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Phytochemicals and Lipase Inhibition of Citronella, Galangal, and Sand Galangal: *In Vitro–In Silico* Approaches

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ABSTRACT

Obesity is a major global health concern, often treated by inhibiting pancreatic lipase to reduce fat absorption. While chemical-based medicine is a widely used synthetic inhibitor, its side effects highlight the need for safer, natural alternatives. This study aimed to characterize the total phenolic content (TPC), total flavonoid content (TFC), antioxidant activity, and pancreatic lipase inhibition of citronella leaf (Cymbopogon nardus), galangal rhizome (Alpinia galanga), and sand galangal rhizome (Kaempferia galanga) through in vitro and in silico analyses and to identify the potential phytochemical compounds responsible for the activity. Citronella showed the highest TPC, TFC, and FRAP values (14.20±0.21 mg GAE/g, 17.36±9.51 mg QE/g, and 92.01±1.88 µmol TE/g, respectively), indicating strong antioxidant potential. Galangal exhibited the highest extraction yield (21.86±5.34%) and DPPH activity (1.09±0.27 µmol TE/g). In vitro lipase inhibition assays revealed galangal and sand galangal had moderate inhibitory effects (IC_{50}= 401.2±18.24 and 374±11.24 µg/mL), while citronella showed weak activity. LC-MS/MS analysis of galangal identified eight compounds, including galangin, eugenol, and galanganol C. Molecular docking showed galangin had the strongest binding affinity (ΔG= -10.239 kcal/mol), interacting with catalytic residues Ser152 and His263 of pancreatic lipase via hydrophobic and electrostatic interactions. These findings suggest that citronella, galangal, and sand galangal possess potential as natural pancreatic lipase inhibitors, with galangal particularly galangin showing the most promising activity for obesity prevention and management.

Keywords: *antioxidant, galangin, in silico, lipase inhibitors, phytochemicals*

INTRODUCTION

The Indonesian Ministry of Health confirmed the surge in obesity cases in 2018, from 15.4% to 21.8% (Kemenkes, 2018). The World Obesity Federation also predicts that in 2030, Indonesia's prevalence will increase to 22%, and they are responsible for 3.1% of Indonesia's GDP for health facilities (WOF, 2023). Obesity is closely related to body fat metabolism and is characterized by a BMI > 30 which stems from an imbalance of intake and calorie expenditure (Gómez-Apo *et al.,* 2021; Liu *et al.,* 2020). Commonly, triglycerides comprise 90% of the main diet and these exogenous fats cannot be directly used as energy. It must be hydrolyzed for absorption, and the important enzyme responsible for lipid absorption is pancreatic lipase (Liu *et al.,* 2020). Pancreatic lipase hydrolyzed 50−70% of triglycerides, producing monoglycerides and free fatty acids. One of the treatments for preventing and managing obesity targets pancreatic lipase such as orlistat. Orlistat reduces 30% of lipid hydrolysis in the intestines (Gómez-Apo *et al.,* 2021). However, orlistat has disadvantageous effects including acute oxalate nephropathy, steatorrhea, pancreatitis, diarrhea, sub-acute hepatic failure, depression, abdominal pain, headache, cholelithiasis, polydipsia, hypertension, and constipation (Rajan *et al.,* 2021). Because of this, an alternative to preventing and managing obesity is urgently needed.

Phytochemicals such as phenols and flavonoids are known to have activity to prevent and manage obesity. Phenols and flavonoids could decrease body weight, triglyceride, total cholesterol, and LDL (low-density lipoprotein) interact with the triad catalytic (Ser156-Asp176-His263) of pancreatic lipase and lead to inhibiting pancreatic lipase activity (Liu *et al.,* 2020). Some Indonesian herbs such as citronella, galangal, and sand galangal are also commonly known as traditional medicine (Hasim *et al.,* 2023). Citronella (*Cymbopogon nardus*) is used for fevers,

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rheumatism, menstrual problems, and intestinal parasites. Citronella reported has citronellal, citronellol, and geraniol as major compounds (Kaur *et al.,* 2021). Citronellal and citronellol are recognized by these effects to decrease weight by decreasing appetite (Batubara *et al.,* 2015). On the other hand, galangal (*Alpinia galanga*) is well-known as an anti-inflammation, anti-microbial, and anti-ulcer (Kumar and Alagawadi, 2013). Galangin found on galangal has been tested on 3T3 cells showing the ability to reduce the accumulation of triglyceride and is adipogenic for regulating CCAAT/enhancer binding protein α, SREBP-1, and PPARγ (Jung *et al.,* 2012). In addition, sand galangal (*Kaempferia galanga* L.) also has activity to prevent obesity by inhibiting the signaling of Akt and mTOR. These are known for their treatment and manage obesity by restraining pancreatic lipase (Iswantini *et al.,* 2010; Shin *et al.,* 2003; Souza *et al.,* 2012). Extract of *Citronella citratus* and sand galangal has been reported to be active in inhibiting pancreatic lipase (Shin *et al.,* 2003; Iswantini *et al.,* 2010). On the other hand, galangin and 3-metylethergalangin were reported on *Alpinia officinarum* to have an activity to inhibit pancreatic lipase (Iswantini *et al.,* 2010; Souza *et al.,* 2012).

Previous investigations have shown that citronella, galangal, and sand galangal have potential to prevent and manage obesity. However, there is a lack of information regarding which of these herbs has the greatest potential to inhibit pancreatic lipase. Therefore, this study aims to explore and characterize citronella, galangal, and sand galangal as natural alternatives for preventing and managing obesity using *in vitro* and *in silico* approaches. Specifically, the evaluation focuses on phytochemical profiles, antioxidant capacity, and pancreatic lipase inhibitory potential. Furthermore, the *in silico* analysis was conducted to predict the interaction between secondary metabolites and pancreatic lipase to validate their inhibitory activity.

MATERIALS AND METHOD

Materials

The materials (citronella, galangal, and sand galangal) were collected from Biofarmaka, IPB University, Bogor Regency, West Java, Indonesia, in August 2023. The 3-dimensional structure of metabolite compounds was downloaded from PubChem, while the structure of pancreatic lipase ID 1LPB target protein was downloaded from RCSB Protein Data Bank in the same month.

Extraction

Citronella, galangal, and sand galangal were procured from Biofarmaka, IPB University, and were washed and dried under sunlight to eliminate moisture content (2.67±0.31% for sand galangal, 2.42±0.10% for galangal, and 5.87±0.00% for citronella). Subsequently, samples were ground then 10 g of sample powder was extracted by ultrasonic method (Decon F5 Major Ultrasonic Processor, New York, USA) using 70% ethanol for 30 min (triplo). Afterward, the extract was filtered and evaporated (Hansin Scientific CO, Seoul, Korea) to remove excess solvent and concentrated biocompounds. Extracts were calculated as yield, transferred to a sealed glass container, and stored at 4 °C to preserve stability (Kousar *et al*., 2023; Wijaya and Noviana, 2022)

Total phenolic content (TPC)

A total of 20 *µ*L extract (1 mg/mL) underwent a reaction with 120 *µ*L Folin Ciocalteu 10% (v/v) and incubated for 5 min. Next, $NaCO_3$ 80 *µ*L was added which was then further incubated for 30 min. Afterward, the phenolic content was measured by a microplate reader (SPECTROstarNano, BMG LABTECH, Ortenberg, Germany) at 750 nm. TPC expressed by mg GAE/g DW (Marlini *et al.,* 2022).

Total flavonoid content (TFC)

Total flavonoid content was quantified by adding 10 *µ*L of extracts (1 mg/mL) combined with 10 *µL* $AlCl_3$ 10% (b/v), 50 *µL* of ethanol p.a, 10 *µL* CH_3COOH p.a, and 120 *µL* distilled water. This mixture was incubated for 30 min and then measured by microplate reader (SPECTROstarNano, BMG LABTECH, Ortenberg, Germany) at 517 nm. TFC expressed by mg QE/g DW (Marlini *et al.,* 2022).

DPPH radical scavenging activity

DPPH (2,2-diphenyl-1-picrylhydrazyl) determines the antioxidant capacity stabilized free radicals along electron transfer (SET) or by donating H^+ (HAT) (Gulcin and Alwasel, 2023). The 100 *µ*L of 125 mM DPPH solution in ethanol was mixed with 100 *µ*L of extracts (1 mg/mL), incubated for 30 min in a dark room and measured at 517 nm which was expressed by mg TE/g DW (Salazar-Aranda *et al.,* 2011).

FRAP

The FRAP (Ferric Reducing Antioxidant Power) assay was performed to determine antioxidant capacity using a freshly prepared FRAP reagent consisting of 1 mM TPTZ in 40 mM HCl, 20 mM $FeCl_3$, and 300 mM acetate buffer (pH 3.6) in a ratio of 1:1:10 (v/v/v). Then, 20 *µ*L of each extract (1 mg/mL) was added to 180 *µ*L of the FRAP reagent and incubated at 37 °C for 15 min. Absorbance was measured at 595 nm, and the results were expressed as mg Trolox equivalents per gram dry weight (mg TE/g DW) (Benzie and Strain, 1999; Sekhon-Loodu *et al*., 2021).

Inhibition of pancreatic lipase assay

The measurement of pancreatic lipase activity was followed by Chedda *et al.* (2016), Lankatillake *et al.* (2021), and Pliego *et al.* (2016) with modification using p-NPB (Sigma Aldrich, St. Loius, USA) (p-nitrophenyl butyrate) as a substrate. The 25 µL extracts were mixed with 100 µL of phosphate buffer saline (PBS, containing 0.5% Triton-X-100 pH 7.2 and 150 mM NaCl), 50 µL enzyme (300 µg/mL in 0.1 M PBS (pH 7.2)), and p-NPB 0.2 M in acetonitrile 25 µL. After, incubated at 37 °C for 30 min. Subsequently reaction was halted by placing the mixture in a hot water bath, then allowing it to cool at room temperature for 10 min. Afterward, the inhibition activity of pancreatic lipase was measured at 415 nm. Orlistat is used as positive control by dissolving 120 mg orlistat with 12 mL of DMSO. The inhibition activity was represented by the percent inhibition calculated by Equation 1.

$$\% \text{ Inhibition} = \frac{(\text{Absorbance blank} - \text{Absorbance of test})}{\text{blank}} \times 100\% \quad \ldots\ldots (1)$$

Statistical analysis

The data were processed using RStudio (version 4.4.0) and analyzed using one-way analysis of variance (ANOVA), followed by Duncan's multiple range test (DMRT) at a 95% confidence level.

LC-MS/MS analysis

Identification of metabolite compounds on galangal using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) incipiently galangal (*Alpinia galanga*) extract 1.4 mg was dissolved in 100 µL of methanol. The mixture was filtered by 0.2 m GHP and injected to the UPLC system. Analysis was performed using a Xeno G2-S resolution with a C-18 column and Xeno G2-S resolution Quadrupole Time-of-Flight (QtoF) mode Electrospray Ionization (ESI) (-) and Multiple-stage Fragmentation (MSE). The mobile phase used was formic acid 0.1% in distilled water and formic acid 0.1% in acetonitrile, operated for 20 min at 100 °C. The gradient elution started with a solvent composition of 70% formic acid 0.1% in distilled water and 30% formic acid 0.1% in acetonitrile during 0–1 min, shifted to 5% formic acid 0.1% in distilled water and 95% formic acid 0.1% in acetonitrile from 6–18 min, and returned to the initial 70:30 ratio with a linear gradient from 19–20 min. Chromatogram data were analyzed using MassLynx 4.1 software, PubChem, and MoNA web server. Compound identification and selection were conducted based on multiple analytical parameters. The primary basis for selection included the molecular ion $[M^+H]^+$ as indicated by the mass-to-charge ratio (m/z), as well as software-assisted matching metrics such as i-Fit, i-Fit Norm, and Fit-Confidence (Widiastuti *et al.,* 2023). Because authentic reference standards were not analyzed, compound identifications are considered putative (MSI Level 2) and are reported accordingly.

Collection of 3D ligand and protein

The 3-dimensional structure of metabolite compounds discovered in galangal and human pancreatic lipase ID 1LPB was downloaded from Pubchem (https://pubchem.ncbi.nlm.nih.gov/) and RSCB Protein Data Bank (https://www.rcsb.org/) in *pdb format. Then they were transferred to *sdf using YASARA Structure. Furthermore, the 1LPB was followed Mudianta *et al.* (2024) prepared by removing water and the experimental ligand. The protein was saved in *pdb format. Meanwhile, the ligands prepared by adding hydrogen atoms and energy minimalization using AMBER 14, ligands were saved in *pdb and *sdf format.

Molecular docking

Molecular docking of human pancreatic lipase ID 1LPB model was conducted using YASARA. The docking involved 50 runs under AMBER14 force field, VINA method, and executed using the "dock_run" macro. Data collected were binding energy and interacted residues. Afterward, data were visualized by Discovery Studio (2D) and Pymol (3D) (Mudianta *et al*., 2024).

RESULTS AND DISCUSSION

Extraction, TPC, and TFC of citronella, galangal, and sand galangal

The extraction of these samples using ultrasonication showed galangal had the highest yield at 21.86±5.34% compared to citronella (13.36±0.92%) and sand galangal (17.63±1.34%) (Table 1). These yields are notably higher than those of previous studies using maceration extraction, such as, Hidayati *et al*. (2023) for galangal (12.47%), Setyowati *et al*. (2025) for citronella (10.64%), and Handayani *et al*. (2015) for sand galangal (14%). Ultrasonication utilizes ultrasonic energy to enhance the extraction of bioactive compounds. The ultrasonic wave caused the negative pressure and led to the formation of cavitation bubbles. The combination of ultrasonic wave and cavitation bubbles results in one or more combinations of fragmentation, local erosion, pore formation, shear force, increasing absorption, and swelling index of the plant matrix (Kumar *et al.,* 2021). These mechanisms contribute to release of bioactive compounds, thereby increasing extraction yields.

The total phenolic content was carried out by calorimetry with Folin-Ciocalteu assay. Folin-

Ciocalteu involved redox reaction based on single-electron transfer (SET) between Folin-Ciocaletu reagent and sample, forming blue color that was quantified by spectrophotometer (Perez *et al.,* 2023). Among samples, citronella exhibited the highest TPC of 14.20±0.21 mg GAE/g, whereas sand galangal had the lowest at 2.52±0.58 mg GAE/g (Table 1). The TPC of citronella was lower than Sankara *et al*. (2024), who conducted TPC values of citronella at 42.69±6.88 mg GAE/g. A similar result was observed in sand galangal, which showed a lower value than the 17.92 mg GAE/g reported by Subaryanti *et al.* (2022). These differences may be attributed to several factors, including the solid-to-solvent ratio, solvent type, extraction method, extraction time, and sample origin (Kiptiyah *et al.,* 2021; Subaryanti *et al.,* 2022).

Table 1. Yield, total phenol, and flavonoid content of citronella, galangal, and sand galangal

	Yield (%)	TPC (mg GAE/g)	TFC (mg QE/g)
Citronella	13.36±0.92^{a}	14.20±0.21^{a}	17.36±9.51^{a}
Galangal	21.86±5.34^{a}	6.02±0.41^{b}	7.53±2.57^{a}
Sand galangal	17.63±1.34^{a}	2.52±0.58^{c}	4.42±0.72^{a}

Note: Mean values in columns marked with different letters indicate significant differences at $p<0.05$; determined by DMRT

Total flavonoid content was quantified using colorimetry method based on color changes that occur between $AlCl_3$ and specific functional groups in flavonoid compounds. The interaction of $AlCl_3$ and keto group at C4, hydroxyl groups at C3/C5, and ortho-dihydroxyl groups on ring A or B caused yellow complex measured by spectrophotometer. On our finding, citronella became the highest flavonoid at 17.36±9.51 mg QE/g and sand galangal displayed the lowest at 4.42±0.72 mg QE/g (Table 1). This value is higher than reported by Mishra and Sharma (2021) which showed sand galangal has 0.813 mg QE/g. Meanwhile, galangal also has lower than Aljobair (2022) 14.12±0.40 QE/g, possibly due to the minor role of flavonoids as constituents in galangal rhizome (Iswantini *et al.,* 2010).

Antioxidant capacity of citronella, galangal, and sand galangal

Results of antioxidant capacity showed, galangal has the highest antioxidant capacity at 1.09±0.27 mg TE/g on the DPPH assay. Moreover, citronella has the highest on FRAP assay at 92.01±1.88 mg TE/g (Table 2). The lowest free radical scavenging capacity both DPPH and FRAP assay were obtained in sand galangal at 0.26±0.01 (DPPH) and 6.81±0.71 *μ*mol TE/g (FRAP). This result may be attributed to the lower TPC and TFC compared to other extracts. Although sand galangal has significant levels of flavonoids, the antioxidant activity of flavonoids can be diminished when they are modified, such as through glycosylation, and lead to reduced anti-oxidant capacity (Ahwan *et al.,* 2024).

Table 2. Antioxidant capacity of citronella, galangal, and sand galangal

	DPPH (*μ*mol TE/g)	FRAP (*μ*mol TE/g)
Citronella	0.82±0.16^{ab}	92.01±1.88^{a}
Galangal	1.09±0.27^{a}	26.10±5.86^{b}
Sand galangal	0.26±0.01^{b}	6.81±0.71^{c}

Note: Mean values in columns marked with different letters indicate significant differences at $p<0.05$; determined by DMRT

Antioxidants play a crucial role in neutralizing free radicals and preventing oxidation through mechanisms such as donating electron or hydrogen transfer (Haerani *et al.,* 2018; Lai-Cheong and McGrath, 2021). Antioxidants protect cells from damage by inhibiting or slowing cellular degradation, preventing the oxidation of proteins, DNA, and lipids, also help to prevent Alzheimer's, amyotrophic lateral sclerosis, Parkinson's, and cardiovascular diseases (Andarina and Djauhari, 2017; Carrera-Julia *et al.,* 2020; Filograna *et al.,* 2016; Mlynarska *et al.,* 2024; Sayuti and Yenrina, 2015; Sinyor *et al.,* 2020;). Antioxidants are found on fruits, mushrooms, beverages, cereals, flowers, and spices (Xu *et al.,* 2017). Quantification of antioxidant capacity could be determined by DPPH and FRAP methods, both methods aim to evaluate the potential compounds to stabilize free radicals. In the DPPH assay, the free radicals could be stabilized either by donating an electron or a hydrogen atom. In contracts, the FRAP assay stabilizes free radicals solely by donating electrons to neutralize the radicals (Kotha *et al*., 2022; Munteanu and Apetrei 2021; Xiao *et al.*, 2020).

Inhibition of pancreatic lipase of citronella, galangal, and sand galangal

The activity of pancreatic lipase inhibition was measured using the calorimetry method using p-nitrophenyl butyrate (p-NPB) as a substrate. p-NPB would be hydrolyzed by pancreatic lipase and produce butyrate and p-nitrophenyl ion, which p-nitrophenyl ion colored yellow (Pliego *et al.,* 2015; Rocha *et al.,* 2023). Orlistat was used as the positive control. Orlistat is used because it is well-known to treat obesity. Orlistat acts by inhibiting pancreatic lipase with a covalent bond on the active side of pancreatic lipase (Qi, 2018). The activity of pancreatic lipase inhibition was expressed by IC_{50}. The IC_{50} indicates how much of inhibitor is needed to achieve half-maximal inhibition (Aykul and Martinez-Hackert, 2016). Batubara *et al*. (2010) classified IC_{50} values at strong (<100 *μ*g/mL), medium (100−450 *μ*g/mL), and weak (450−700 *μ*g/mL). The inhibition of pancreatic

lipase showed sand galangal (374±11.24 μg/mL) and galangal (401.2±18.24 μg/mL) showed medium inhibition, and citronella had weak (527.2±47.33 μg/mL) (Figure 1). However, sand galangal and galangal have no significant differences (p<0.05), but these samples are weaker than orlistat, which has IC_{50} of 48.9±4.4 μg/mL.

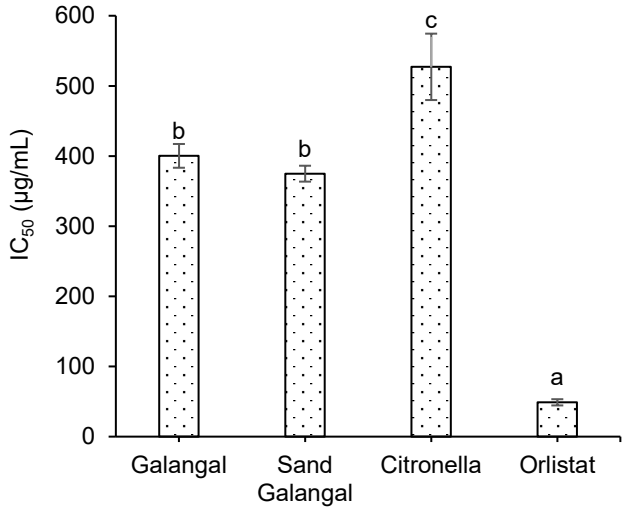

Figure 1. The activity of pancreatic lipase of citronella, galangal, sand galangal, and orlistat, expressed by IC_{50}. Mean values in columns marked with different letters indicate significant differences at p<0.05; determined by DMRT

The correlation matrix is presented in Figure 2. Analysis using the Pearson method revealed a strong positive correlation between the phenolic and flavonoid contents and both antioxidant capacity (DPPH and FRAP) and pancreatic lipase inhibition. This suggests that phenolic and flavonoid compounds may contribute to the observed inhibition. However, considering that higher phenolic/flavonoid levels did not consistently correspond to lower IC_{50} values, it is possible that some of these compounds exert antagonistic effects, or that other non-phenolic compounds play a more direct role in lipase inhibition. Based on the IC_{50} values and antioxidant profiles, galangal was selected as the extract with the most promising bioactivity. Therefore, it was subjected to LC-MS/MS analysis to identify its constituent bio-active compounds, followed by *in silico* docking to validate their interaction with pancreatic lipase.

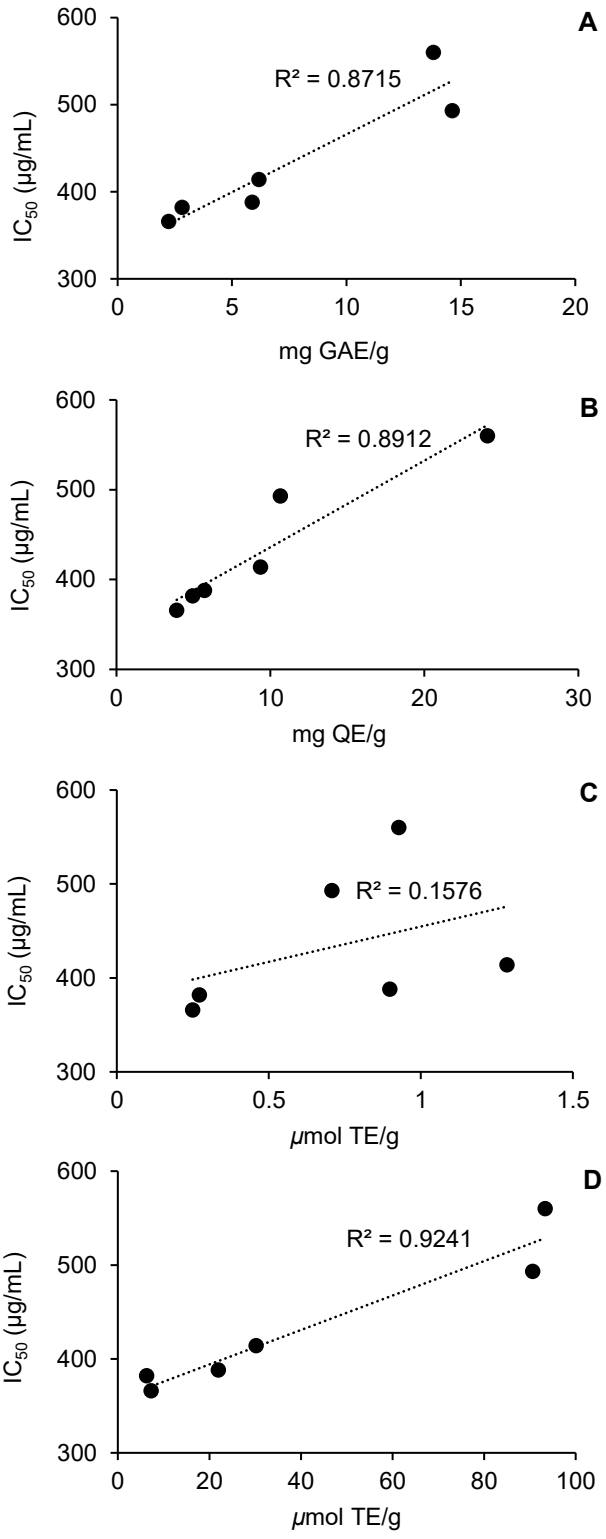

Figure 2. Correlation of TPC (A), TFC (B), and antioxidant capacity by DPPH (C), FRAP (D) of crude extracts of citronella, galangal, and sand galangal with the inhibition of pancreatic lipase determined by Pearson

LC-MS/MS of galangal extract

Galangal extract was identified by LC-MS/MS showed, nine compounds found on galangal (*Alpinia galanga*), reported identifications are putative and supported by the observed precursor m/z, retention times, MS/MS fragment ions, and library match scores (Table 3), such as cinnamaldehyde (Figure 3).

Table 3. Biocompounds of galangal extract

Retention Time	Formula	Biocompound	Group	Molecular Weight (g/mol)
1.87	$C_{14}H_{11}BrO_2$	4-(benzyloxy)-3-bromobenzaldehyde	-	291.14
3.08	C_9H_8O	Cinnamaldehyde	Polyphenol	134.06
5.45	$C_9H_8O_2$	Trans-p-hydroxycinnamaldhye	Polyphenol	148.06
7.06	$C_{27}H_{28}O_5$	Galanganol C	Terpene	399.16
9.78	$C_3H_3ClN_4O_2$	3-Chloro-N-hydroxy-1H-1,2,4-triazole-5-carboxamide	-	162.07
10.20	$C_{10}H_{12}O_2$	Eugenol	Phenol	164.20
10.64	$C_{15}H_{10}O_5$	Galangin	Flavonoid	271.24
10.66	$C_{20}H_{30}O_3$	Galanal A	Terpene	318.28
19.44	$C_7H_6O_6$	p-Formylphenol	Phenol	122.12

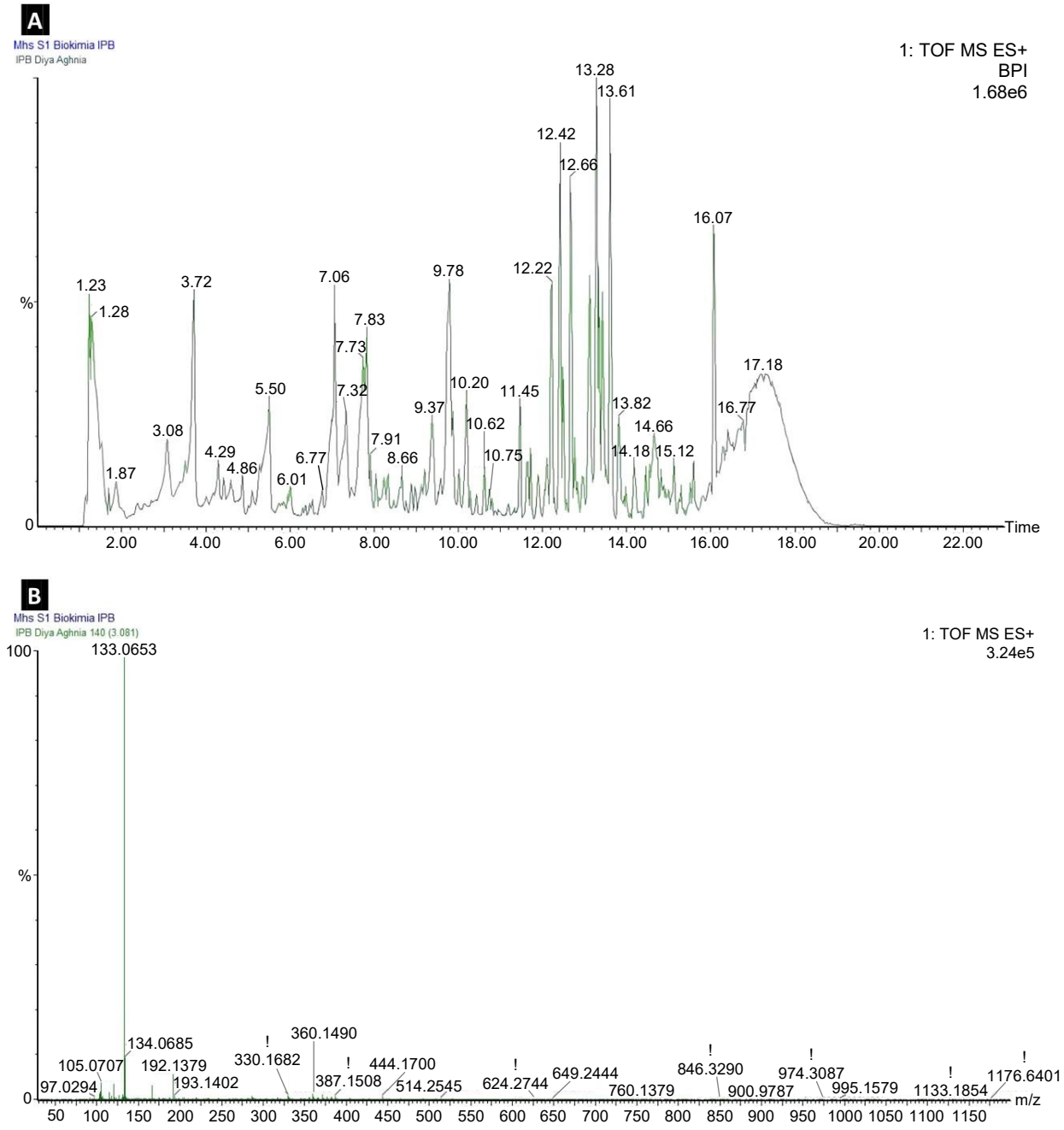

Figure 3. Chromatogram of galangal extract (A) and spectra of cinnamaldehyde found in galangal (B)

Phenol was the most common group found in galangal extract. Other compounds such as 1'-acetoxychavicol acetate, 1'-acetoxyeugenol acetate, 1'-hydroxychavicol acetate, alpha-farnesene borneol, bornyl acetate, ferulic acid, galangal A, galangal B, galanganal, leuteolin may be conducted in galangal (Das *et al.,* 2020; Khairullah *et al.,* 2020; Manse *et al.,* 2016; Morikawa *et al.,* 2005; Morita and Itokawa, 1998). It could happen because of the different solvents that are used. Several compounds found on galangal were identified as polyphenols, flavonoids, and terpenes, which are known as antioxidants. Phenolic and flavonoids are known as antioxidants because the benzene ring in their structure allows them to stabilize when they react to free radicals and the position of hydroxyl groups (Zeb, 2020).

LC-MS/MS QToF is an advanced analytic technique that combines liquid chromatography with QToF mass spectrometry for the identification and quantification of metabolite in the sample. The technique utilized a quadrupole mass analyzer to obtain the parent ions mass to charge ratio (m/z) and time-of-flight analysis to determine the product ion m/z (Harmita *et al*., 2019). The LC system uses ultra-performance liquid chromatography (UPHL) with a C18 column. HPLC is used in the identification of active compounds in samples based on their polarity.

Molecular docking

The molecular docking of galangal extract used YASARA Structure within 42 ligands, 8 ligands identified by LC-MS/MS, 2 comparative ligands (orlistat and metoxyundecylphoshinic acid (MUP)), and 32 ligands identified by the literature studied. Molecular docking is used to analyze the interaction between ligands and protein receptor by contacting residues and binding affinity values, or Gibbs free energy (ΔG) (Agu *et al.,* 2023; Winarsih *et al.,* 2024). MUP is used as a comparative ligand because it binds by 1LPB structures as an experimental ligand that binds the active side (Egloff *et al.,* 1995). As a result, galangin came as the most potential inhibitor that binds active site and triad catalytic residues followed by isorhamnetin, kaempferide, kaempferol, luteolin, galaganol C, and galaganolacetone.

Galangin binds with Arg256 as an active site and Ser152 and His263 as the catalytic site by hydrogen, carbon-hydrogen, and pi-cation interaction (Figure 4). Triad catalytic (Ser152-Asp176-His) of pancreatic lipase plays a major role in inhibiting pancreatic lipase. Ser152 acts as interface recognition, contributing to the regulation of lipase adsorption. The adsorption process induces significant conformation changes in lipase, affecting the movement of the lid domain and surface loops, thereby facilitating substrate access to the catalytic site (Hussain, 2014). Asp176 residue is responsible for 80% of hydrolyzing substrates and the mutation of His263 leads to the loss of the catalytic function of the enzyme (Kumar and Chauchan, 2021). It's related to the interaction of ligand and the catalytic site may lead to the inhibition of pancreatic lipase.

Galangin also has the highest Gibbs free energy (ΔG) at -10.239 kcal/mol compared with orlistat (-6.703 kcal/mol) and MUP (-5.662 kcal/mol) (Table 4). The interaction of contacted residues could represent Gibbs energy values such as hydrogen bond, van der Waals, hydrophobic, and electrostatic interaction (Syahbanu *et al.,* 2021). Galangin showed hydrogen bonds on Ser152 residues, hydrogen bonds are dipole-dipole interactions that are more stable than other interactions and lead to lower Gibbs free energy and increased binding affinity (Du *et al.,* 2016). Taken together, galangal was selected due to its moderate IC_{50} (401.2 *μ*g/mL), high antioxidant capacity, and notable phenolic and flavonoid content. LC-MS/MS analysis identified key compounds such as galangin, eugenol, and galanganol C. Among them, galangin showed the strongest binding affinity (ΔG = -10.239 kcal/mol) and interacted with catalytic residues Ser152 and His263. This consistency across *in vitro* inhibition, compound profiling, and *in silico* docking supports galangin as the major bioactive compound in galangal.

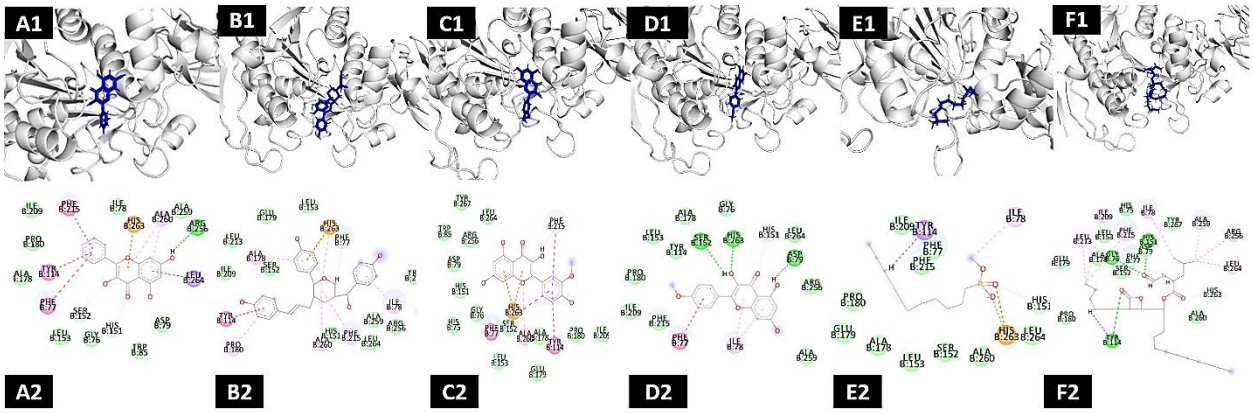

Figure 4. Molecular docking of phytochemicals found in galangal to pancreatic lipase (A) galangin; (B) galanganol C; (C) isoharmetine; (D) kaempferide; (E) orlistat; (F) MUP

Table 4. Molecular docking of galangal

Compounds	Gibbs free energy (ΔG) (kcal/mol)	Contacting Residues
Galangin	-10.239	Gly76, Phe77, Ile78, Asp79, Trp85, Try114, His151, Ser152, Leu153, Ala178, Pro180, Ile209, Phe215, Arg256, Ala260, His263, Leu264
Isoharmnetin	-9.179	His75, Gly76, Phe77, Asp79, Trp85, Try114, His151, Ser152, Leu153, Ala178, Glu179, Pro180, Ile209, Phe215, Arg256, Ala260, His263, Leu264, Try267
Kaempferide	-8.990	Gly76, Phe77, Ile79, Asp79, Try114, His151, Ser152, Leu153, Ala178, Pro180, Ile209, Phe215, Arg256, Ala259, His263, Leu264
Kaempferol	-8.990	Gly76, Phe,77, Ile78, Asp79, Try114, His151, Ser152, Pro180, Ile209, Phe215, Arg256, Ala259, His263, Leu264
Luteolin	-8.990	Gly76, Phe,77, Ile78, Asp79, Try114, His151, Ser152, Pro180, Ile209, Phe215, Arg256, Ala259, His263, Leu264
Orlistat	-6.702	His75, Gly76, Phe77, Ile78, Asp79, Try114, His151, Ser152, Leu153, Ala178, Glu179, Pro180, Ile209, Leu213, Phe215, Arg256, Ala259, Ala260, His263, Leu264, Try267
MUP	-5.662	Phe77, Ile78, Try114, His151, Ser152, Leu153, Ala78, Glu179, Pro180, Ile209, Phe215, Ala260, His263, Leu264

CONCLUSION

Among the tested extracts, galangal demonstrated the strongest potential as a natural pancreatic lipase inhibitor. It had the highest extraction yield (21.86±5.34%), strong antioxidant activity (1.09±0.27 *μ*mol TE/g), and moderate lipase inhibition (IC_{50}= 401.2±18.24 *μ*g/mL). Although citronella exhibited higher TPC, TFC, and FRAP values, its lipase inhibition was weak (IC_{50}= 527.2±47.33 *μ*g/mL). LC-MS/MS analysis identified nine bioactive compounds in galangal, with galangin showing the most negative binding affinity (ΔG= -10.239 kcal/mol) and interaction with key catalytic residues (Ser152, His263). However, compound identifications from LC-MS/MS were based on spectral library matching and are therefore tentative (MSI Level 2). Confirmation using authentic reference standards or co-injection experiments is required to unequivocally identify the compounds and will be performed in follow-up studies. These findings consistently support galangin as the lead inhibitor candidate. Further studies should explore terpenoid compounds and validate IC_{50} values of individual metabolites. This study has some limitations, as the assays were restricted to *in vitro* and *in silico* models and the LC-MS/MS identifications were tentative due to the absence of authentic standards. Nevertheless, the findings suggest practical applications, with galangal and its key metabolite galangin serving as promising natural sources of pancreatic lipase inhibitors that could be developed into functional foods, nutraceuticals, or complementary therapies for obesity management.

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