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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]

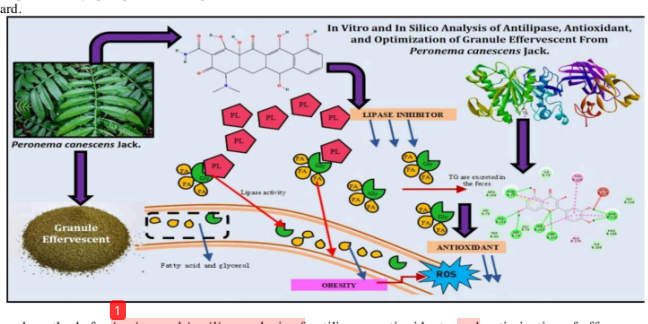

Figure 1: Research methods for *in vitro* and *in silico* analysis of antilipase, antioxidants, and optimization of effervescent granules from PC.

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 4-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 80°C, an injection temperature of 300°C, and an ion source temperature of 250°C. The oven temperature was gradually increased to 330°C at 6°C 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

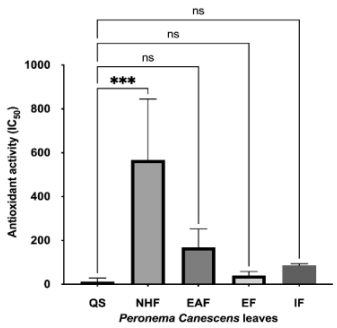

Figure 2: Antioxidant activity of PC fractions measured by DPPH assay, ns = not Significant (p>0,0***(p<0,001), QS (Quercetin standard), NHF (n-Hexane fraction, EAF (Ethyl acetate fraction), EF (Ethanol fraction), IF (Insoluble fraction).

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 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, inhi 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 e nol 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.

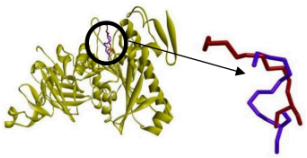

Figure 3: 3D structure of the pancreatic lipase enzyme (PDB ID 1LPB) showing an overlay of the blue (before) and red (after) molecular docking of the native ligand.

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 Activity (PSA)	Voltage
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.

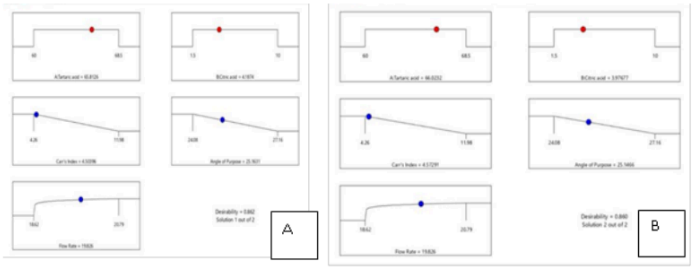

Figure 4: Design Expert optimization (A) Formula 1 Solution (B) Formula 2 Solution

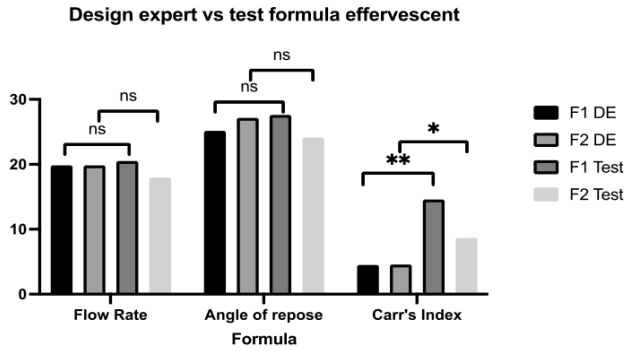

Figure 5: Formula optimization using Design expert vs test, ns = not Significant (p>0,05), *(p<0,05), F1 DE (Formula 1 Design Expert), F2 DE (Formula 2 Design Expert), F1 Test (Formula 1 test), F2 Test (Formula 2 test).

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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