent Granules from PC Extract.

Ingredient	Formula				
	A	B	C	D	E
PC Extract	10%	10%	10%	10%	10%
Tartaric Acid	12.72%	12.30%	13.58%	14%	13.15%
Citric Acid	1.58%	2%	0.72%	0.30%	1.15%
Na. Bicarbonate	14.30%	14.30%	14.30%	14.30%	14.30%
Sucrose	60.40%	60.40%	60.40%	60.40%	60.40%
PVP	1%	1%	1%	1%	1%

Statistical analysis

The data were expressed as the mean ± standard deviation (SD) of experiments in triplicate. This statistical analysis in this study was carried out with one-way anova using a GraphPad Prism (Version 9.5.1 (528), 2023. Graph Pad Inc. software San Diego, CA, USA). IC_{50} value represented the concentration of the test sample causing 50% inhibition, in which the value <0.05 was considered significant.

Results and Discussion

Antioxidant Activity (DPPH)

The antioxidant activity was determined using the DPPH method, with the results expressed as the Inhibition Concentration 50 (IC_{50}). According to,[21] a compound is classified as a powerful antioxidant if its IC_{50} is less than 50 µg/mL, strong if IC_{50} is less than 100 µg/mL, medium if IC_{50} is less than 150 µg/mL, weak if IC_{50} is less than 200 µg/mL, and very weak if IC_{50} is greater than 200 µg/mL. The IC_{50} values obtained in this study for the PC fractions are shown in Figure 2. Quercetin as a positive control had the highest antioxidant activity with

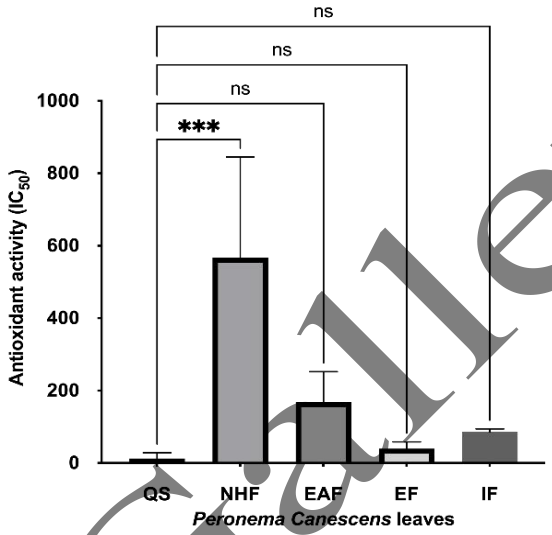

Figure 2: Antioxidant activity of PC fractions measured by DPPH assay, ns = not Significant (p>0,05), ***(p<0,001), QS (Quercetin standard), NHF (n-Hexane fraction, EAF (Ethyl acetate fraction), EF (Ethanol fraction), IF (Insoluble fraction).

an IC_{50} value of 23.77 µg/mL, which is classified as very strong according to the criteria established by,[21] and the ethanol fraction with C50 of 47.27 ± 1.90 µg/mL, which is categorized as very strong exhibits stronger antioxidant activity than the other fraction. In contrast, the ethyl acetate fraction exhibited a much weaker antioxidant potential with an IC_{50} of 201.89 ± 20.08 µg/mL, classified as very weak. The N-hexane fraction showed the highest IC_{50} value at 685.70 ± 32.15 µg/mL, indicating very weak antioxidant activity. The insoluble fraction had an IC_{50} value of 86.09 ± 7.94 µg/mL, falling under the strong category for antioxidant activity. with the order being Ethanol fraction > ethanol extract > insoluble fraction > ethyl acetate fraction > n-hexane fraction. The OH group on quercetin can function as a hydrogen donor. Quercetin can donate hydrogen atoms to neutralize free radicals, reducing the potential for cell oxidative damage.[21] Polar molecules such as flavonoids, phenolics, and glycosides are known for their antioxidant properties. The Ethanol fraction, having the lowest IC_{50} value, shows a significant difference, as denoted by four stars, when compared to the ethyl acetate and n-hexane fractions. Polar fractions, such as the ethanol and insoluble fractions, contain a higher number of substances capable of donating hydrogen atoms, leading to the formation of a reduced (nonradical) form, which is indicated by the loss of the purple color, as described in reference,[21] This process reduces 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals into a stable nonradical hydrazine derivative, resulting in a color change. The DPPH antioxidant activity of the PC fractions is presented in Figure 2.

In vitro Pancreatic Antilipase Activity

The inhibition of pancreatic lipase involves the interaction between lipase enzymes and their substrates. This test uses PNPB (P-nitrophenyl butyrate) as the substrate and Porcine Pancreatic Lipase (PPL) as the enzyme. The inhibitory effect is assessed by measuring the hydrolysis of P-nitrophenyl butyrate to P-nitrophenol at a wavelength of 405 nm using an ELISA reader. Pancreatic lipase inhibition by PC was tested at a concentration of 200 µg/mL, with PPL solution in phosphate buffer (pH 7.2) and PNPB solution. One unit of activity is defined as the reaction rate that produces 1 µmol of p-nitrophenol in 10 minutes at 37°C. The inhibition of lipase activity is expressed as the percentage reduction in activity when PPL is incubated with the test compound. PPL was chosen as the enzyme model due to its similarities with human pancreatic lipase (HPL), exhibiting comparable kinetics and enzyme characteristics.[22] According to,[18] antilipase activity is robust when the inhibition percentage exceeds 50%. The results of the PC fraction at a concentration of 200 µg/mL are shown in Table 2. Similar to our findings, a recent study demonstrated that flavonoid-rich plant extracts exhibit strong antioxidant and antilipase activities, making them potential candidates for anti-obesity therapy.[5]

Table 2: Pancreatic antilipase activity of the PC fraction

Sample	% inhibition of ±SD	Types of Antilipase [2]
Orlistat Standard	61.64±9.11	Strong
N-hexane fraction	18.66±5.21	Weak
Ethyl Acetate Fraction	67.65 ±8.04	Strong
Ethanol Fraction	14.22±4.69	Weak
Insoluble fraction	6.45±1.13	Weak

Obesity triggers inflammatory processes in excess lipogenesis, inhibits lipolysis, and increases adipocyte apoptosis. This matter increases the release of Reactive Oxygen Species (ROS) and will cause oxidative stress. Oxidative stress caused by obesity can result in damage to cells

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and tissues and trigger the emergence of degenerative diseases.[23] Antioxidants help neutralize radicals and reduce the risk of complications from degenerative diseases. Therefore, supplementation with antioxidants will reduce the risk of obesity-related complications and oxidative stress.[24] The ethanol fraction proved the most potent antioxidant agent, while ethyl acetate showed pancreatic antilipase activity. Differences in the compounds that guide these two activities. These results follow research on other materials that show that the ethyl acetate fraction has higher anti-obesity activity than the ethanol fraction. The ethanol fraction has stronger antioxidant activity than the ethyl acetate fraction. Regarding compound content, the ethanol fraction has higher total phenolic content and total flavonoid content than the ethyl acetate fraction.[25] It is necessary to prove the levels of PC leaf extracts and fractions regarding the levels of compounds, not only phenolics and flavonoids but also other groups of compounds. So, to support both activities, an effervescent preparation will be made from PC leaves extract. Effervescent granules are preferred because they are easy to use, dissolve easily in water, and taste better. Compared to tablet preparations, effervescent granules reduce stomach irritation, which sometimes occurs when tablet preparations are swallowed directly, and reduce the risk of blockage in the esophagus because they are completely dissolved in liquid before consumption.

In silico Pancreatic Antilipase Activity

Molecular docking validation is performed by redocking. The redocking results of the native ligands are shown in Figure 3. The blue structure represents the initial conformation of the enzyme-ligand complex before molecular docking. In contrast, the red structure shows the optimized docking pose of the native ligand after computational refinement. The close alignment between the pre-and post-docking structures, indicated by a root mean square deviation (RMSD) value below 2 Å, confirms the reliability and accuracy of the docking method used in this study.

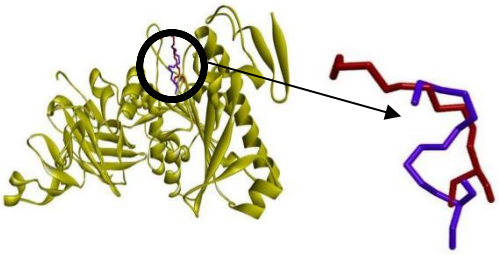

Figure 3: 3D structure of the pancreatic lipase enzyme (PDB ID 1LPB) showing an overlay of the blue (before) and red (after) molecular docking of the native ligand.

The GC-MS identification revealed that the primary compound in the active fraction was Trilaurin, accounting for 54.83% of the area and a similarity index of 59%. The three compounds identified by GC-MS were then prepared for further in silico molecular docking tests. Molecular docking of the quercetin standard, Orlistat, and the three GC-MS compounds was performed to compare the compounds obtained from pancreatic antilipase testing with the standards known to exhibit pancreatic antilipase activity, as reported in previous studies. The results of the molecular docking are presented in Table 3.

The most promising compound is Dimethyl Tetracycline, which exhibits the lowest binding energy and inhibition coefficient values compared to the Orlistat standard. The Dimethyl Tetracycline has antilipase activity similar to Orlistat. In addition to the binding energy and inhibition coefficient, pancreatic antilipase activity is evaluated based on its interaction with the amino acid serine 152. After analyzing the GC-MS-identified compounds through in silico tests, any compounds that bind to amino acid residues can potentially serve as alternative ligands to replace Orlistat. The next step is to assess whether these compounds can be used as oral drugs by evaluating them according to Lipinski's Rule of Five, as shown in Table 4.

Table 3: Molecular docking scores and interactions of the identified compounds.

No	Structure Name	Binding Energy	Amino Acid Bonds
1	Orlistat	-6.62	Gly 76, Phe 77, Ile 78, Asp 79, Tyr 114, His 151, **Ser 152,** Leu 153, Ala 178, Glu 179, Pro 180, Ile 209, Phe 215, Arg 256, Ala 259, His 263, Leu 264
2	Quercetin	-8.28 (Run of 83)	His B:75, Gly B:76, Phe B:77, Ile B:78, Asp B:79, Trp B:85, Tyr B:114, His B:151, **Ser B:152,** Ala B:178, Glu B:179, Pro B:180, Ile B:209, Phe B:215, Gly B:216, Arg B:256, His B:263.
3	Dimethyl Tetracycline	-7.78 (Run of 14)	Gly B:76, Phe B:77, Ile B:78, Asp B:79, Tyr B:114, His B:151, **Ser B:152,** Leu B:153, Ala B:178, Pro B:180, Phe B:215, Arg B:256, Asp B:257, Ala B:259, Ala B:260, His B:263, Leu B:264.
4	2-methoxy-5H-indole[2,3-b]quinoxaline	-7.25 (Run of 77)	His B:75, Gly B:76, Phe B:77, Ile B:78, Asp B:79, Trp B:85, Tyr B:114, His B:151, **Ser B:152,** Leu B:153, Ala B:178, Pro B:180, Ile B:209, Phe B:215, His B:263, Leu B:264.
5	Trilaurin	-3.52 (Run of 7)	Ile B:78, Tyr B:114, His B:151, **Ser B:152,** Leu B:153, Ala B:178, Pro B:180, Ile B: 209, Leu B:213, Phe B:215, Trp B:252, Thr B:255, Arg B:256, Ala B:259, Ala B:260, His B:263, Leu B:264.

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Table 4: Predicted Lipinski's Rule of Five for the Ligands.

No	Molecular Name	Molecular Weight	Log P	Hydrogen Bond Donor (HBD)	Hydrogen Bond Acceptor (HBA)	Polar Voltage Activity (PSA)
1	Quercetin	302.24	1.99	5	7	122.11
2	Dimethyl Tetracycline	430.41	-0.55	6	9	176.06
3	2-methoxy-5H-indolo[2,3-b]quinoxaline	249.27	3.27	1	3	108.60
4	Trilaurin	639.02	11.75	0	6	278.43

Table 4 indicates that the natural ligand candidates suitable for use are Quercetin, Dimethyl Tetracycline, and 2-methoxy-5H-indolo[2,3-b]quinoxaline. These compounds meet Lipinski's Rule of Five, with molecular weights under 500 Da, hydrogen bond donors not exceeding 5, hydrogen bond acceptors not exceeding 10, partition coefficients (log P) under 5, and polar surface areas (PSA) under 1025 Å², making them suitable for oral administration. In addition to adhering to Lipinski's Rule, candidate compounds must also pass pharmacokinetic and toxicity assessments conducted using pkCSM software. The results of drug-likeness analysis, along with absorption, distribution, metabolism, excretion, and toxicity (ADMET) predictions, are presented in Table 5. A compound is considered to have blood-brain barrier (BBB) permeability if its log BB value in the distribution phase is greater than 0.3. Molecules with a log BB value below 0.1 are not effectively distributed in the brain. CYP2D6 metabolic parameters predict whether cytochrome P450 will likely metabolize a given molecule. The total clearance (CLtot) parameter indicates excretion rates in log (ml/min/kg). Drug clearance primarily occurs through renal and hepatic clearance (kidney excretion) (liver metabolism and bile excretion). Ames toxicity testing is a commonly used method to evaluate the mutagenic potential of compounds through bacterial assays. Among the candidates, Dimethyl Tetracycline meets both Lipinski's rule of five and ADMET prediction criteria.

The Evaluation of granule preparations includes tests for flow rate, angle of repose, bulk density, tapped density, Carr's compressibility index, and Hausner ratios.[26]

Table 5: ADMET Prediction for Compounds from the Ethyl Acetate Fraction of PC

No	Molecular Name	Absorption (CaCO2 Permeability) (log Papp in 10-6 cm/sec)	Distribution (VDss (human)) (log L/kg)	Metabolism (CYP2D6) (YES/NO)	Excretion (Total clearance) (log ml/min/kg)	AMES Toxicity (YES/NO)	Hepatotoxicity	Skin Sensitization
1	Orlistat	0.40	-1.02	No	1.68	No	Yes	No
2	Quercetin	-0.28	0.06	No	0.46	No	No	No
3	Dimethyl tetracycline	-0.01	0.61	No	0.35	No	No	No
4	2-methoxy-5H-indolo[2,3-b]quinoxaline	1.30	-0.01	No	0.77	Yes	Yes	No
5	Trilaurin	0.14	-0.82	No	2.23	No	No	No

In this study, optimization using design experts focuses on flow rate, angle of repose, and Carr's index. Good flow characteristics are defined by the ability of particles to flow independently without clumping, influenced by gravitational force.[27] The flow rate test indicates that all the effervescent granules produced exhibit excellent flow, with a suitable flow time greater than 10 grams per second. The flow rate results of the effervescent granules for each formula are presented in Table 6. Based on the observations for Formula 3 and Formula 4 in Table 6, these formulas exhibit a faster flow time due to a higher tartaric acid content than Formula 1 and Formula 2. Tartaric acid has a higher density than citric acid, which allows granules with a greater tartaric acid content to flow more rapidly because of the increased gravitational force.[27] The angle of repose is the stable angle formed between a pile of cone-shaped particles and a horizontal plane. If the angle is less than 30°, the material is considered to flow easily. Conversely, if the angle is 40° or greater, the material will likely be difficult to flow. The shape of the granules can influence the value of the angle of repose.[28]

Table 6: Flow rate, angle of repose, and bulk density of the effervescent granules from PC.

Formula	Flow rate	Angle of repose	Bulk density (g/ml)	Tapped density (g/ml)	Hausner ratios	Carr's compressibility index (%)
A	18.66	25.05	0.52	0.56	1.07	6.25
B	20.43	25.85	0.53	0.55	1.04	4.26

C	20.79	27.16	0.53	0.56	1.07	6.38
D	20.63	24.09	0.50	0.56	1.14	11.98
E	18.74	26.03	0.49	0.53	1.06	5.99

Table 6 presents the results of the stationary angle test for formulas 1-5, all of which are below 30°. A stationary angle of no more than 30° indicates excellent flow properties, meaning all the formulas demonstrate good flow behavior. The granules flow more quickly and easily with less friction and tensile force between them. Furthermore, smaller granule sizes increase cohesiveness, reducing the flow velocity and resulting in a higher stationary angle.[29] Determining bulk density includes measuring the actual weight, compressive weight, Hausner factor, and percent compressibility. The Hausner factor is used to compare the actual and compressive weights, helping to assess the flow or free-flowing properties of the powder. All seven formulas meet the qualification of having a Hausner factor of less than 1.25, indicating good flow characteristics. Granule compressibility refers to the ability of the granules to maintain compactness under pressure. Factors such as porosity, type density, particle shape, and moisture content can affect the flow properties of the granules. Good flow properties ensure easier molding of the granules and help maintain uniform weight. The results for the Hausner factor and compressibility are shown in Table 6. The percent compressibility results indicated that Carr's index ranged from 4.26% to 14.59%, which aligns with the literature stating that granules with a Carr's index value below 15% demonstrate good flowability.

The optimal formula using Design Expert is intended to generate the most efficient formula based on the response data from the prepared parameters. The response data, analyzed through ANOVA in Design Expert, is processed to identify the optimal formula.[10] The ideal formula is the one with a desirability value closest to 1. Using the simplex lattice design method in the Design Expert software, the optimal formula was determined to have 65.81 mg of tartaric acid and 4.19 mg of citric acid, with a desirability value of 0.86. Before finalizing, the optimal formula requires verification. The results of design expert optimization formula 1 solution and formula 2 solution are shown in Figure 4, with the formula test results from the design expert optimization provided in Table 7. This study utilized a Design Expert to optimize the effervescent granule formulation containing PC extract Figure 4. This software allows integrated analysis to evaluate interactions between formulation variables and determine the optimal combination of ingredients used. The optimization of the effervescent granule formulation resulted in two optimal formulas. Formula 1: 13.16% tartaric acid and 0.84% citric acid. Formula 2: 13.21% tartaric acid and 0.80% citric acid. The desirability score for both formulas was 0.862, indicating a high optimization level. The flow rate and angle of repose parameters from the optimized formulas showed no significant differences compared to laboratory experimental results ($p > 0.05$), suggesting the predictive model's accuracy in Figure 5. Based on the GraphPad Version 9.5.1 (528), 2023 statistical analysis Figure 5, the flow rate and angle of repose values from Formula 1 and Formula 2 in both the Design Expert optimization and the actual test results showed no significant difference, indicating that the optimization and laboratory test produced similar outcomes.

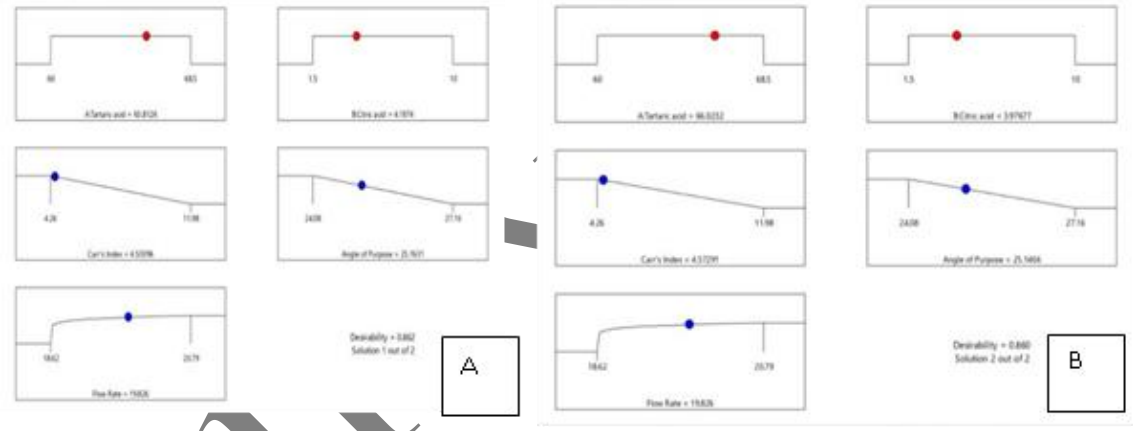

Figure 4: Design Expert optimization (A) Formula 1 Solution (B) Formula 2 Solution

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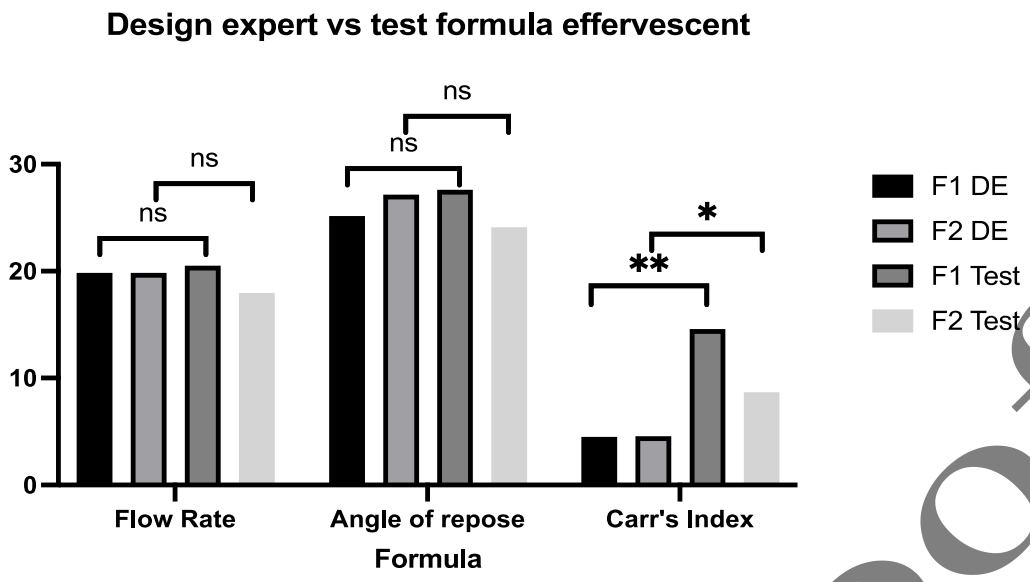

Figure 5: Formula optimization using Design expert vs test, ns = not Significant (p>0,05), *(p<0,05), F1 DE (Formula 1 Design Expert), F2 DE (Formula 2 Design Expert), F1 Test (Formula 1 test), F2 Test (Formula 2 test).

Table 7: Results of the formula test from Design Expert Optimization.

Formula	Flow rate	Angle of repose	Bulk density (g/ml)	Tapped density (g/ml)	Hausner ratios	Carr's compressibility index (%)
1	20.50	27.61	0.52	0.60	1.17	14.59
2	17.96	24.10	0.54	0.59	1.10	8.68

However, Carr's index test revealed a discrepancy between the Design Expert optimization and the test results, as effervescent granules are highly sensitive to room temperature, which may have influenced the test outcomes. Additional research is needed to isolate compounds from PC based on the results of the in silico data. An integrated study of network pharmacology and component analysis should be conducted to explore the molecular mechanisms of PC extract in treating obesity.[30] In silico anti-obesity activity should be explored using additional receptor targets, as the anti-obesity mechanism extends beyond pancreatic lipase. Central nervous system mechanisms can be investigated, targeting receptors such as GLP-1 (liraglutide), 5-HT2c (lorcaserin), and TAAR-1 (phentermine). In vivo, testing is also recommended to validate the efficacy and safety of these compounds in animal models and clinical settings. Furthermore, advanced formulations, such as nanocarrier systems, could be developed to enhance PC-based products' bioavailability and therapeutic potential. Future studies should focus on the antioxidant properties of the sample using comprehensive methods.[31] These include determination of hydrogen peroxide scavenging capacity, determination of ferric reducing power, determination of nitric oxide (NO) scavenging activity, determination of ascorbic acid, determination of vitamin e, and assessment of lipid peroxidation inhibition. These assays will provide a deeper understanding of the antioxidant potential and the mechanisms by which the sample mitigates oxidative stress.

Conclusion

The antioxidant activity of the PC fraction, evaluated using the DPPH method, revealed that the ethanol fraction exhibited significantly stronger antioxidant activity (IC_{50} = 47.27 ± 1.90 µg/mL) compared to the ethyl acetate fraction (IC_{50} = 201.89 ± 20.08 µg/mL, $p < 0.05$). This difference highlights the greater presence of polar compounds, such as flavonoids and phenolics, in the ethanol fraction. Among the identified compounds, dimethyl tetracycline showed the lowest binding energy (-7.78 kcal/mol) in molecular docking studies, suggesting its potential as a strong pancreatic lipase inhibitor. This was further supported by its adherence to Lipinski's Rule of Five, indicating good oral bioavailability. Formula optimization using the Design Expert software resulted in two formulas. The flow rate and angle of repose values from the design expert and the laboratory tests did not show significant differences, indicating that the optimization and experimental results aligned. However, differences were observed in the Carr's Index test between the design expert optimization and the lab results.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors affirm that the work presented in this article is original, and they accept full responsibility for any claims related to the article's content.

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Original Research Article

In Vitro and *in Silico* Analysis of Antilipase, Antioxidant, and Optimization of Granule Effervescent from *Peronema canescens* Jack

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ABSTRACT

Obesity results from prolonged energy imbalance, with anti-obesity treatment targeting pancreatic lipase inhibition. *Peronema canescens* Jack. (PC) known as Sungkai, has traditionally been used to treat various ailments. This study aimed to assess PC antioxidant and antilipase activities and optimize effervescent granule formulations. Phytochemical screening and thin-layer chromatography (TLC) were performed, followed by antioxidant analysis using 2,2-diphenyl-1-picrylhydazyl (DPPH) and pancreatic antilipase activity, using the p-NPB substrate, were employed. The ethanol fraction of PC demonstrated potent antioxidant activity (IC_{50} = 47.27 µg/mL), while the insoluble fraction showed the highest pancreatic antilipase activity (67.65%). Gas chromatography-mass spectrometry (GC-MS) identified active compounds, including dimethyl tetracycline, 2-methoxy-5H-indolo[2,3-b] quinoxaline, and trilaurin, with molecular docking study indicating dimethyl tetracycline was the most effective antilipase candidate, binding to the pancreatic receptor (PDB ID: 1LPB). This compound also met Lipinski's Rule of Five and ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity), suggesting favorable pharmacokinetics and safety. Evaluation of effervescent granules included angle of repose, bulk density, and tapped density. Optimization of tartaric and citric acid concentration using Design Expert 13 yielding two optimal formulas: Formula 1 with 13.16% tartaric acid and 0.84% citric acid, and Formula 2 with 13.21% tartaric acid and 0.80% citric acid. PC leaves have the potential to be an antioxidant and anti-obesity and can be developed into effervescent formula.

Keywords: *Peronema Canescens* Jack., Antioxidant, Effervescent granules, Molecular docking, Pancreatic antilipase.

Introduction

The increasing prevalence of degenerative diseases in Indonesia, alongside infectious diseases, indicates changing health challenges, with obesity emerging as a major concern. RISKESDAS (Indonesia's basic health research) data reveal a rise in obesity rates from 14.8% in 2013 to 21% in 2018.[1] Factors that contribute to obesity encompass environmental factors, urban living, and eating patterns. Diets high in fats and sugars but low in fiber cause an energy imbalance, which, when combined with triglyceride buildup, this imbalance triggers oxidative stress and inflammatory responses within the body.[2] This ongoing inflammation, fat accumulation, and suppression of fat breakdown causes adipocyte apoptosis, producing Reactive Oxygen Species (ROS) that harm cells and tissues, raising the risk of degenerative diseases.[3] Antioxidants are essential for neutralizing ROS, helping to reduce the risk of degenerative diseases linked to oxidative stress.[4] In the context of medical treatments, FDA-approved drugs for obesity aim to either decrease calorie absorption or control appetite.

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Central nervous system (CNS) suppressants, including lorcaserin, liraglutide, phentermine-topiramate, and naltrexone/bupropion, work by targeting appetite-regulating receptors such as 5HT2c, GLP-1, and TAAR-1. On the other hand, Orlistat acts as a lipase inhibitor, reducing the absorption of dietary fats by approximately 30%.[5] People in Indonesia prefer using herbal medicine due to its natural properties, which are perceived as safer and less likely to cause unwanted side effects. In general, herbal medicines are more affordable than synthetic drugs. They also contain a variety of plant-based ingredients. Herbal medicine is considered effective for targeting multiple health issues. Conversely, Orlistat is a therapeutic agent for obesity that reduces calorie absorption in the intestinal tract.[5] Nevertheless, the effectiveness of Orlistat is constrained by side effects such as gastrointestinal problems, including oily stools, flatulence, and rectal discharge.[6] These limitations highlight the importance of seeking complementary or alternative treatments, especially natural ones with fewer side effects and potential long-term benefits. Herbal medicine presents a promising alternative to synthetic drugs for managing obesity, thanks to its safety, availability, and ability to target multiple mechanisms. *Peronema canescens* Jack. (PC), locally known as Sungkai, has attracted attention for its potential therapeutic benefits. Traditionally utilized in Indonesian medicine, the leaves of PC contain secondary metabolites like phenols, triterpenoids, flavonoids, tannins, alkaloids, steroids, and saponins, which have been reported to exhibit anti-inflammatory, antioxidant, antidiabetic, and immune-boosting properties.[7] The bioactive compounds in PC position it as a promising candidate for anti-obesity treatments, primarily by inhibiting pancreatic lipase, which helps reduce lipid absorption. Recent studies have highlighted the potential of plant-based compounds for pancreatic lipase inhibition, particularly in

treating obesity.[8] For example, in silico modeling allows for structural predictions and identifying binding sites, enhancing target interaction in drug development.[9] Moreover, effervescent granules offer a convenient dosage form by combining acidic and alkaline compounds that release CO_2 upon dissolution. These granules provide high solubility, ease of use, and rapid absorption, making them an ideal delivery system for antioxidants and antilipase agents.[10] Given the therapeutic potential of PC, developing a granule formulation can enhance the accessibility and effectiveness of its bioactive components. While traditional treatments like GLP-1 receptor agonists have proven effective in managing obesity, they are especially beneficial for patients with comorbidities such as type 2 diabetes. Other plant-based studies indicate that appetite suppression may occur by activating the 5-HT2C receptor.[11] Additionally, TAAR1 agonists present the potential to address maladaptive eating behaviors associated with metabolic disorders.[12] Inhibitors targeting the lipase enzyme, such as those aimed at PDB proteins 1LPB and 5ZUN, further reinforce the potential of lipase inhibition as a therapeutic target for anti-obesity drugs.[13]

The methods employed in this study, including phytochemical screening, DPPH antioxidant assay, pancreatic antilipase activity testing, and molecular docking, are specifically chosen to assess the bioactive compounds in PC and their potential for obesity treatment. These approaches are highly relevant as they combine experimental and computational techniques to identify promising antilipase candidates. This is the first study to comprehensively evaluate PC antioxidant and antilipase activities while optimizing effervescent granule formulations. The integration of in vitro and in silico approaches in this study provides a novel insight into the potential therapeutic uses of PC in combating obesity. This holistic approach highlights the potential of PC as a safe, accessible, and effective therapy for obesity.

Materials and Methods

Materials

Rotary evaporator (Heidolph-G3), Silica Gel F254 plates, UV lamps (254 nm and 366 nm, Evaco GL 220V 50Hz T8 15W), micropipettes (Socorex & Dragon Lab), vortex mixers, UV-Vis Spectrophotometer (Shimadzu UV-1780, Shimadzu Corporation, Japan), ELISA reader (Synergy HTX, Agilent, USA), GC-MS (Shimadzu QP 2010 SE, Shimadzu Corporation, Japan). *Peronema canescens* Jack (PC), ethanol, n-hexane, ethyl acetate, $FeCl_3$, $MgSO_4$, hydrochloric-ethanolic acid mixture (1:1), hydrochloric acid, Liebermann-Burchard reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH, Sigma-Aldrich, USA, 97% purity, analytical grade) was used for antioxidant assays, quercetin, p.a. methanol, crude porcine pancreatic lipase (PPL), P-nitrophenyl butyrate (p-NPB, Sigma-Aldrich, USA, 98% purity, analytical grade) was used for pancreatic antilipase activity, phosphate buffer (pH 7.2), DMSO, and orlistat standard.

Hardware and Software

Some of the software used, including the receptors for the test, can be downloaded from the RCSB PDB website (https://www.rcsb.org/). The ligands used in the test are available for download from the PubChem website (https://pubchem.ncbi.nlm.nih.gov/). Test ligands and receptors were created using ChemDraw Professional 15.0, Chem3D 15.0, Biovia Discovery Studio 2021, Command Prompt, and AutoDock Tools 1.5.6. Docking visualizations were performed using Biovia Discovery Studio 2021. Lipinski's Rule of Five testing was conducted using the Lipinski rules available at (http://www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp). Pharmacokinetics and toxicology testing were performed using the pk-CSM website (https://biosig.lab.uq.edu.au/pkcsm/). Molecular docking simulations were conducted on a laptop (Acer Aspire A314-35, Laptop-ML0EUN2).

Methods

The general research methods for the in vitro and in silico analysis of antilipase, antioxidants, and optimization of effervescent granules from PC are outlined in Figure 1.

Sample preparation, extraction, and fractionation

The sample used in this study was *Peronema canescens*, Jack (PC), sourced from Kayutanam in Padang Pariaman District, West Sumatra, Latitude : 0°29'46.8312", Longitude : 100°20'7.638", Altitude: Located at an altitude of between 100 to 1000 meters above sea level. Harvested between May and July 2021 from trees measuring 6-7 meters in height. The maceration process was conducted for 3 days (3x24 hours), with occasional stirring and repeated solvent changes using 96% ethanol. The resulting macerate was then filtered and concentrated using a rotary vacuum evaporator, followed by thickening in a water bath at approximately 40°C.[14–16]

Twenty grams of the PC ethanol extract were placed in a beaker with a stir bar and magnetic stirrer. The fractionation process began by adding 100 mL of n-hexane, followed by stirring to separate the liquid from the insoluble extract. This step was repeated 5-6 times, adding 100 mL of n-hexane each time until a clear n-hexane fraction was obtained. Next, 100 mL of ethyl acetate was added to the insoluble n-hexane extract, and the fractionation was repeated until a distinct ethyl acetate fraction was obtained. Subsequently, 100 mL of ethanol solution was used to fractionate the insoluble ethyl acetate extract, repeating the process 5-6 times until a precise ethanol fraction was obtained. The remaining insoluble fraction, treated with ethanol, was designated as the insoluble fraction. The fractions were concentrated using a rotary vacuum evaporator, and the final thickening was performed in a water bath at approximately 50°C to yield a viscous fraction.[14]

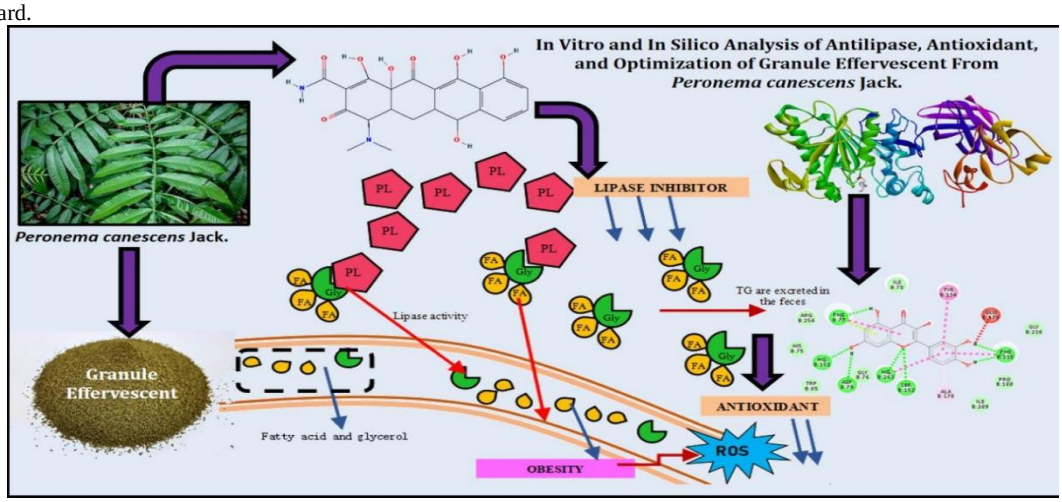

Figure 1: Research methods for *in vitro* and *in silico* analysis of antilipase, antioxidants, and optimization of effervescent granules from PC.

Antioxidant Activity
The PC fraction was dissolved in methanol and prepared at 10, 20, 30, 40, 50, and 60 µg/mL.[17] The antioxidant activity was determined by adding 1.0 mL of the PC fraction solution to a test tube containing 4.0 mL of 0.1 mM DPPH for each concentration. The mixture was homogenized using a vortex for 1 minute and allowed to stand for the designated time for each test solution. The absorbance of the solution was then measured wavelength at 516.0 nm using a UV-Vis spectrophotometer (Shimadzu UV-1780, Shimadzu Corporation, Japan). The same procedure was followed to measure the absorbance of the quercetin standard series.

Pancreatic Antilipase Activity
The pancreatic antilipase inhibition activity of the n-hexane, ethyl acetate, ethanol, and insoluble fractions was assessed using 96-well plates and an ELISA reader (Synergy HTX, Agilent, USA). The enzyme stock concentration was approximately 0.1 µg/mL, prepared by dissolving 1 mg of solid porcine pancreatic lipase (PPL) powder in 1 mL of buffer solution (a). The fraction was prepared at a concentration of 500 µg/mL (b), and p-NPB was dissolved in 1% DMSO (c) and subsequently diluted with a 50 mM phosphate buffer (pH 7.2, 0.5%) to a final concentration of 2.5 mM in 100 µL (d). Solutions (a), (b), and (d) were mixed and incubated at 37°C for 10 minutes. Each sample was tested in triplicate. Orlistat was used as a positive control, and 1% DMSO was the negative control without inhibitors. One unit of activity is defined as the reaction rate that generates 1 µmol of p-nitrophenyl butyrate at 37°C. Lipase activity inhibition was expressed as the percentage reduction in activity when PPL was incubated with the test compound.[18]

Identification of compounds in the active fraction of PC using GC-MS.
GC-MS analysis was conducted at the integrated laboratory of Universitas Islam Indonesia. The active fraction, prepared at a concentration of 500 µg/mL, was injected in a volume of 1.0 µL for analysis using Gas Chromatography coupled with a Flame Ionization Detector (FID) and Mass Spectrometry (MS) (Shimadzu QP 2010 SE, Shimadzu Corporation, Japan). The mobile phase consisted of chloroform: ethanol mixture (1:1), and the analysis was performed using an Rtx-5 MS column (5% diphenyl / 95% dimethyl polysiloxane) with specifications of 0.25 µm thickness, 30.0 m length, and 0.25 mm inner diameter. The instrument settings included an initial temperature of 80ºC, an injection temperature of 300ºC, and an ion source temperature of 250ºC. The oven temperature was gradually increased to 330ºC at 6ºC per minute. The column flow rate was set to 0.74 mL/min with a pressure of 42.3 kPa.[19]

Molecular Docking
The receptors used in this study were obtained from the Protein Data Bank in 3D structure format or were drawn using ChemDraw software. These receptors, which are protein macromolecules, were isolated from any irrelevant molecules along with the ligands. The isolation process was performed using Discovery Studio 2021, and the files were saved in pdb format. Optimization involved adding hydrogen atoms, merging nonpolar hydrogens, and calculating Gasteiger charges using AutodockTools 1.5.6. The resulting file was saved in pdbqt format. For ligand preparation, 2D and 3D structures of the selected ligands were created to determine their molecular structure using ChemDraw Pro 12.0 software. The ligands were then prepared using AutoDockTools 1.5.6, where the compound structures were corrected, and Gasteiger charges were added. The prepared ligands were saved and ready for docking.[9,13]

Evaluation of Drug Likeliness and ADMET
Assessing the drug-likeness of compounds is based on Lipinski's Rule of Five, which utilizes both experimental and computational approaches to evaluate solubility and permeability in drug discovery and *development*.[20] The Rule of Five suggests that poor absorption and permeability are likely when the molecular weight exceeds 500, the number of hydrogen bond acceptors is greater than 10, the number of hydrogen bond donors exceeds 5, and the calculated log P (ClogP) is higher than 5 (or MlogP > 4.15). ADMET predictions encompass absorption (CaCO2 permeability), distribution (BBB permeability), metabolism (CYP2D6 substrate), excretion (total clearance), and toxicity (AMES toxicity).[9]

Effervescent formulation
The effervescent formula consists of five different formulations. Each ingredient is weighed and sifted through mesh 30. After sifting, the ingredients are added, extracted, and homogenized. The homogeneous mixture is gradually combined with 95% ethanol until granules are formed. The granules are then sifted through mesh 20/30 and dried. The effervescent formula containing PC extract is presented in Table 1.

Table 1: Formulation of Effervescent Granules from PC Extract.

Ingredient	**Formula**				
	A	**B**	**C**	**D**	**E**
PC Extract	10%	10%	10%	10%	10%
Tartaric Acid	12.72%	12.30%	13.58%	14%	13.15%
Citric Acid	1.58%	2%	0.72%	0.30%	1.15%
Na. Bicarbonate	14.30%	14.30%	14.30%	14.30%	14.30%
Sucrose	60.40%	60.40%	60.40%	60.40%	60.40%
PVP	1%	1%	1%	1%	1%

Statistical analysis
The data were expressed as the mean ± standard deviation (SD) of experiments in triplicate. This statistical analysis in this study was carried out with one-way anova using a GraphPad Prism (Version 9.5.1 (528), 2023. Graph Pad Inc. software San Diego, CA, USA). IC_{50} value represented the concentration of the test sample causing 50% inhibition, in which the value <0.05 was considered significant.

Results and Discussion

Antioxidant Activity (DPPH)
The antioxidant activity was determined using the DPPH method, with the results expressed as the Inhibition Concentration 50 (IC_{50}). According to,[21] a compound is classified as a powerful antioxidant if its IC_{50} is less than 50 µg/mL, strong if IC_{50} is less than 100 µg/mL, medium if IC_{50} is less than 150 µg/mL, weak if IC_{50} is less than 200 µg/mL, and very weak if IC_{50} is greater than 200 µg/mL. The IC_{50} values obtained in this study for the PC fractions are shown in Figure 2. Quercetin as a positive control had the highest antioxidant activity with

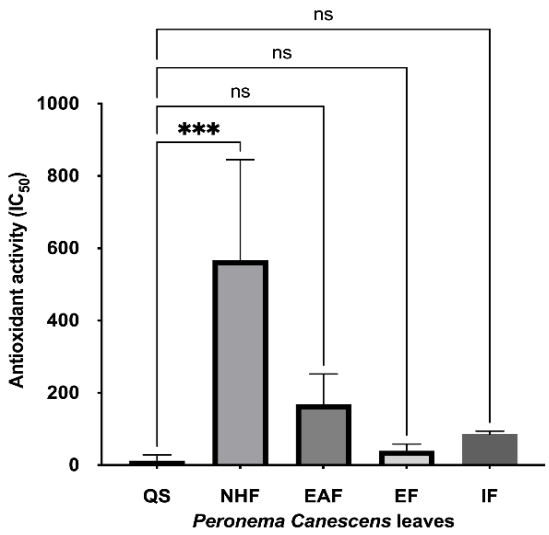

Figure 2: Antioxidant activity of PC fractions measured by DPPH assay, ns = not Significant (p>0,05), ***(p<0,001), QS (Quercetin standard), NHF (n-Hexane fraction, EAF (Ethyl acetate fraction), EF (Ethanol fraction), IF (Insoluble fraction).

an IC_{50} value of 23.77 μg/mL, which is classified as very strong according to the criteria established by,[21] and the ethanol fraction with C50 of 47.27 ± 1.90 μg/mL, which is categorized as very strong exhibits stronger antioxidant activity than the other fraction. In contrast, the ethyl acetate fraction exhibited a much weaker antioxidant potential with an IC_{50} of 201.89 ± 20.08 μg/mL, classified as very weak. The N-hexane fraction showed the highest IC_{50} value at 685.70 ± 32.15 μg/mL, indicating very weak antioxidant activity. The insoluble fraction had an IC_{50} value of 86.09 ± 7.94 μg/mL, falling under the strong category for antioxidant activity. with the order being Ethanol fraction > ethanol extract > insoluble fraction > ethyl acetate fraction > n-hexane fraction. The OH group on quercetin can function as a hydrogen donor. Quercetin can donate hydrogen atoms to neutralize free radicals, reducing the potential for cell oxidative damage.[21] Polar molecules such as flavonoids, phenolics, and glycosides are known for their antioxidant properties. The Ethanol fraction, having the lowest IC_{50} value, shows a significant difference, as denoted by four stars, when compared to the ethyl acetate and n-hexane fractions. Polar fractions, such as the ethanol and insoluble fractions, contain a higher number of substances capable of donating hydrogen atoms, leading to the formation of a reduced (nonradical) form, which is indicated by the loss of the purple color, as described in reference,[21] This process reduces 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals into a stable nonradical hydrazine derivative, resulting in a color change. The DPPH antioxidant activity of the PC fractions is presented in Figure 2.

In vitro Pancreatic Antilipase Activity

The inhibition of pancreatic lipase involves the interaction between lipase enzymes and their substrates. This test uses PNPB (P-nitrophenyl butyrate) as the substrate and Porcine Pancreatic Lipase (PPL) as the enzyme. The inhibitory effect is assessed by measuring the hydrolysis of P-nitrophenyl butyrate to P-nitrophenol at a wavelength of 405 nm using an ELISA reader. Pancreatic lipase inhibition by PC was tested at a concentration of 200 μg/mL, with PPL solution in phosphate buffer (pH 7.2) and PNPB solution. One unit of activity is defined as the reaction rate that produces 1 μmol of p-nitrophenol in 10 minutes at 37°C. The inhibition of lipase activity is expressed as the percentage reduction in activity when PPL is incubated with the test compound. PPL was chosen as the enzyme model due to its similarities with human pancreatic lipase (HPL), exhibiting comparable kinetics and enzyme characteristics.[22] According to,[18] antilipase activity is robust when the inhibition percentage exceeds 50%. The results of the PC fraction at a concentration of 200 μg/mL are shown in Table 2. Similar to our findings, a recent study demonstrated that flavonoid-rich plant extracts exhibit strong antioxidant and antilipase activities, making them potential candidates for anti-obesity therapy.[5]

Table 2: Pancreatic antilipase activity of the PC fraction

Sample	% inhibition of ±SD	Types of Antilipase [2]
Orlistat Standard	61.64±9.11	Strong
N-hexane fraction	18.66±5.21	Weak
Ethyl Acetate Fraction	67.65 ±8.04	Strong
Ethanol Fraction	14.22±4.69	Weak
Insoluble fraction	6.45±1.13	Weak

Obesity triggers inflammatory processes in excess lipogenesis, inhibits lipolysis, and increases adipocyte apoptosis. This matter increases the release of Reactive Oxygen Species (ROS) and will cause oxidative stress. Oxidative stress caused by obesity can result in damage to cells and tissues and trigger the emergence of degenerative diseases.[23] Antioxidants help neutralize radicals and reduce the risk of complications from degenerative diseases. Therefore, supplementation with antioxidants will reduce the risk of obesity-related complications and oxidative stress.[24] The ethanol fraction proved the most potent antioxidant agent, while ethyl acetate showed pancreatic antilipase activity. Differences in the compounds that guide these two activities. These results follow research on other materials that show that the ethyl acetate fraction has higher anti-obesity activity than the ethanol fraction. The ethanol fraction has stronger antioxidant activity than the ethyl acetate fraction. Regarding compound content, the ethanol fraction has higher total phenolic content and total flavonoid content than the ethyl acetate fraction.[25] It is necessary to prove the levels of PC leaf extracts and fractions regarding the levels of compounds, not only phenolics and flavonoids but also other groups of compounds. So, to support both activities, an effervescent preparation will be made from PC leaves extract. Effervescent granules are preferred because they are easy to use, dissolve easily in water, and taste better. Compared to tablet preparations, effervescent granules reduce stomach irritation, which sometimes occurs when tablet preparations are swallowed directly, and reduce the risk of blockage in the esophagus because they are completely dissolved in liquid before consumption.

In silico Pancreatic Antilipase Activity

Molecular docking validation is performed by redocking. The redocking results of the native ligands are shown in Figure 3. The blue structure represents the initial conformation of the enzyme-ligand complex before molecular docking. In contrast, the red structure shows the optimized docking pose of the native ligand after computational refinement. The close alignment between the pre-and post-docking structures, indicated by a root mean square deviation (RMSD) value below 2 Å, confirms the reliability and accuracy of the docking method used in this study.

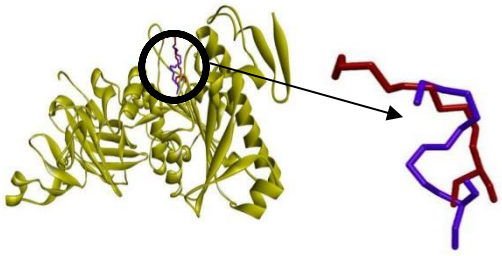

Figure 3: 3D structure of the pancreatic lipase enzyme (PDB ID 1LPB) showing an overlay of the blue (before) and red (after) molecular docking of the native ligand.

The GC-MS identification revealed that the primary compound in the active fraction was Trilaurin, accounting for 54.83% of the area and a similarity index of 59%. The three compounds identified by GC-MS were then prepared for further in silico molecular docking tests. Molecular docking of the quercetin standard, Orlistat, and the three GC-MS compounds was performed to compare the compounds obtained from pancreatic antilipase testing with the standards known to exhibit pancreatic antilipase activity, as reported in previous studies. The results of the molecular docking are presented in Table 3.

The most promising compound is Dimethyl Tetracycline, which exhibits the lowest binding energy and inhibition coefficient values compared to the Orlistat standard. The Dimethyl Tetracycline has antilipase activity similar to Orlistat. In addition to the binding energy and inhibition coefficient, pancreatic antilipase activity is evaluated based on its interaction with the amino acid serine 152. After analyzing the GC-MS-identified compounds through in silico tests, any compounds that bind to amino acid residues can potentially serve as alternative ligands to replace Orlistat. The next step is to assess whether these compounds can be used as oral drugs by evaluating them according to Lipinski's Rule of Five, as shown in Table 4.

Table 3: Molecular docking scores and interactions of the identified compounds.

No	Structure Name	Binding Energy	Amino Acid Bonds
1	Orlistat	-6.62	Gly 76, Phe 77, Ile 78, Asp 79, Tyr 114, His 151, **Ser 152,** Leu 153, Ala 178, Glu 179, Pro 180, Ile 209, Phe 215, Arg 256, Ala 259, His 263, Leu 264
2	Quercetin	-8.28 (Run of 83)	His B:75, Gly B:76, Phe B:77, Ile B:78, Asp B:79, Trp B:85, Tyr B:114, His B:151, **Ser B:152,** Ala B:178, Glu B:179, Pro B:180, Ile B:209, Phe B:215, Gly B:216, Arg B:256, His B:263.
3	Dimethyl Tetracycline	-7.78 (Run of 14)	Gly B:76, Phe B:77, Ile B:78, Asp B:79, Tyr B:114, His B:151, **Ser B:152,** Leu B:153, Ala B:178, Pro B:180, Phe B:215, Arg B:256, Asp B:257, Ala B:259, Ala B:260, His B:263, Leu B:264.
4	2-methoxy-5H-indole[2,3-b]quinoxaline	-7.25 (Run of 77)	His B:75, Gly B:76, Phe B:77, Ile B:78, Asp B:79, Trp B:85, Tyr B:114, His B:151, **Ser B:152,** Leu B:153, Ala B:178, Pro B:180, Ile B:209, Phe B:215, His B:263, Leu B:264.
5	Trilaurin	-3.52 (Run of 7)	Ile B:78, Tyr B:114, His B:151, **Ser B:152,** Leu B:153, Ala B:178, Pro B:180, Ile B: 209, Leu B:213, Phe B:215, Trp B:252, Thr B:255, Arg B:256, Ala B:259, Ala B:260, His B:263, Leu B:264.

Table 4: Predicted Lipinski's Rule of Five for the Ligands.

No	Molecular Name	Molecular Weight	Log P	Hydrogen Bond Donor (HBD)	Hydrogen Bond Acceptor (HBA)	Polar Voltage Activity (PSA)
1	Quercetin	302.24	1.99	5	7	122.11
2	Dimethyl Tetracycline	430.41	-0.55	6	9	176.06
3	2-methoxy-5H-indolo[2,3-b]quinoxaline	249.27	3.27	1	3	108.60
4	Trilaurin	639.02	11.75	0	6	278.43

Table 4 indicates that the natural ligand candidates suitable for use are Quercetin, Dimethyl Tetracycline, and 2-methoxy-5H-indolo[2,3-b]quinoxaline. These compounds meet Lipinski's Rule of Five, with molecular weights under 500 Da, hydrogen bond donors not exceeding 5, hydrogen bond acceptors not exceeding 10, partition coefficients (log P) under 5, and polar surface areas (PSA) under 1025 Å², making them suitable for oral administration. In addition to adhering to Lipinski's Rule, candidate compounds must also pass pharmacokinetic and toxicity assessments conducted using pkCSM software. The results of drug-likeness analysis, along with absorption, distribution, metabolism, excretion, and toxicity (ADMET) predictions, are presented in Table 5. A compound is considered to have blood-brain barrier (BBB) permeability if its log BB value in the distribution phase is greater than 0.3. Molecules with a log BB value below 0.1 are not effectively distributed in the brain. CYP2D6 metabolic parameters predict whether cytochrome P450 will likely metabolize a given molecule. The total clearance (CLtot) parameter indicates excretion rates in log (ml/min/kg). Drug clearance primarily occurs through renal and hepatic clearance (kidney excretion) (liver metabolism and bile excretion). Ames toxicity testing is a commonly used method to evaluate the mutagenic potential of compounds through bacterial assays. Among the candidates, Dimethyl Tetracycline meets both Lipinski's rule of five and ADMET prediction criteria.

The Evaluation of granule preparations includes tests for flow rate, angle of repose, bulk density, tapped density, Carr's compressibility index, and Hausner ratios.[26]

Table 5: ADMET Prediction for Compounds from the Ethyl Acetate Fraction of PC

No	Molecular Name	Absorption (CaCO2 Permeability) (log Papp in 10-6 cm/sec)	Distribution (VDss (human)) (log L/kg)	Metabolism (CYP2D6) (YES/NO)	Excretion (Total clearance) (log ml/min/kg)	AMES Toxicity (YES/NO)	Hepatotoxicity	Skin Sensitization
1	Orlistat	0.40	-1.02	No	1.68	No	Yes	No
2	Quercetin	-0.28	0.06	No	0.46	No	No	No
3	Dimethyl tetracycline	-0.01	0.61	No	0.35	No	No	No
4	2-methoxy- 5H-indolo[2,3-b]quinoxaline	1.30	-0.01	No	0.77	Yes	Yes	No
5	Trilaurin	0.14	-0.82	No	2.23	No	No	No

In this study, optimization using design experts focuses on flow rate, angle of repose, and Carr's index. Good flow characteristics are defined by the ability of particles to flow independently without clumping, influenced by gravitational force.[27] The flow rate test indicates that all the effervescent granules produced exhibit excellent flow, with a suitable flow time greater than 10 grams per second. The flow rate results of the effervescent granules for each formula are presented in Table 6. Based on the observations for Formula 3 and Formula 4 in Table 6, these formulas exhibit a faster flow time due to a higher tartaric acid content than Formula 1 and Formula 2. Tartaric acid has a higher density than citric acid, which allows granules with a greater tartaric acid content to flow more rapidly because of the increased gravitational force.[27] The angle of repose is the stable angle formed between a pile of cone-shaped particles and a horizontal plane. If the angle is less than 30°, the material is considered to flow easily. Conversely, if the angle is 40° or greater, the material will likely be difficult to flow. The shape of the granules can influence the value of the angle of repose.[28]

Table 6: Flow rate, angle of repose, and bulk density of the effervescent granules from PC.

Formula	Flow rate	Angle of repose	Bulk density (g/ml)	Tapped density (g/ml)	Hausner ratios	Carr's compressibility index (%)
A	18.66	25.05	0.52	0.56	1.07	6.25
B	20.43	25.85	0.53	0.55	1.04	4.26
C	20.79	27.16	0.53	0.56	1.07	6.38
D	20.63	24.09	0.50	0.56	1.14	11.98
E	18.74	26.03	0.49	0.53	1.06	5.99

Table 6 presents the results of the stationary angle test for formulas 1-5, all of which are below 30°. A stationary angle of no more than 30° indicates excellent flow properties, meaning all the formulas demonstrate good flow behavior. The granules flow more quickly and easily with less friction and tensile force between them. Furthermore, smaller granule sizes increase cohesiveness, reducing the flow velocity and resulting in a higher stationary angle.[29] Determining bulk density includes measuring the actual weight, compressive weight, Hausner factor, and percent compressibility. The Hausner factor is used to compare the actual and compressive weights, helping to assess the flow or free-flowing properties of the powder. All seven formulas meet the qualification of having a Hausner factor of less than 1.25, indicating good flow characteristics. Granule compressibility refers to the ability of the granules to maintain compactness under pressure. Factors such as porosity, type density, particle shape, and moisture content can affect the flow properties of the granules. Good flow properties ensure easier molding of the granules and help maintain uniform weight. The results for the Hausner factor and compressibility are shown in Table 6. The percent compressibility results indicated that Carr's index ranged from 4.26% to 14.59%, which aligns with the literature stating that granules with a Carr's index value below 15% demonstrate good flowability.

The optimal formula using Design Expert is intended to generate the most efficient formula based on the response data from the prepared parameters. The response data, analyzed through ANOVA in Design Expert, is processed to identify the optimal formula.[10] The ideal formula is the one with a desirability value closest to 1. Using the simplex lattice design method in the Design Expert software, the optimal formula was determined to have 65.81 mg of tartaric acid and 4.19 mg of citric acid, with a desirability value of 0.86. Before finalizing, the optimal formula requires verification. The results of design expert optimization formula 1 solution and formula 2 solution are shown in Figure 4, with the formula test results from the design expert optimization provided in Table 7. This study utilized a Design Expert to optimize the effervescent granule formulation containing PC extract Figure 4. This software allows integrated analysis to evaluate interactions between formulation variables and determine the optimal combination of ingredients used. The optimization of the effervescent granule formulation resulted in two optimal formulas. Formula 1: 13.16% tartaric acid and 0.84% citric acid. Formula 2: 13.21% tartaric acid and 0.80% citric acid. The desirability score for both formulas was 0.862, indicating a high optimization level. The flow rate and angle of repose parameters from the optimized formulas showed no significant differences compared to laboratory experimental results ($p > 0.05$), suggesting the predictive model's accuracy in Figure 5. Based on the GraphPad Version 9.5.1 (528), 2023 statistical analysis Figure 5, the flow rate and angle of repose values from Formula 1 and Formula 2 in both the Design Expert optimization and the actual test results showed no significant difference, indicating that the optimization and laboratory test produced similar outcomes.

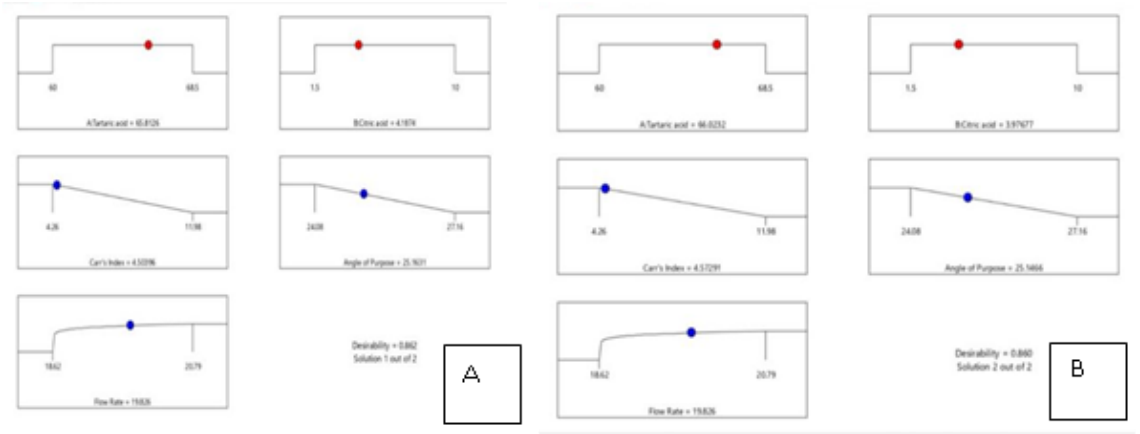

Figure 4: Design Expert optimization (A) Formula 1 Solution (B) Formula 2 Solution

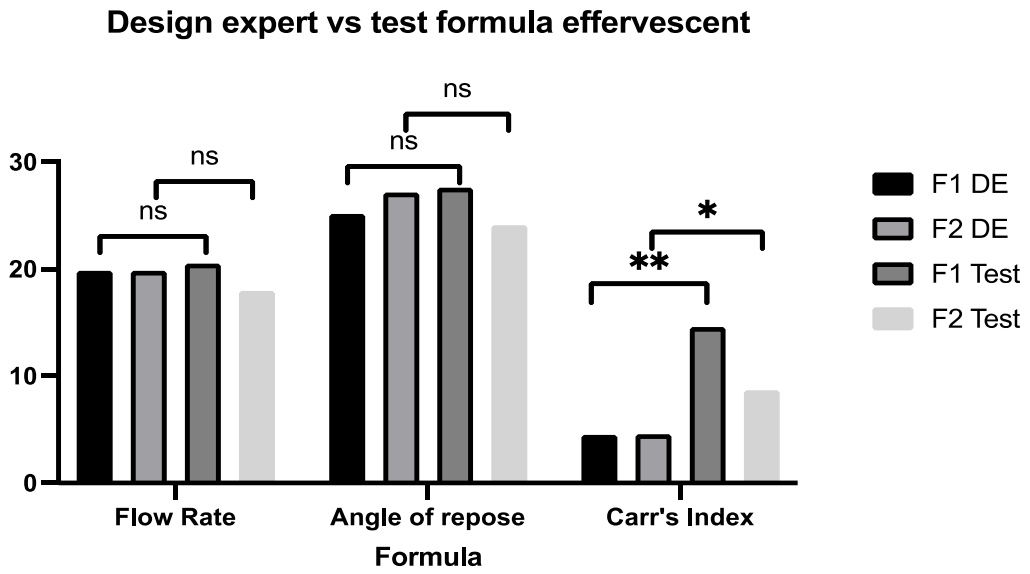

Figure 5: Formula optimization using Design expert vs test, ns = not Significant (p>0,05), *(p<0,05), F1 DE (Formula 1 Design Expert), F2 DE (Formula 2 Design Expert), F1 Test (Formula 1 test), F2 Test (Formula 2 test).

Table 7: Results of the formula test from Design Expert Optimization.

Formula	Flow rate	Angle of repose	Bulk density (g/ml)	Tapped density (g/ml)	Hausner ratios	Carr's compressibility index (%)
1	20.50	27.61	0.52	0.60	1.17	14.59
2	17.96	24.10	0.54	0.59	1.10	8.68

However, Carr's index test revealed a discrepancy between the Design Expert optimization and the test results, as effervescent granules are highly sensitive to room temperature, which may have influenced the test outcomes. Additional research is needed to isolate compounds from PC based on the results of the in silico data. An integrated study of network pharmacology and component analysis should be conducted to explore the molecular mechanisms of PC extract in treating obesity.[30] In silico anti-obesity activity should be explored using additional receptor targets, as the anti-obesity mechanism extends beyond pancreatic lipase. Central nervous system mechanisms can be investigated, targeting receptors such as GLP-1 (liraglutide), 5-HT2c (lorcaserin), and TAAR-1 (phentermine). In vivo, testing is also recommended to validate the efficacy and safety of these compounds in animal models and clinical settings. Furthermore, advanced formulations, such as nanocarrier systems, could be developed to enhance PC-based products' bioavailability and therapeutic potential. Future studies should focus on the antioxidant properties of the sample using comprehensive methods.[31] These include determination of hydrogen peroxide scavenging capacity, determination of ferric reducing power, determination of nitric oxide (NO) scavenging activity, determination of ascorbic acid, determination of vitamin e, and assessment of lipid peroxidation inhibition. These assays will provide a deeper understanding of the antioxidant potential and the mechanisms by which the sample mitigates oxidative stress.

Conclusion

The antioxidant activity of the PC fraction, evaluated using the DPPH method, revealed that the ethanol fraction exhibited significantly stronger antioxidant activity (IC_{50} = 47.27 ± 1.90 µg/mL) compared to the ethyl acetate fraction (IC_{50} = 201.89 ± 20.08 µg/mL, $p < 0.05$). This difference highlights the greater presence of polar compounds, such as flavonoids and phenolics, in the ethanol fraction. Among the identified compounds, dimethyl tetracycline showed the lowest binding energy (-7.78 kcal/mol) in molecular docking studies, suggesting its potential as a strong pancreatic lipase inhibitor. This was further supported by its adherence to Lipinski's Rule of Five, indicating good oral bioavailability. Formula optimization using the Design Expert software resulted in two formulas. The flow rate and angle of repose values from the design expert and the laboratory tests did not show significant differences, indicating that the optimization and experimental results aligned. However, differences were observed in the Carr's Index test between the design expert optimization and the lab results.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors affirm that the work presented in this article is original, and they accept full responsibility for any claims related to the article's content.

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