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Anticholesterolemic Evaluation of *Physalis angulata* L. Ethanolic Extract Using In Vitro, GC-MS, and Docking Approaches

Evaluasi Antikolesterol Ekstrak Etanol *Physalis angulata* L. Menggunakan Pendekatan *In Vitro*, GC-MS, dan Penambatan Molekuler

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ABSTRACT

Physalis angulata L. is traditionally used as a medicinal plant and contains various pharmacologically active metabolites. This study evaluated the anticholesterolemic potential of *P. angulata* ethanolic extract using in vitro assays, gas chromatography–mass spectrometry (GC-MS), and molecular docking. The extract obtained by remaceration contained flavonoids, phenolics, tannins, saponins, and steroids/triterpenoids. Thin-layer chromatography indicated the presence of terpenoids, flavonoids, essential oils, and saponins. Quantitative analysis showed flavonoid, phenolic, tannin, and sterol contents of 34.86 ± 1.11 mg RE/g, 5.14 ± 0.15 mg GAE/g, 0.99 ± 0.01%, and 0.072 ± 0.0004 mg CE/g, respectively. Antioxidant IC_{50} values were 209.55 ± 9.10 ppm for DPPH and 220.18 ± 4.23 ppm for FRAP. The Liebermann–Burchard assay showed cholesterol inhibition with an IC_{50} of 96.11 ppm. GC-MS tentatively identified several compounds, while molecular docking suggested favorable interactions of hydroxydehydrostevic acid and farnesiferol C with HMG-CoA reductase.

Kata kunci:
Inhibisi kolesterol
GC-MS
Penambatan molekuler
P. angulata
Skrining fitokimia

ABSTRAK

Physalis angulata L. secara tradisional digunakan sebagai tanaman obat dan mengandung berbagai metabolit aktif farmakologis. Penelitian ini mengevaluasi potensi antikolesterol ekstrak etanol *P. angulata* menggunakan uji in vitro, kromatografi gas–spektrometri massa (GC-MS), dan penambatan molekuler. Ekstrak yang diperoleh melalui remaserasi mengandung flavonoid, fenolik, tanin, saponin, dan steroid/triterpenoid. Kromatografi lapis tipis menunjukkan adanya terpenoid, flavonoid, minyak atsiri, dan saponin. Analisis kuantitatif menunjukkan kadar flavonoid, fenolik, tanin, dan sterol masing-masing sebesar 34,86 ± 1,11 mg RE/g, 5,14 ± 0,15 mg GAE/g, 0,99 ± 0,01%, dan 0,072 ± 0,0004 mg CE/g. Nilai IC_{50} antioksidan adalah 209,55 ± 9,10 ppm untuk DPPH dan 220,18 ± 4,23 ppm untuk FRAP. Uji Liebermann–Burchard menunjukkan inhibisi kolesterol dengan IC_{50} sebesar 96,11 ppm. GC-MS mengidentifikasi beberapa senyawa secara tentatif, sedangkan penambatan molekuler menunjukkan interaksi yang baik antara hydroxydehydrostevic acid dan farnesiferol C dengan HMG-CoA reduktase.

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1. INTRODUCTION

Cholesterol imbalance, particularly elevated low-density lipoprotein (LDL) levels, is a major risk factor for cardiovascular disease (CVD), which remains one of the leading causes of death worldwide (Pirillo & Norata, 2023). In 2019, the World Health Organization reported that CVD accounted for more than 17 million deaths annually, with hypercholesterolemia being one of the major contributors to this burden. In Indonesia, the Basic Health Research report showed that the prevalence of high cholesterol levels among adults reached 35.9% (Riskesdas, 2018). Statins are commonly used to manage hypercholesterolemia by inhibiting HMG-CoA reductase, a key enzyme in cholesterol biosynthesis (Agosto et al., 2024). However, long-term statin use may be associated with adverse effects, including hepatotoxicity, myopathy, and metabolic complications (Nawata, 2023). These limitations, together with concerns regarding treatment cost and accessibility, have encouraged the exploration of safer and more affordable alternatives from natural sources (Ekor, 2014).

Herbal medicines have gained increasing attention as complementary sources of bioactive compounds for managing lipid metabolism disorders (Karimi et al., 2015). Plant-derived compounds, including flavonoids, phenolics, steroids, saponins, and terpenoids, have been reported to influence cholesterol metabolism through antioxidant activity, inhibition of lipid oxidation, modulation of lipid absorption, and interaction with cholesterol-regulating enzymes (Posadzki et al., 2013). *Physalis angulata* L., commonly known as ciplukan in Indonesia, has traditionally been used to treat various conditions, including infections, inflammation, and metabolic disorders (Novitasari et al., 2024). Previous studies have reported that *P. angulata* contains secondary metabolites such as flavonoids, steroids, and withanolides, which may contribute to its pharmacological properties, including its potential role in lipid regulation (Liu et al., 2011).

Recent advances in phytochemical analysis and computer-aided drug discovery have improved the evaluation of plant-based compounds against disease-relevant molecular targets. Gas chromatography–mass spectrometry (GC-MS) enables the identification of volatile and semi-volatile bioactive compounds, while molecular docking can be used to predict ligand interactions with target proteins such as HMG-CoA reductase (Meng et al., 2011). The integration of in vitro bioassays, phytochemical profiling, and in silico analysis provides a broader preliminary assessment of bioactivity and possible mechanisms. Although the pharmacological properties of *P. angulata* have been previously reported, studies that specifically combine in vitro cholesterol inhibition, GC-MS-based compound profiling, and molecular docking against HMG-CoA reductase remain limited.

Therefore, this study aimed to evaluate the anticholesterolemic potential of *P. angulata* ethanolic extract using an integrated in vitro, GC-MS, and molecular docking approach. The extract was assessed through phytochemical screening, thin-layer chromatography, quantitative determination of selected secondary metabolites, antioxidant assays, and an in vitro cholesterol inhibition assay. GC-MS analysis was conducted to identify major compounds in the extract, while molecular docking was used to predict their binding interactions with HMG-CoA reductase. This approach was applied to provide preliminary scientific evidence regarding the potential of *P. angulata* ethanolic extract as a natural source of anticholesterolemic compounds.

2. METHODS

2.1. Plant Material Collection and Preparation

Roots, stems, leaves, flowers, and fruits of *P. angulata* were collected from Sleman, Indonesia. The plant material was identified and authenticated at the Laboratory of Plant Biology, Sekolah Tinggi Ilmu Farmasi Yayasan Pharmasi Semarang, Indonesia, No: 016/EL-AFM/II/2025. Fresh plant materials, obtained from five-month-old plants, were thoroughly washed, oven-dried at 50 °C, powdered using a blender, sieved through 40/60 mesh, and stored in airtight containers until extraction.

2.2. Ethanolic Extraction and Extract Characterization

A total of 50 g of dried powdered *P. angulata* herb was extracted by remaceration using 500 mL of 95% ethanol (Merck, Darmstadt, Germany) in an amber airtight container. The mixture was intermittently stirred during the first 6 h and then allowed to stand for 18 h. The extraction process was repeated daily using fresh solvent for five consecutive days until the filtrate became colorless. The combined filtrates were concentrated under reduced pressure using a rotary evaporator (Heidolph, Schwabach, Germany) at 50 °C to obtain a viscous ethanolic extract. The organoleptic characteristics of the extract, including appearance, color, odor, and taste, were recorded according to Rahardhian et al. (2025).

2.3. Phytochemical Screening

Preliminary phytochemical screening was performed on both powdered material and ethanolic extract using standard colorimetric methods to detect alkaloids, flavonoids, tannins, saponins, phenolics, polyphenols, and steroids/triterpenoids. Thin-layer chromatography (TLC) was performed using silica gel 60 F_{254} plates (Merck, Darmstadt, Germany). The chromatograms were developed using appropriate mobile phases and visualized with specific detection reagents according to Putri et al. (2022).

2.4. Physicochemical Evaluation

Physicochemical evaluation was conducted to determine non-specific quality parameters, including specific gravity, moisture content, drying loss, total ash, and acid-insoluble ash. Moisture content was determined using the toluene distillation method, while other parameters were assessed according to previously reported procedures (Wigati & Rahardhian, 2018). Microbial contamination was evaluated using potato dextrose agar (PDA) and plate count agar (PCA) media (Merck, Darmstadt, Germany)

to determine yeast and mold counts and total plate counts, respectively.

2.5. Determination of Total Flavonoid, Phenolic, Tannin, and Sterol Contents

Total flavonoid content was determined using the aluminum chloride colorimetric method. Rutin 99% (Sigma-Aldrich, St. Louis, United States) was used as the reference standard, and the results were expressed as milligrams of rutin equivalents per gram of extract (mg RE/g extract) (Suharsanti et al., 2019). Total phenolic content was determined using the Folin–Ciocalteu colorimetric method, with gallic acid 99% (Sigma-Aldrich, St. Louis, United States) as the standard. The results were expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/g extract) (Rahardhian et al., 2019). Tannin content was determined by potassium permanganate titration. Total sterol content was measured using a UV-Vis spectrophotometer 1700 PC (Shimadzu, Kyoto, Japan) at 272 nm, with cholesterol as the standard, and the results were expressed as milligrams of cholesterol equivalents per gram of extract (mg CE/g extract).

2.6. Antioxidant Assays

The antioxidant activity of the ethanolic extract was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and ferric reducing antioxidant power (FRAP) assays. Absorbance was measured at 517 nm for the DPPH assay and 720 nm for the FRAP assay using a UV-Vis spectrophotometer 1700 PC (Shimadzu, Kyoto, Japan). The percentage of inhibition was calculated from the absorbance values, and IC_{50} values were obtained using linear regression analysis (Kedare & Singh, 2011; Ilyasov et al., 2020). All measurements were performed in triplicate, and the results were expressed as mean ± standard deviation.

2.7. In Vitro Cholesterol Inhibition Assay

The in vitro cholesterol inhibition assay was performed using the Liebermann–Burchard colorimetric method with slight modification according to Rahardhian et al. (2020). Briefly, 50 mg of the ethanolic extract was dissolved in chloroform p.a. (Merck, Darmstadt, Germany) to prepare a 1000 ppm stock solution, followed by serial dilution to obtain the required test concentrations. Each dilution was mixed with 250 ppm cholesterol standard solution and homogenized using a vortex mixer (Thermo Fisher Scientific, Waltham, United States). Subsequently, 2.0 mL of anhydrous acetic acid and 0.1 mL of concentrated H_2SO_4 were added to the mixture. After incubation in the dark, the absorbance of the reaction mixture was measured at 668 nm using a double-beam UV–Vis spectrophotometer (Shimadzu, Kyoto, Japan). The assay used cholesterol standard solution as the control, while no reference anticholesterolemic drug was included as a positive control. The percentage of cholesterol inhibition was calculated using the following equation:

$$Cholesterol\ inhibition\ (\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

where Acontrol is the absorbance of the cholesterol control solution and Asample is the absorbance of the cholesterol solution treated with the extract. The IC_{50} value, defined as the extract concentration required to inhibit 50% of cholesterol, was calculated using linear regression analysis of concentration versus percentage inhibition. The assay was performed in triplicate, and the results were expressed as mean values.

2.8. GC-MS Analysis

Gas chromatography–mass spectrometry (GC-MS) analysis was performed using an Agilent GC/MS system equipped with MassHunter Acquisition software version 10.2.530.3 (Agilent Technologies, California, United States). Sample injection volumes ranged from 0.1 to 1.0 µL, and the injector temperature was maintained at 260–300 ˚C. Helium was used as the carrier gas at a flow rate of 1 mL/min. The oven temperature was programmed from 60–70 ˚C to 270–290 ˚C at a rate of 4–10 ˚C/min. The detector and column temperatures were set at 250 ˚C and 325 ˚C, respectively. Compound identification was performed by comparing mass spectra with the instrument library database, and the results were reported as tentative identifications based on library match scores (Ibrahim et al., 2021).

2.9. In Silico Molecular Docking

Molecular docking analysis was performed using AutoDock GPU software (CCSB, Scripps, La Jolla, United States). The three-dimensional structure of HMG-CoA reductase receptor protein (PDB ID: 1HWK) was obtained from the RCSB Protein Data Bank and prepared using AutoDock Tools version 1.5.6 (CCSB, Scripps, La Jolla, United States). Ligand structures were obtained from PubChem and prepared using Chem3D (PerkinElmer, Waltham, United States) and AutoDock Tools. The docking grid was set at coordinates X = 2.330, Y = −9.463, and Z = −12.171. A total of 1000 ligand conformations were generated for each compound. The best docking pose was selected based on the lowest binding energy, and the interaction profile was used to predict ligand binding affinity toward HMG-CoA reductase (Agosto et al., 2024).

3. RESULTS AND DISCUSSION

3.1. Extraction Yield, Phytochemical Profile, and Extract Quality

Extraction of 301 g of dried *P. angulata* powder produced 36.60 g of viscous ethanolic extract, corresponding to a yield of 12.16%. This value exceeded the minimum extract yield requirement of 9.6%, indicating that remaceration with 95% ethanol was effective in recovering extractable constituents from the plant material. Ethanol is suitable for extracting semi-polar constituents and may recover several secondary metabolite groups, including phenolics, flavonoids, saponins, steroids, and terpenoid-related compounds.

Phytochemical screening showed that the ethanolic extract contained phenolics, polyphenols, flavonoids, tannins, saponins, and steroids/triterpenoids, whereas alkaloids and anthraquinones were not detected. The presence of these metabolites is relevant because phenolic and flavonoid compounds may contribute to

antioxidant-related activity, while saponins and steroids/triterpenoids are often associated with lipid-modulating effects (Bonciolini et al., 2023; Timilsena et al., 2023; Toma et al., 2020). These findings provide an initial chemical basis for evaluating the extract's antioxidant and anticholesterol activities.

Thin-layer chromatography (TLC) further supported the phytochemical screening results. Flavonoids were detected using chloroform:ethyl acetate (60:40) with ammonia vapor, showing spots with Rf values of 0.93 and 0.98. Essential oils were detected using toluene:ethyl acetate (93:7) and anisaldehyde–H_2SO_4, with Rf values of 0.87 and 0.95. Terpenoids were detected using hexane:ethyl acetate (1:1) and $SbCl_3$ in chloroform, producing an Rf value of 0.90. Saponins were detected using chloroform:methanol:water (64:50:10) and anisaldehyde–H_2SO_4, with an Rf value of 0.94. The absence of alkaloids and anthraquinones in TLC was consistent with the preliminary phytochemical screening. TLC remains useful for preliminary confirmation of metabolite groups in herbal extracts because it enables rapid separation and visualization of phytochemical constituents (Harborne, 1998; Putri et al., 2022; Ramonah et al., 2020). The complete phytochemical screening and TLC profile of the ethanolic extract are presented in **Table 1**.

The physicochemical evaluation showed that the extract had a specific gravity of 1.47 ± 0.05 g/mL, indicating a dense extractive preparation. The moisture content of the crude drug and extract was 7.94 ± 0.01% and 4.84 ± 0.01%, respectively, both below the required limit of <11.7%. Low moisture content is important for maintaining herbal extract stability because excessive water may promote hydrolysis, microbial growth, and degradation of active constituents (WHO, 2011). The loss on drying was 5.36 ± 0.10%, further indicating acceptable physical stability of the concentrated extract.

The total ash and acid-insoluble ash values were 35.17 ± 0.88% and 1.02 ± 0.03%, respectively, both exceeding the recommended limits of <12.2% and <0.7%. These elevated values may indicate the presence of residual inorganic material, soil-derived minerals, siliceous matter, or contamination introduced during harvesting, washing, drying, grinding, or processing. Ash parameters are important in herbal standardization because they reflect inorganic residues and possible contamination in crude drugs and extracts (WHO, 2011; Wigati & Rahardhian, 2018). Therefore, although the extract showed acceptable moisture and microbial quality, the high ash values indicate that future preparation should include stricter cleaning and handling of plant material.

Microbial contamination remained within acceptable limits, with a total plate count of 1.0×10^1 CFU/g and a yeast and mold count of 1.8×10^2 CFU/g. The extract was organoleptically characterized as thick, greenish yellow, with a characteristic odor and bitter taste. The water-soluble extractive value was 60.19 ± 1.66%, whereas the ethanol-soluble extractive value was higher at 79.61 ± 1.64%. This difference supports the suitability of ethanol for extracting major constituents from *P. angulata*. The physicochemical, microbial, organoleptic, and extractive parameters of the ethanolic extract are summarized in **Table 2**.

3.2. Quantitative Phytochemical Contents and Antioxidant Activity

Quantitative analysis showed that the ethanolic extract contained total flavonoids of 34.86 ± 1.11 mg RE/g extract, total phenolics of 5.14 ± 0.15 mg GAE/g extract, tannins of 0.99 ± 0.01%, and total sterols of 0.072 ± 0.0004 mg CE/g extract. Among the measured constituents, flavonoids were present at the highest concentration, suggesting that this group may be one of the dominant quantified secondary metabolites in the extract. The use of rutin and gallic acid as reference standards allowed the results to be expressed as rutin equivalents and gallic acid equivalents, respectively, following established colorimetric approaches (Rahardhian et al., 2019; Suharsanti et al., 2019). The total flavonoid, phenolic, tannin, and sterol contents of the ethanolic extract are summarized in **Table 3**.

Table 1. Phytochemical screening and TLC profile of *Physalis angulata* L. ethanolic extract

Chemical compound	Reagent / eluent	Detection method	Result	Rf	Observation
Phenolic	$FeCl_3$	Color reaction	+	–	Green
Polyphenol	$FeCl_3$ + $K_3Fe(CN)_6$	Color reaction	+	–	Blue-black
Flavonoid	Mg, HCl, amyl alcohol	Color reaction	+	0.93, 0.98	Orange; positive with ammonia vapor
Tannin	0.5% gelatin solution	Precipitate formation	+	–	Insoluble precipitate
Alkaloid	HCl + Dragendorff reagent	Precipitate formation	–	–	Negative
Saponin	Shaking + 2 N HCl	Foam formation	+	0.94	Stable foam 0.5–1 cm; positive with anisaldehyde–H_2SO_4
Steroid/Triterpenoid	Ether + acetic anhydride + H_2SO_4	Color reaction	+	–	Blue-green
Essential oil	Toluene:ethyl acetate (93:7)	Anisaldehyde–H_2SO_4	+	0.87, 0.95	Positive
Terpenoid	Hexane:ethyl acetate (1:1)	$SbCl_3$ in chloroform	+	0.90	Positive
Anthraquinone	Ethyl acetate:methanol:water (100:13.5:10)	KOH in 90% ethanol	–	–	Negative

Note: (+) = detected; (–) = not detected; Rf = retention factor; TLC = thin-layer chromatography.

Table 2. Physicochemical, microbial, organoleptic, and extractive parameters of *Physalis angulata* L. ethanolic extract

Parameter	Value	Requirement
Specific gravity (g/mL)	1.47 ± 0.05	–
Moisture content of crude drug (%)	7.94 ± 0.01	<11.7
Moisture content of extract (%)	4.84 ± 0.01	<11.7
Loss on drying (%)	5.36 ± 0.10	–
Total ash (%)	35.17 ± 0.88*	<12.2
Acid-insoluble ash (%)	1.02 ± 0.03*	<0.7
Total plate count (CFU/g)	1.0×10^1	$<10^4$
Yeast and mold count (CFU/g)	1.8×10^2	$<10^3$
Form	Thick extract	–
Color	Greenish yellow	–
Odor	Characteristic	–
Taste	Bitter	–
Water-soluble extract (%)	60.19 ± 1.66	–
Ethanol-soluble extract (%)	79.61 ± 1.64	–

Note: Values are presented as mean ± SD. CFU = colony-forming unit. *Values exceeding the recommended threshold.

The antioxidant activity of the extract was evaluated using DPPH radical scavenging and ferric reducing antioxidant power (FRAP) assays. The extract exhibited IC_{50} values of 209.55 ± 9.10 ppm in the DPPH assay and 220.18 ± 4.23 ppm in the FRAP assay. In comparison, rutin showed markedly lower IC_{50} values of 55.75 ppm and 55.91 ppm, respectively. Since lower IC_{50} values indicate stronger antioxidant activity, the extract demonstrated weaker antioxidant activity than rutin in both assays. The DPPH assay measures radical scavenging ability, whereas the FRAP assay evaluates reducing power; therefore, both assays provide complementary information on antioxidant behavior (Ilyasov et al., 2020; Kedare & Singh, 2011). The antioxidant activity of the extract and rutin standard is presented in **Table 4**.

The IC_{50} values above 200 ppm indicate that the antioxidant activity of the ethanolic extract can be categorized as weak to very weak. This finding suggests that although the extract contains flavonoids and phenolic compounds, their concentration, chemical form, or interaction with other crude extract constituents may limit the overall antioxidant response. Therefore, antioxidant activity should be interpreted as a supporting property of the extract rather than the primary explanation for the observed anticholesterol activity.

3.3. In Vitro Cholesterol Inhibition Activity

The in vitro cholesterol inhibition assay showed that the ethanolic extract of *P. angulata* exhibited an IC_{50} value of 96.11 ppm. This result indicates that the extract was able to inhibit cholesterol under Liebermann–Burchard assay conditions at a lower concentration than that required for its antioxidant effect. The difference between antioxidant IC_{50} and cholesterol inhibition IC_{50} suggests that the anticholesterol activity may not depend solely on free-radical scavenging or reducing capacity. Instead, other metabolite groups detected in the extract, including saponins, sterols, steroids/triterpenoids, flavonoids, and phenolics, may contribute through different chemical interactions.

The observed cholesterol inhibition is chemically plausible because plant-derived compounds have been reported to influence lipid metabolism and cholesterol-related processes through multiple mechanisms (Posadzki et al., 2013). The presence of saponins and steroid/triterpenoid compounds is particularly relevant because these metabolite groups may interact with sterol structures or lipid-related pathways. The Liebermann–Burchard method used in this study provides a colorimetric basis for evaluating cholesterol reduction in vitro and has been applied in previous anticholesterol screening of plant extracts (Rahardhian et al., 2020). Nevertheless, this assay does not directly demonstrate systemic cholesterol-lowering efficacy.

The absence of a positive control drug should be acknowledged as a limitation. The assay used cholesterol standard solution as the control for calculating inhibition, but no reference anticholesterolemic drug, such as simvastatin, atorvastatin, or ezetimibe, was included. Therefore, the IC_{50} value of 96.11 ppm should be interpreted as preliminary evidence of in vitro cholesterol inhibition rather than as proof of potency comparable to standard lipid-lowering agents.

Table 3. Total flavonoid, phenolic, tannin, and sterol contents of *P. angulata* ethanolic extract

Parameter	Value	Unit / Equivalent
Total flavonoid content	34.86 ± 1.11	mg RE/g extract
Total phenolic content	5.14 ± 0.15	mg GAE/g extract
Tannin content	0.99 ± 0.01	%
Total sterol content	0.072 ± 0.0004	mg CE/g extract

Note: Values are presented as mean ± standard deviation. RE = rutin equivalent; GAE = gallic acid equivalent; CE = cholesterol equivalent.

Table 4. Antioxidant activity of *Physalis angulata* L. ethanolic extract and rutin standard using DPPH and FRAP assays

Sample	DPPH IC_{50} (ppm)	FRAP IC_{50} (ppm)	Interpretation
P. angulata ethanolic extract	209.55 ± 9.10	220.18 ± 4.23	Weak to very weak antioxidant activity
Rutin standard	55.75	55.91	Stronger antioxidant activity than extract

Note: IC_{50} = concentration required to inhibit or reduce 50% of activity; DPPH = 2,2-diphenyl-1-picrylhydrazyl; FRAP = ferric reducing antioxidant power. Lower IC_{50} values indicate stronger antioxidant activity.

3.4. GC-MS Profiling of the Ethanolic Extract

GC-MS analysis tentatively identified several compounds in the ethanolic extract based on library matching scores. The detected compounds included hydroxydehydrostevic acid, cedroxyde, phytol, farnesiferol C, flourensadiol, and uvidin C. Among these, farnesiferol C showed the highest library match score of 67.04, followed by phytol at 63.59, hydroxydehydrostevic acid at 62.49, cedroxyde at 62.41, uvidin C at 61.51, and flourensadiol at 61.07 and 60.90. These compounds represent a mixture of diterpenoid-, sesquiterpenoid-, and sterol-like constituents that may be relevant to the observed biological activity. The compounds tentatively identified by GC-MS analysis are listed in **Table 5**.

The GC-MS profile provides preliminary information regarding the lipophilic and semi-volatile compounds present in the extract. However, the library match scores obtained in this study were moderate, indicating that the identified compounds should be regarded as tentative rather than definitive. GC-MS-based identification should ideally be supported by stronger library similarity, retention indices, authentic standards, or complementary analytical techniques to improve confidence in compound assignment (Ibrahim et al., 2021). This limitation is important because subsequent molecular docking interpretation depends on the reliability of the selected ligand structures.

3.5. Molecular Docking against HMG-CoA Reductase

Molecular docking was performed to explore the possible interaction between selected GC-MS-identified compounds and HMG-CoA reductase, a key enzyme involved in cholesterol biosynthesis. The native ligand showed the strongest predicted binding affinity, with a binding energy of −10.86 kcal/mol, and was used as the reference. Among the tested phytoconstituents, hydroxydehydrostevic acid showed the lowest binding energy at −7.86 kcal/mol, followed by farnesiferol C at −7.63 kcal/mol, flourensadiol at −6.61 kcal/mol, cedroxyde at −6.19 kcal/mol, phytol at −5.89 kcal/mol, and uvidin C at −5.31 kcal/mol. These values suggest that hydroxydehydrostevic acid and farnesiferol C had relatively stronger predicted affinity toward HMG-CoA reductase than the other tested compounds. The molecular docking parameters of the native ligand and selected compounds against HMG-CoA reductase are presented in **Table 6**.

Table 5. GC-MS profile of tentatively identified compounds in *P. angulata* ethanolic extract

Peak	Retention time (min)	Tentatively identified compound	Molecular formula	Molecular weight (g/mol)	Peak area (%)	Library match score
19	43.525	Hydroxydehydrostevic acid	$C_{20}H_{30}O_3$	318.45	1.78	62.49
23	47.783	Cedroxyde	$C_{15}H_{24}O$	220.35	6.46	62.41
25	48.337	Phytol	$C_{20}H_{40}O$	296.53	14.22	63.59
32	54.190	Farnesiferol C	$C_{24}H_{30}O_4$	382.50	1.60	67.04
36	56.858	Flourensadiol	$C_{15}H_{26}O_2$	238.37	3.27	61.07
39	59.146	Uvidin C	$C_{15}H_{26}O_3$	254.37	2.20	61.51
41	59.607	Farnesol	$C_{15}H_{26}O$	222.37	2.50	60.90

Note: Compound identities are tentative because they were assigned based on GC-MS library matching scores.

Table 6. Molecular docking parameters of native ligand and selected compounds against HMG-CoA reductase

Ligand	Estimated binding energy (kcal/mol)	Intermolecular energy (kcal/mol)	Electrostatic energy (kcal/mol)	Internal energy (kcal/mol)	Torsional free energy (kcal/mol)
Native ligand	−10.86	−16.23	−2.23	−3.41	5.37
Cedroxyde	−6.19	−6.19	−0.16	0.00	0.00
Farnesiferol C	−7.63	−9.42	−0.11	−0.86	1.79
Flourensadiol	−6.61	−7.20	−0.40	−0.05	0.60
Hydroxydehydrostevic acid	−7.86	−8.75	−2.40	−0.01	0.89
Phytol	−5.89	−10.36	−0.10	−2.04	4.47
Uvidin C	−5.31	−6.21	−0.34	−0.78	0.89

Note: More negative binding energy values indicate stronger predicted ligand–receptor affinity. Docking results are predictive and do not confirm biological inhibition.

The docking results provide a plausible computational explanation for the in vitro cholesterol inhibition observed in this study. HMG-CoA reductase is a central enzyme in cholesterol biosynthesis and is commonly used as a molecular target in cholesterol-lowering studies (Agosto et al., 2024). The use of molecular docking may assist in predicting potential ligand–target interactions and prioritizing compounds for further biological evaluation (Meng et al., 2011). In this study, hydroxydehydrostevic acid and farnesiferol C showed more favorable binding energies than the other tested constituents, indicating that these compounds may warrant further investigation as candidate ligands.

The interpretation of docking findings should remain cautious. Molecular docking estimates ligand–receptor interaction under computational assumptions and does not confirm enzyme inhibition, cellular activity, bioavailability, toxicity, or in vivo cholesterol-lowering effects. Moreover, because the GC-MS compound assignments were tentative, the docking analysis is also dependent on structural identities that require confirmation. Therefore, the docking results should be considered supportive evidence that complements the in vitro assay rather than confirmatory proof of mechanism. The predicted 2D and 3D ligand–receptor interactions are summarized in **Table 7**, while the superimposition of ligand conformations before and after docking is shown in **Figure 1**.

Table 7. Selected ligands from GC-MS analysis and their predicted 2D and 3D interactions with HMG-CoA reductase

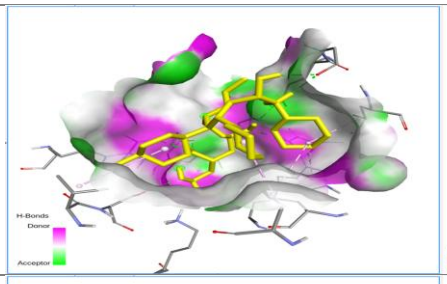
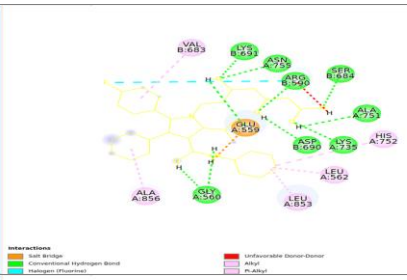
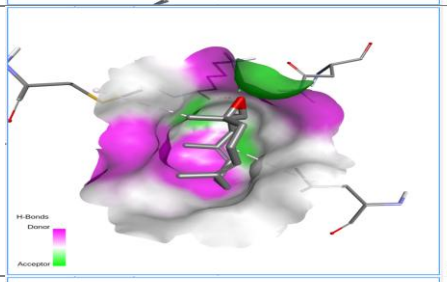
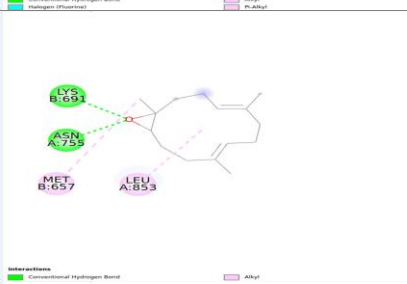
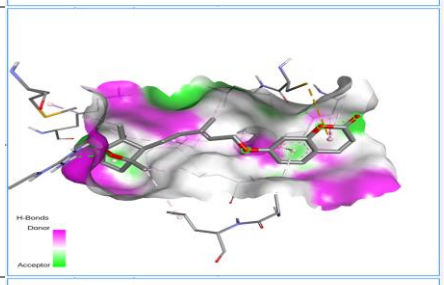
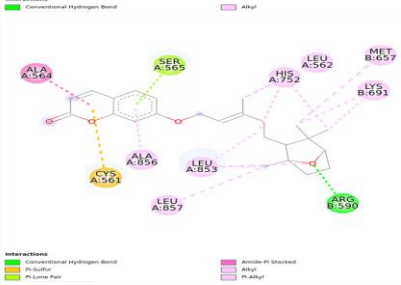
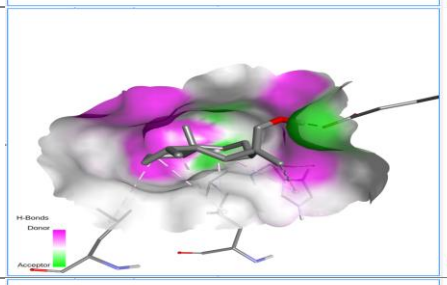
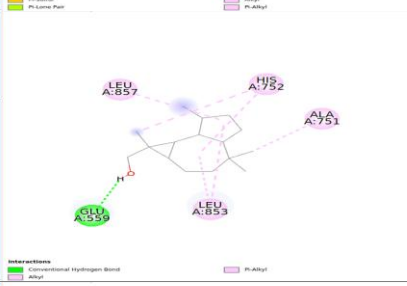
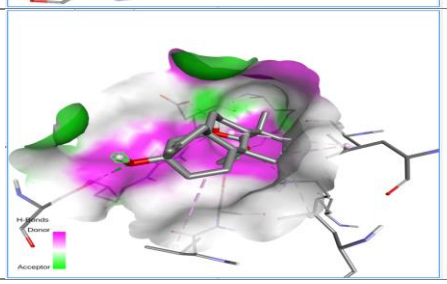
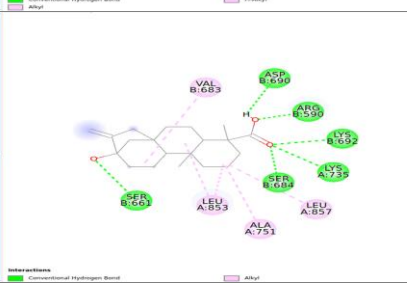
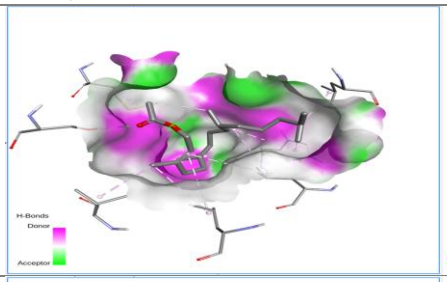
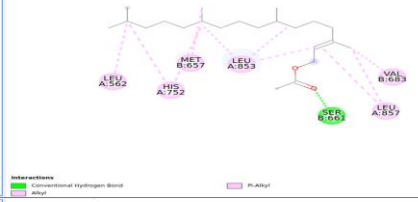
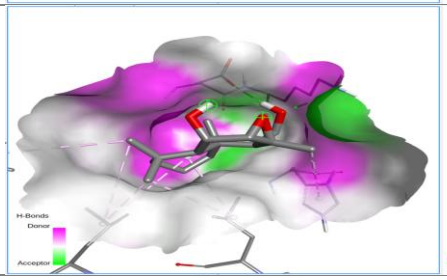
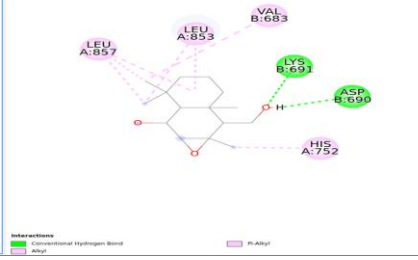
Ligand	3D and 2D Ligand Receptor Interaction	
Native		
Cedroxyde		
Fernesiferol C		
Flourensadiol		
Hydroxydehydrostevic acid		

Table 7. Selected ligands from GC-MS analysis and their predicted 2D and 3D interactions with HMG-CoA reductase (*continue*)

Phytol		
Uvidin C		

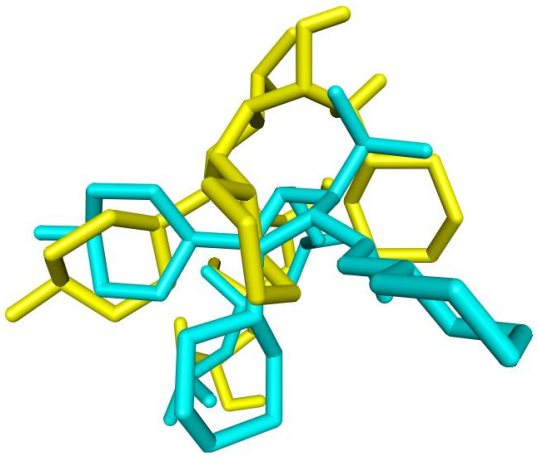


Figure 1. Superimposition of ligand conformations before and after docking. Yellow indicates the pre-docking conformation, whereas turquoise indicates the post-docking conformation.

The integration of phytochemical screening, quantitative metabolite analysis, antioxidant assays, in vitro cholesterol inhibition, GC-MS profiling, and molecular docking indicates that *P. angulata* ethanolic extract possesses a complex chemical profile with preliminary anticholesterol potential. The extract showed weak to very weak antioxidant activity but more notable cholesterol inhibition, suggesting that non-antioxidant mechanisms may contribute to its activity. The presence of flavonoids, saponins, steroids/triterpenoids, and terpenoid-like compounds provides a reasonable chemical basis for this effect. Nevertheless, the elevated ash values, absence of a positive control in the cholesterol assay, tentative GC-MS identification, and predictive nature of molecular docking should be acknowledged as limitations. These considerations are important to avoid overclaiming and to position the findings as preliminary evidence that requires confirmation through compound isolation, structural validation, direct enzyme inhibition assays, and biological evaluation in more representative models.

4. CONCLUSION

The ethanolic extract of *P. angulata* showed a broad phytochemical profile and meaningful preliminary anticholesterol activity, as indicated by the presence of flavonoids, phenolics, tannins, saponins, and steroids/triterpenoids, with total flavonoid content of 34.86 ± 1.11 mg RE/g extract, total phenolic content of 5.14 ± 0.15 mg GAE/g extract, tannin content of 0.99 ± 0.01%, and total sterol content of 0.072 ± 0.0004 mg CE/g extract. Although the extract showed weak to very weak antioxidant activity, with IC_{50} values of 209.55 ± 9.10 ppm for DPPH and 220.18 ± 4.23 ppm for FRAP, it demonstrated notable in vitro cholesterol inhibition with an IC_{50} value of 96.11 ppm. GC-MS analysis tentatively identified hydroxydehydrostevic acid, cedroxyde, phytol, farnesiferol C, flourensadiol, and uvidin C, while molecular docking suggested that hydroxydehydrostevic acid and farnesiferol C had relatively favorable predicted interactions with HMG-CoA reductase. These findings provide a useful scientific basis for further investigation of *P. angulata* as a natural source of anticholesterolemic compounds and support the relevance of integrating phytochemical evaluation, in vitro assays, GC-MS profiling, and molecular docking in early-stage medicinal

plant research. However, because GC-MS identification was based on library matching, the cholesterol assay did not include a positive control drug, and docking analysis remains predictive, further studies involving compound isolation, structural confirmation, direct HMG-CoA reductase inhibition assays, and cellular or in vivo evaluation are required to validate its pharmacological relevance and safety.

AUTHOR CONTRIBUTIONS

Conceptualization, methodology, investigation, resources, data curation, supervision, and project administration, E.S.H.; software, formal analysis, visualization, and funding acquisition, M.R.R.R.; validation and writing—review and editing, E.S.H., M.R.R.R., and ES ; writing—original draft preparation, E.S.H. All authors have read and agreed to the published version of the manuscript.

INSTITUTIONAL REVIEW BOARD STATEMENT

Not applicable.

INFORMED CONSENT STATEMENT

Not applicable.

DATA AVAILABILITY STATEMENT

Data supporting the findings of this study are available upon reasonable request from the corresponding author.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

ROLE OF FUNDERS

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DECLARATION OF GENERATIVE ARTIFICIAL INTELLIGENCE (AI) USE

During the preparation of this manuscript, the authors used ChatGPT (OpenAI) to assist in improving the clarity, structure, grammar, and readability of the text. After using this tool, the authors thoroughly reviewed, edited, and verified the entire content to ensure that it accurately represents their own ideas, data, interpretations, and scientific conclusions. The authors take full responsibility for the integrity and originality of the published work.

REFERENCES

Agosto, N. J., Alambatin, P. B., Bacalso, J., Cabisada, J., & Carating, B. D. (2024). The evaluation of *Citrus bergamia* phytochemicals as potential cholesterol-lowering agents against HMG-CoA reductase: An in silico molecular docking study. *Biology and Life Sciences Forum, 35*, Article 7. https://doi.org/10.3390/blsf2024035007

Bonciolini, A., Cantele, C., Salgarella, N. I., Zeppa, G., Bertolino, M., & Cardenia, V. (2023). Effect of tannins on cholesterol content and its oxidation in egg pasta as related to different pasta shapes. *Food and Bioprocess Technology, 16*(7), 1541–1554. https://doi.org/10.1007/s11947-023-03016-1

Ekor, M. (2014). The growing use of herbal medicines: Issues relating to adverse reactions and challenges in monitoring safety. *Frontiers in Pharmacology, 4*, Article 177. https://doi.org/10.3389/fphar.2013.00177

Harborne, J. B. (1998). *Phytochemical methods: A guide to modern techniques of plant analysis* (3rd ed.). Chapman & Hall.

Ibrahim, H., Uttu, A. J., Sallau, M. S., & Iyun, O. R. A. (2021). Gas chromatography–mass spectrometry (GC–MS) analysis of ethyl acetate root bark extract of *Strychnos innocua* (Delile). *Beni-Suef University Journal of Basic and Applied Sciences, 10*, Article 61. https://doi.org/10.1186/s43088-021-00156-1

Ilyasov, I. R., Beloborodov, V. L., Selivanova, I. A., & Terekhov, R. P. (2020). ABTS/PP decolorization assay of antioxidant capacity reaction pathways. *International Journal of Molecular Sciences, 21*(3), Article 1131. https://doi.org/10.3390/ijms21031131

Karimi, A., Majlesi, M., & Rafieian-Kopaei, M. (2015). Herbal versus synthetic drugs: Beliefs and facts. *Journal of Nephropharmacology, 4*(1), 27–30.

Kedare, S. B., & Singh, R. P. (2011). Genesis and development of DPPH method of antioxidant assay. *Journal of Food Science and Technology, 48*(4), 412–422. https://doi.org/10.1007/s13197-011-0251-1

Liu, Z. L., Liu, J. P., Zhang, A. L., Wu, Q., Ruan, Y., Lewith, G., & Visconte, D. (2011). Chinese herbal medicines for hypercholesterolemia. *Cochrane Database of Systematic Reviews, 2011*(7), Article CD008305. https://doi.org/10.1002/14651858.CD008305.pub2

Meng, X.-Y., Zhang, H.-X., Mezei, M., & Cui, M. (2011). Molecular docking: A powerful approach for structure-based drug discovery. *Current Computer-Aided Drug Design, 7*(2), 146–157. https://doi.org/10.2174/157340911795677602

Nawata, K. (2023). Risk factors for heart, cerebrovascular, and kidney diseases: Evaluation of potential side effects of medications to control hypertension, hyperglycemia, and hypercholesterolemia. *Frontiers in Cardiovascular Medicine, 10*, Article 1103250. https://doi.org/10.3389/fcvm.2023.1103250

Novitasari, A., Rohmawaty, E., & Rosdianto, A. M. (2024). *Physalis angulata* Linn. as a medicinal plant: A review. *Biomedical*

Reports, 20(3), Article 38. https://doi.org/10.3892/br.2024.1735

Pirillo, A., & Norata, G. D. (2023). The burden of hypercholesterolemia and ischemic heart disease in an ageing world. *Pharmacological Research, 193*, Article 106814. https://doi.org/10.1016/j.phrs.2023.106814

Posadzki, P., Watson, L. K., & Ernst, E. (2013). Adverse effects of herbal medicines: An overview of systematic reviews. *Clinical Medicine, 13*(1), 7–12. https://doi.org/10.7861/clinmedicine.13-1-7

Putri, C. N., Rahardhian, M. R. R., & Ramonah, D. (2022). Effect of extraction methods for total phenolic content, total flavonoid content and antibacterial activity of ethanolic extract insulin leaves (*Smallanthus sonchifolius*). *JPSCR: Journal of Pharmaceutical Science and Clinical Research, 7*(1), 15–27. https://doi.org/10.20961/jpscr.v7i1.43465

Rahardhian, M. R. R., Ardiansyah, F., Susilawati, Y., Wilar, G., Sumiwi, S. A., Levita, J., & Muchtaridi, M. (2025). Immunomodulatory and acute toxicity studies of *Peronema canescens* Jack leaves: In vivo hematological analysis and in vitro IL-6 gene expression inhibition. *The Indonesian Biomedical Journal, 17*(3), 295–306. https://doi.org/10.18585/inabj.v17i3.3577

Rahardhian, M. R. R., Suharsanti, R., Sugihartini, N., & Lukitaningsih, E. (2019). In vitro assessment of total phenolic, total flavonoid and sunscreen activities of crude ethanolic extract of belimbing wuluh (*Averrhoa bilimbi*) fruits and leaves. *Journal of Global Pharma Technology, 11*(4), 308–313.

Rahardhian, M. R. R., Yuniarti, N., Ariani, L. W., & Suharsanti, R. (2020). In vitro determination of antioxidant activity, total phenolics, total flavonoid, and anti-cholesterol activity of saffron (*Crocus sativus*) extracts. *Journal of Global Pharma Technology, 12*(9), 223–230.

Ramonah, D., Rahardhian, M. R. R., & Putri, C. N. (2020). Determinasi total flavonoid, total fenolik, dan aktivitas antibakteri ekstrak etanol daun insulin (*Smallanthus sonchifolius*) dengan metode perkolasi. *Media Farmasi Indonesia, 15*(1), 1585–1592.

Riskesdas. (2018). *Laporan nasional Riskesdas 2018*. Lembaga Penerbit Badan Penelitian dan Pengembangan Kesehatan. http://repository.bkpk.kemkes.go.id/3514/1/Laporan%20Riskesdas%202018%20Nasional.pdf

Suharsanti, R., Sugihartini, N., Lukitaningsih, E., & Rahardhian, M. R. R. (2019). Effect of different solvent on total phenolic, total flavonoid, and sun protection factor of belimbing wuluh (*Averrhoa bilimbi* Linn.) fruits fraction. *Journal of Global Pharma Technology, 11*(1), 154–162.

Timilsena, Y. P., Phosanam, A., & Stockmann, R. (2023). Perspectives on saponins: Food functionality and applications. *International Journal of Molecular Sciences, 24*(17), Article 13538. https://doi.org/10.3390/ijms241713538

Toma, L., Sanda, G. M., Niculescu, L. S., Deleanu, M., Sima, A. V., & Stancu, C. S. (2020). Phenolic compounds exerting lipid-regulatory, anti-inflammatory and epigenetic effects as complementary treatments in cardiovascular diseases. *Biomolecules, 10*(4), Article 641. https://doi.org/10.3390/biom10040641

Wigati, D., & Rahardhian, M. R. R. (2018). Penetapan standarisasi non-spesifik ekstrak etanol hasil perkolasi umbi bawang dayak (*Eleutherine palmifolia* (L.) Merr). *Jurnal Ilmu Farmasi dan Farmasi Klinik, 15*(2), 36–40.

WHO (World Health Organization). (2011). *Quality control methods for herbal materials*. World Health Organization. https://www.who.int/publications/i/item/9789241500739