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In Vitro Screening of Cholesterol-Lowering Activity in Jamaican Cherry (*Muntingia calabura*), Basil (*Ocimum basilicum* L.), and Red Gedi (*Abelmoschus manihot* L. Medik) Leaf Extracts

Margaretha Brigithania Mandagi[1*], Mario Walean[2], Novita N. G. Tumiwa[3]
[1-3] Universitas Prisma Manado, Indonesia
Email: brigithania13@gmail.com[1]

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Abstract

*Dyslipidemia remains a significant cardiovascular health concern, encouraging investigation of plant-derived compounds with potential cholesterol-lowering activity. This study aimed to identify secondary metabolites and compare the in vitro cholesterol-associated activity of ethanolic leaf extracts of Jamaican cherry (*Muntingia calabura *L.), basil (*Ocimum basilicum *L.), and red gedi (*Abelmoschus manihot *L. Medik). A quantitative experimental design was conducted using maceration with 96% ethanol, qualitative phytochemical screening, and the Liebermann–Burchard spectrophotometric method. Extracts were evaluated at 50, 100, and 150 ppm, with simvastatin as the positive reference. Activity was assessed through absorbance measurements, percentage inhibition, and estimated IC_{50} values. All extracts tested positive for alkaloids, flavonoids, tannins, and steroids, whereas saponins were detected only in Jamaican cherry. At 150 ppm, Jamaican cherry exhibited 98.36% inhibition, compared with 22.87% for red gedi and 8.98% for basil. The reported IC_{50} values were 81.220, 173.377, and 219.592 ppm, respectively. However, the latter two estimates exceeded the tested concentration range. Jamaican cherry demonstrated the strongest colorimetric response, supporting its prioritization for further investigation. These preliminary findings require analytical validation and biological confirmation before therapeutic efficacy can be established.*

Keywords: *basil leaves, cholesterol-lowering activity, Jamaican cherry leaves, Liebermann–Burchard method, phytochemical screening, red gedi leaves.*

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INTRODUCTION

Dyslipidemia remains a major public health concern because elevated circulating cholesterol, particularly low-density lipoprotein cholesterol, contributes to atherosclerotic cardiovascular disease and its associated morbidity and mortality. The clinical significance of cholesterol dysregulation extends beyond abnormal laboratory measurements, reflecting disturbances in lipid homeostasis that contribute to progressive vascular injury and increase the risk of coronary artery disease and ischemic stroke (Mayo Clinic, 2025). In Indonesia, the 2018 Basic Health Research survey reported that 28.8% of individuals aged 15 years and older had total cholesterol concentrations of at least 200 mg/dL, indicating a substantial population burden requiring preventive and therapeutic attention (Riskesdas, 2018). Pharmacological management has historically relied on statins, whose development represented a major advancement in lipid-lowering therapy through inhibition of 3-hydroxy-3-methylglutaryl coenzyme A reductase, a principal enzyme involved in hepatic cholesterol biosynthesis (Endo & Beppu, 2010). Simvastatin remains an established representative of this pharmacological class, although its clinical mechanism should be distinguished from cholesterol interactions evaluated through chemical assays conducted outside biological systems (Talreja & Cassagnol, 2023). Contemporary lipid-management research has increasingly emphasized individualized therapeutic strategies because statin intolerance, variation in treatment response, and residual cardiovascular risk may complicate long-term management (Banach et al., 2023). Advances in nonstatin lipid-lowering agents have expanded therapeutic possibilities while reinforcing the scientific relevance of investigating additional molecular structures capable of interacting with cholesterol metabolism (Kayani et al., 2024). Plant-derived compounds have attracted attention

because their structural diversity includes flavonoids, alkaloids, saponins, tannins, and sterol-related constituents with distinct physicochemical properties and biological activities. Investigating these compounds requires careful differentiation between direct physicochemical interactions with cholesterol, activity detected in experimental screening systems, and clinically meaningful lipid-lowering effects. The leaves of Jamaican cherry (Muntingia calabura L.), basil (Ocimum basilicum L.), and red gedi (Abelmoschus manihot L. Medik) represent relevant botanical materials for preliminary comparative investigation because their documented secondary metabolites provide a biochemical rationale for evaluating their responses in standardized in vitro cholesterol assays.

Existing phytochemical and pharmacological investigations provide several complementary lines of evidence concerning the potential relevance of plant secondary metabolites to cholesterol-related research. Flavonoids constitute a structurally diverse group of polyphenolic compounds whose antioxidant properties and biological activities depend on molecular substitution patterns, functional groups, and interactions with cellular targets, although these properties cannot independently establish cholesterol-lowering efficacy (Kumar & Pandey, 2013; Panche et al., 2016). Research on citrus flavanones has further connected selected flavonoid constituents with cardiovascular pharmacology, supporting investigation of their possible relevance to lipid-associated processes without implying that all flavonoid-containing extracts produce equivalent biological effects (Testai & Calderone, 2017). Alkaloid research similarly demonstrates that specific plant constituents, including berberine, can influence lipid metabolism through mechanisms that depend on chemical identity and biological exposure rather than the mere detection of an alkaloid class (Wang & Zidichouski, 2018). Saponins exhibit amphiphilic characteristics and may interact with biological membranes and sterol-containing systems, whereas the properties and potential applications of individual saponins differ according to their molecular composition (Francis et al., 2002; Moghimipour & Handali, 2015). Investigations of phytosterols and isolated plant sterol compounds provide an additional chemical basis for examining sterol-related structures, although evidence concerning anticancer properties or the activity of isolated compounds cannot be directly generalized to crude-extract cholesterol reduction (Shahzad et al., 2017; Muharni et al., 2021). Within the three botanical materials selected for the present investigation, studies of basil have documented phytochemical constituents and antioxidant-related characteristics, while investigations of ethanolic basil extracts and extract standardization highlight the relevance of plant identity, material preparation, and extraction procedures to phytochemical evaluation (Kumalasari & Adiarna, 2020; Nadeem et al., 2022; Krismayadi et al., 2024). Research on Jamaican cherry leaves has characterized phenolic, flavonoid, and tannin constituents, reviewed the plant's pharmacological properties, and examined cholesterol-related activity in male mice, collectively supporting further investigation while maintaining a distinction between chemical composition and in vivo outcomes (Anisa & Najib, 2022; Sumarni et al., 2022; Natasha et al., 2024). Red gedi has been examined through the isolation of an organic acid constituent and the phytochemical screening of ethanolic leaf extracts, providing botanical and chemical information but more limited direct evidence concerning cholesterol-related assay responses (Mamahit & Soekamto, 2010; Sari & Parbuntari, 2025). These research streams establish the biochemical plausibility of investigating all three plants while indicating that phytochemical detection, isolated-compound activity, and whole-extract biological effects represent different levels of evidence.

Despite the expanding literature on plant-derived bioactive compounds, a methodological and comparative research gap remains evident in the available studies relevant to these three leaf extracts. Previous cholesterol-related investigations have examined diverse botanical materials, extraction solvents, and experimental systems, including methanolic parijoto fruit extract, parijoto extract formulated in chitosan nanoparticles, ethanolic pakcoy extracts, and methanolic green tea preparations (Amin, 2015; Luhurningtyas et al., 2020; Andriani & Anggraini, 2023; Naufalzuhdi, 2019). More recent studies have extended this approach to flavonoid-containing Dutch teak leaf extracts, different solvent fractions of pulai bark, ethyl acetate fractions of bajakah tampala, and buni leaf fractions, demonstrating continued interest in measuring cholesterol-associated responses using in vitro experimental methods (Parbuntari & Yuliandari, 2023; Pramesty et al., 2023; Chicade et al., 2025; Darojat et al., 2026). Although these investigations collectively demonstrate the applicability of plant-extract screening, differences in plant species, extraction techniques, concentrations, and analytical procedures limit direct comparison of the reported activities across separate experiments. The available references do not establish a controlled, simultaneous comparison of ethanolic Jamaican

cherry, basil, and red gedi leaf extracts using the same Liebermann–Burchard reaction conditions, concentration range, and analytical framework. This constitutes a specific comparative and methodological gap rather than evidence that the individual plants have never been investigated. A further analytical limitation arises from the chemical characteristics of the Liebermann–Burchard reaction, which involves acid-mediated transformations of cholesterol and the formation of colored products whose measured absorbance depends on the reaction environment (Xiong et al., 2007). Consequently, changes in absorbance following the addition of crude plant extracts cannot automatically be attributed to cholesterol removal or inhibition of biological cholesterol synthesis because extract pigments, reactive constituents, and matrix interactions may influence the measured signal. Spectrophotometric quantification requires attention to wavelength selection, reaction stability, reference measurements, and potential analytical interference, while concentration–response interpretation requires appropriate estimation procedures rather than an assumption of linear activity across all tested concentrations (Mettler-Toledo, 2015; Sebaugh, 2011). The unresolved question is therefore not merely whether these plants contain secondary metabolites, but how their crude ethanolic extracts compare under common experimental conditions and to what extent the observed analytical responses support a defensible preliminary ranking of cholesterol-associated activity.

The identified gap has scientific implications for natural-product screening because the absence of directly comparable measurements can encourage unsupported conclusions regarding the relative potency of botanical preparations. Extraction conditions determine which constituents enter the experimental sample, and differences in solvent characteristics, plant matrices, and processing procedures may substantially influence the chemical composition of crude extracts; consequently, standardized extraction is necessary when comparing different botanical materials (Zhang et al., 2018). Preliminary phytochemical analysis provides a complementary means of identifying broad metabolite classes, although qualitative color and precipitation tests do not establish the concentration, molecular identity, or pharmacological contribution of individual compounds (Buitrago D et al., 2016; Ngibad, 2019; Shaikh & Patil, 2020). The interpretation of colorimetric screening also requires recognition that reaction products can arise from chemical interactions between reagents and multiple constituents within an extract. Spectroscopic investigations of tannic acid and related polyphenols demonstrate the importance of considering chemical complex formation and reaction-dependent optical characteristics when interpreting absorbance-based measurements, although their findings cannot be treated as direct evidence of cholesterol-binding activity (Espina et al., 2022). Research examining the color chemistry of anthocyanins provides a further illustration of how molecular structure and chemical environment influence visible color responses, reinforcing the need to distinguish analytical signal formation from biological activity (Smellie et al., 2026). Such considerations are particularly relevant when the test materials contain phenolic constituents, pigments, and other compounds capable of altering spectrophotometric measurements. The broader literature on plant extracts also indicates that an experimentally observed biological response may concern a target unrelated to cholesterol metabolism; for example, the larvicidal activity investigated in papaya leaf extract research illustrates the need to specify the actual experimental endpoint rather than generalize activity across pharmacological applications (Ilham et al., 2019). Without this distinction, preliminary laboratory screening may be incorrectly interpreted as evidence of therapeutic effectiveness. A comparative investigation that combines standardized phytochemical screening with concentration-dependent Liebermann–Burchard measurements can provide a more consistent evidentiary basis for prioritizing botanical extracts for subsequent chemical characterization. Its practical relevance lies primarily in guiding laboratory research and candidate selection, not in recommending unvalidated plant extracts as substitutes for established lipid-lowering treatment.

The present research is positioned at the intersection of comparative pharmacognosy, phytochemical characterization, and analytical screening of cholesterol-associated activity. Its proposed novelty is not the discovery of previously unknown medicinal plants or a new cholesterol-lowering mechanism, but the integration of three botanically distinct leaf extracts into a common experimental framework designed to produce directly comparable preliminary evidence. Existing studies provide phytochemical information on basil, Jamaican cherry, and red gedi separately, while related cholesterol-screening research has investigated other plant extracts and solvent fractions under experimental conditions that are not necessarily equivalent (Nadeem et al., 2022; Natasha et al., 2024; Sari & Parbuntari, 2025; Chicade et al., 2025; Darojat et al., 2026). In contrast, the present

investigation employs ethanolic extracts of all three plants and assesses their responses at identical extract concentrations of 50, 100, and 150 ppm using the Liebermann–Burchard method, with simvastatin included as an experimental reference. Combining qualitative detection of alkaloids, flavonoids, saponins, tannins, and steroids with spectrophotometric measurements provides two complementary dimensions of assessment: the broad phytochemical characteristics of each extract and its concentration-dependent response in the cholesterol-containing reaction system. The methodological framework draws on established phytochemical screening and extraction principles while recognizing that the presence of a particular metabolite class cannot independently establish its contribution to the observed response (Shaikh & Patil, 2020; Zhang et al., 2018). The use of percentage inhibition enables the analytical responses to be compared relative to a common reference, while IC_{50} estimation offers an additional concentration-based comparison that must be interpreted according to the observed dose–response range and the appropriateness of the fitted model (Parbuntari & Yuliandari, 2023; Sebaugh, 2011). The inclusion of simvastatin provides an internal reference for the chemical assay but does not establish equivalence between its pharmacological inhibition of hepatic cholesterol biosynthesis and the effects of crude plant extracts on a reagent-based reaction. This distinction is central to the study's contribution because it preserves the separation between preliminary analytical activity and demonstrated therapeutic efficacy. The originality of the investigation consequently resides in its controlled cross-species comparison and integrated interpretation of phytochemical profiles and in vitro responses, addressing a clearly delimited gap without extending its claims beyond the experimental design.

This study aims to identify the principal classes of secondary metabolites present in ethanolic leaf extracts of Jamaican cherry (Muntingia calabura L.), basil (Ocimum basilicum L.), and red gedi (Abelmoschus manihot L. Medik) and to comparatively evaluate their in vitro cholesterol-associated activity using the Liebermann–Burchard spectrophotometric method. The specific objectives are to characterize the qualitative occurrence of alkaloids, flavonoids, saponins, tannins, and steroids in each extract; determine the analytical responses of the extracts at concentrations of 50, 100, and 150 ppm; calculate the corresponding percentage inhibition values; and estimate their IC_{50} values to support comparison of concentration-dependent activity under standardized experimental conditions. Simvastatin is incorporated as a positive reference to characterize assay responses, while interpretation remains restricted to the chemical measurement system employed. The investigation seeks to establish a comparative profile of the three botanical preparations and identify which extract warrants priority in subsequent phytochemical isolation and experimental validation. Its scientific contribution is expected to consist of directly comparable preliminary evidence connecting qualitative secondary-metabolite profiles with measured in vitro activity, thereby extending the available literature beyond separate investigations of individual botanical materials. Its methodological contribution lies in applying a common extraction and analytical approach while explicitly distinguishing spectrophotometric responses from established biochemical mechanisms or physiological cholesterol reduction. The practical contribution concerns the identification of promising natural-product research candidates for further investigation rather than the confirmation of therapeutic efficacy or clinical applicability. The study is therefore designed to provide a scientifically bounded foundation for subsequent work involving compound identification, analytical interference assessment, mechanistic evaluation, and biological validation of potential cholesterol-related activity.

RESEARCH METHODS

This study employed a quantitative experimental design involving comparative in vitro screening of cholesterol-associated activity in ethanolic leaf extracts of Jamaican cherry (Muntingia calabura L.), basil (Ocimum basilicum L.), and red gedi (Abelmoschus manihot L. Medik). The research was conducted from March to April 2026 at the Basic Laboratory of Prisma University, using plant materials collected from Manembo-nembo Atas Village, Matuari District, Bitung City, North Sulawesi, Indonesia. The experimental materials consisted of 1 kg of leaves from each plant species, which were subjected to sorting, washing under running water, air-drying away from direct sunlight, and grinding to obtain dried powdered material. Extraction was performed using 50 g of powdered leaves from each species through maceration in 96% ethanol for 24 hours, repeated three times using fresh solvent with occasional stirring. The resulting macerates were filtered using ordinary filter paper and Whatman No. 42 filter paper, combined, and concentrated by evaporation at

approximately 45°C to obtain crude ethanolic extracts. The extraction procedure was consistent across the three botanical materials to support comparative evaluation, while the use of ethanol followed established approaches for recovering multiple classes of secondary metabolites from plant matrices (Zhang et al., 2018). Qualitative phytochemical screening was subsequently performed to identify alkaloids, flavonoids, saponins, tannins, and steroids in each extract. Alkaloids were examined using Dragendorff's reagent, flavonoids using magnesium and concentrated hydrochloric acid, saponins through the formation of persistent foam, tannins using 1% ferric chloride, and steroids using chloroform and concentrated sulfuric acid. The qualitative identification procedures were based on observable precipitation, foam formation, and reagent-induced color changes associated with the respective phytochemical classes (Ngibad, 2019; Shaikh & Patil, 2020). Laboratory equipment included an analytical balance, blender, glassware, micropipettes, water bath, hot plate, and ultraviolet–visible (UV–Vis) spectrophotometer. The reagents and experimental materials comprised the three ethanolic plant extracts, 96% ethanol, cholesterol standard, chloroform, simvastatin, acetic anhydride, concentrated sulfuric acid, and the reagents required for phytochemical screening. The principal experimental factors were botanical extract type and extract concentration, whereas the measured responses consisted of qualitative phytochemical reactions, spectrophotometric absorbance, percentage inhibition, and estimated half-maximal inhibitory concentration (IC_{50}).

In vitro cholesterol-associated activity was evaluated using the Liebermann–Burchard colorimetric method, in which cholesterol reacts with acetic anhydride and concentrated sulfuric acid to form colored reaction products measurable by UV–Vis spectrophotometry (Xiong et al., 2007). The three ethanolic extracts were evaluated at concentrations of 50, 100, and 150 ppm, while simvastatin served as the positive reference at concentrations of 5, 7.5, and 10 ppm. Stock solutions of the plant extracts were prepared by dissolving 10 mg of each extract in 10 mL of chloroform to obtain a concentration of 1,000 ppm, followed by dilution to the designated experimental concentrations. The simvastatin reference preparation involved dissolving 21 mg of powdered simvastatin tablets in 10 mL of chloroform, followed by preparation of the specified reference concentrations. A cholesterol solution designated as 200 ppm was used for the reaction system, although the original preparation record described dissolving 2 mg of cholesterol in 20 mL of chloroform, which corresponds mathematically to 100 ppm; the reported concentration designation was retained without assuming an undocumented additional preparation step. Wavelength determination was performed by reacting 1 mL of cholesterol solution with 0.5 mL of acetic anhydride and 0.025 mL of concentrated sulfuric acid, followed by spectrophotometric examination within the 400–700 nm range. The analytical wavelength was set at 420 nm, and reaction operating time was assessed by monitoring absorbance at 16, 18, 20, 22, and 24 minutes, with 22 minutes selected as the measurement time based on the original stability assessment. The negative control comprised 1.25 mL of cholesterol solution, 0.5 mL of acetic anhydride, and 0.025 mL of concentrated sulfuric acid, while the reagent blank contained 2.5 mL of chloroform, 0.5 mL of acetic anhydride, and 0.025 mL of concentrated sulfuric acid. Each experimental reaction mixture was prepared using 1.25 mL of extract solution and 1.25 mL of cholesterol solution, followed by the addition of 0.5 mL of acetic anhydride and 0.025 mL of concentrated sulfuric acid. The reaction mixtures were covered with aluminum foil and incubated for 15 minutes before spectrophotometric measurement at 420 nm, using the established operating-time assessment as the analytical reference. Percentage inhibition was calculated as $\mathrm{Inhibition}\,(\%)=[(A_{\mathrm{control}}A_{\mathrm{sample}})/A_{\mathrm{control}}]\times100$, where A_{control} represents negative-control absorbance and A_{sample} represents absorbance measured in the presence of the extract or reference preparation. IC_{50} values were estimated by fitting a linear regression equation, $y=ax+b$, between extract concentration and percentage inhibition and solving for the concentration corresponding to 50% inhibition, following the concentration–response estimation framework described by Sebaugh (2011). Data were processed using Microsoft Excel and evaluated descriptively through comparisons of phytochemical screening outcomes, absorbance measurements, percentage inhibition, and estimated IC_{50} values, with the findings organized into tables and graphs. The documented procedures included reagent blanks, a negative control, a positive reference, and wavelength and operating-time assessments; experimental replicate numbers, statistical significance testing, and formal analytical validation parameters were not specified in the available methodological record. Accordingly, the resulting measurements were interpreted as preliminary comparative responses within a chemical

assay rather than direct evidence of enzymatic inhibition, physiological cholesterol reduction, or clinical lipid-lowering efficacy.

RESULTS AND DISCUSSION

Sample Collection, Preparation of Dried Plant Material, and Extraction

The preparation of botanical materials constituted the initial experimental stage for evaluating the comparative cholesterol-associated activity of Jamaican cherry (Muntingia calabura L.), basil (Ocimum basilicum L.), and red gedi (Abelmoschus manihot L. Medik) leaf extracts. Plant samples were collected from Manembo-nembo Atas Village, Matuari District, Bitung City, North Sulawesi, Indonesia, with an initial fresh material weight of 1 kg for each species. The selection of these botanical materials was supported by previous investigations documenting secondary metabolites in Jamaican cherry, basil, and red gedi leaves, although their chemical compositions and reported biological properties differ across species (Anisa & Najib, 2022; Nadeem et al., 2022; Sari & Parbuntari, 2025). The use of identical initial fresh weights established a common starting quantity for sample preparation, while subsequent differences in recovered dried material reflected species-specific sample characteristics and the effects of processing.

The collected leaves underwent wet sorting and washing under running water to remove visible impurities before being subjected to air-drying away from direct sunlight for approximately one week. This preparation was intended to reduce moisture content while limiting unnecessary thermal exposure that could affect the stability of temperature-sensitive phytochemical constituents. Zhang et al. (2018) emphasized that the preparation and processing of botanical materials influence the subsequent extraction of natural products because changes in plant structure and constituent stability may affect the composition of recovered extracts. The drying procedure was applied consistently to the three species, although the absence of documented final moisture measurements prevents a quantitative comparison of drying efficiency or residual water content.

Following drying, the botanical materials were subjected to dry sorting and grinding to produce powdered samples suitable for extraction. The processing generated 321 g of dried basil leaf powder, 164 g of Jamaican cherry leaf powder, and 196 g of red gedi leaf powder, indicating differences in material recovery despite identical initial fresh weights. These variations may reflect differences in original moisture content, leaf morphology, or processing losses, but the available measurements do not establish the relative contribution of each factor. Studies examining the standardization of basil simplicia and ethanolic extracts have emphasized the relevance of raw-material characteristics and preparation procedures to the consistency of botanical preparations (Krismayadi et al., 2024).

The mass of recovered dried powder represented 32.1% of the initial fresh basil material, compared with 16.4% for Jamaican cherry and 19.6% for red gedi, based on the recorded preparation weights. These percentages describe mass recovery after processing rather than independently determined dry-matter content, since moisture measurements and processing-loss assessments were not documented. Differences in recovered powder mass demonstrate that equal fresh sample weights do not necessarily produce equivalent quantities of material available for solvent extraction. This distinction is relevant to comparative pharmacognostic studies because extraction performance should be evaluated relative to the actual mass of processed botanical material rather than the initial fresh weight alone (Zhang et al., 2018).

The quantitative characteristics of the preparation and extraction stages are presented in Table 1, including initial fresh material, recovered dried powder, extracted powder mass, concentrated extract mass, and calculated extraction yield. Each species was extracted using 50 g of dried powdered material, allowing the extraction yield to be expressed relative to the same starting mass. The extraction yield was calculated as the mass of concentrated extract divided by the mass of powdered material subjected to maceration, multiplied by 100%, consistent with the distinction between raw-material recovery and extract recovery in natural-product processing (Zhang et al., 2018). This calculation provides a descriptive measure of extract recovery without establishing the relative abundance of individual secondary metabolites.

Table 1. Preparation Characteristics and Extraction Yields of the Three Leaf Samples

Parameter	Jamaican cherry	Basil	Red gedi
Initial fresh leaf weight (g)	1,000	1,000	1,000
Recovered dried powder (g)	164	321	196
Powder recovery (%)	16.40	32.10	19.60
Powder used for extraction (g)	50	50	50
Concentrated extract obtained (g)	3.50	4.20	3.20
Extraction yield (% w/w)	7.00	8.40	6.40
Extract appearance	Dark greenish-black	Dark green	Comparatively lighter dark green

Source: Primary research data (2026); percentage values calculated from the recorded sample and extract masses.

The results in Table 1 indicate that basil produced the greatest concentrated extract mass, reaching 4.20 g from 50 g of powdered material, corresponding to an extraction yield of 8.40%. Jamaican cherry produced 3.50 g of extract with a yield of 7.00%, whereas red gedi generated 3.20 g with the lowest yield of 6.40%. These differences indicate variation in the quantities of ethanol-extractable material recovered under the documented extraction conditions, without demonstrating that the extract with the highest yield contained the greatest concentration of biologically active constituents. Previous phytochemical investigations of basil have identified multiple constituent classes, illustrating why crude extraction yield should be distinguished from chemical composition and pharmacological activity (Kumalasari & Adiarna, 2020; Nadeem et al., 2022).

Extraction was conducted through maceration using 96% ethanol for 24 hours and repeated three times with fresh solvent and occasional stirring. The procedure was applied to 50 g of dried powdered material from each species, followed by filtration and evaporation at approximately 45°C to obtain concentrated extracts. Ethanol is widely employed in natural-product extraction because its solvent properties permit the recovery of various phytochemical constituents, although extraction selectivity remains dependent on compound polarity, plant-matrix composition, and processing conditions (Zhang et al., 2018). The standardized maceration procedure reduced variation attributable to the documented extraction protocol, but the available records do not include measurements of extraction completeness or chemical recovery efficiency.

Visual examination of the concentrated extracts revealed differences in appearance, with Jamaican cherry yielding a dark greenish-black extract, basil producing a dark green extract, and red gedi exhibiting a comparatively lighter dark green coloration. These observations are compatible with differences in extract composition, although visual characteristics alone cannot establish the presence or relative concentration of specific secondary metabolites. Investigations of polyphenolic compounds have demonstrated that chemical structure and interactions within reaction systems can influence observable coloration, emphasizing the limited specificity of visual assessment as an indicator of chemical identity (Espina et al., 2022). The documented color variations were therefore treated as descriptive characteristics of the recovered materials rather than quantitative evidence of phytochemical abundance or cholesterol-associated activity.

The observed differences in extract recovery also require interpretation in relation to the subsequent experimental comparison of cholesterol-associated responses. Basil exhibited the highest extraction yield, while Jamaican cherry and red gedi produced lower quantities of concentrated extract, establishing that the three botanical matrices differed in their recovery of crude soluble material. Research on plant-derived compounds indicates that biological or chemical activity depends on the identity and properties of constituent molecules rather than crude extract mass alone, particularly when multiple metabolite classes are present in the same preparation (Kumar & Pandey, 2013; Francis et al., 2002). Consequently, extraction yield provides information about material recovery and laboratory processing but cannot independently predict the percentage inhibition or IC_{50} values subsequently measured using the Liebermann–Burchard method.

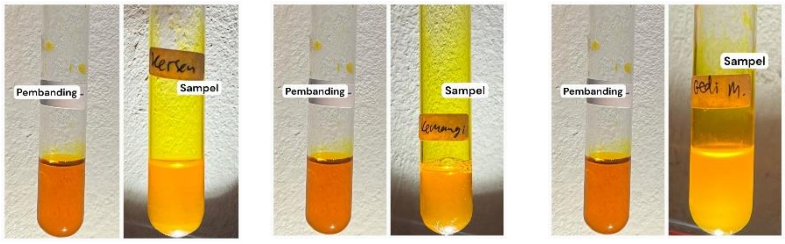
The preparation and extraction results establish the experimental basis for comparing the three ethanolic leaf extracts under a common analytical procedure. Differences in dried material recovery,

concentrated extract mass, and visual appearance demonstrate the heterogeneity of the botanical samples while preserving the documented consistency of the extraction mass, solvent concentration, maceration duration, and evaporation temperature. Previous research on extraction methodology and phytochemical characterization supports the use of these preparation characteristics as essential information for evaluating the comparability and reproducibility of natural-product investigations (Zhang et al., 2018; Shaikh & Patil, 2020). The concentrated extracts obtained from this stage constituted the materials for subsequent qualitative phytochemical screening and spectrophotometric evaluation, through which differences in secondary-metabolite profiles and cholesterol-associated analytical responses could be examined without assuming a direct relationship between extraction yield and pharmacological potency.

Phytochemical Screening of Jamaican Cherry, Basil, and Red Gedi Leaf Extracts

Qualitative phytochemical screening was performed to characterize the major secondary metabolite classes present in ethanolic leaf extracts of Jamaican cherry (Muntingia calabura L.), basil (Ocimum basilicum L.), and red gedi (Abelmoschus manihot L. Medik). The examination focused on alkaloids, flavonoids, saponins, tannins, and steroids using reagent-based reactions identified through precipitation, foam formation, and characteristic color changes. These metabolite classes were selected because previous investigations have documented their occurrence in medicinal plants and established their relevance to diverse biological and physicochemical activities (Kumar & Pandey, 2013; Francis et al., 2002; Shaikh & Patil, 2020). Qualitative screening was used to identify the presence of broad chemical groups rather than determine individual molecular structures or absolute metabolite concentrations, providing a preliminary chemical profile for interpreting subsequent cholesterol-associated assay responses.

The alkaloid screening results indicated weakly positive reactions in all three ethanolic leaf extracts, as demonstrated by precipitate formation following the addition of Dragendorff's reagent. The comparable qualitative responses suggest that alkaloid-reactive constituents were detectable in Jamaican cherry, basil, and red gedi extracts, although the observations do not establish equivalent concentrations or identical alkaloid compositions. Preliminary phytochemical investigations have demonstrated the applicability of reagent-induced precipitation for identifying alkaloid-containing botanical preparations, while emphasizing that such reactions provide presumptive evidence requiring instrumental confirmation for specific compound identification (Ngibad, 2019; Shaikh & Patil, 2020). The detection of alkaloid-reactive constituents is relevant to cholesterol-related research because selected plant alkaloids, including berberine, exhibit lipid-metabolism-associated biological activities, although these findings cannot be generalized to unidentified alkaloids present in the investigated extracts (Wang & Zidichouski, 2018).



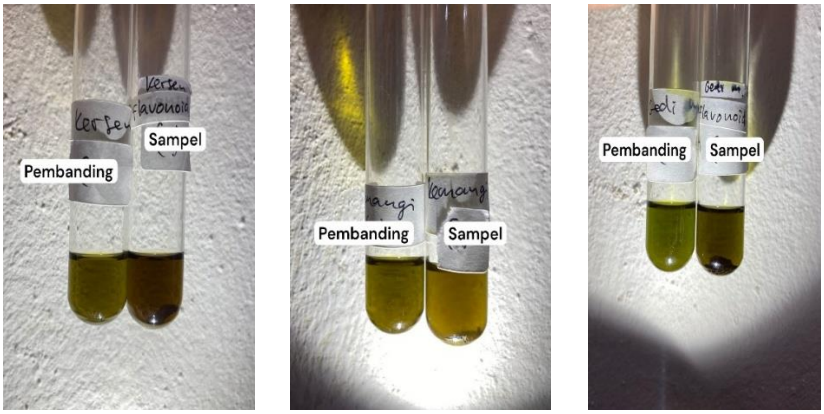
(a) Jamaican cherry (b) basil (c) red gedi

Figure 1. Alkaloid test results: (a) Jamaican cherry leaves, (b) basil leaves, and (c) red gedi leaves.

Source: Authors' documentation (2026).

Flavonoid screening produced strongly positive (+++) qualitative reactions in Jamaican cherry, basil, and red gedi extracts following treatment with magnesium and concentrated hydrochloric acid. The recorded color changes were interpreted as positive responses in the applied flavonoid screening procedure, indicating the presence of constituents capable of participating in the relevant reagent-induced reactions. Previous chemical investigations have established that flavonoids possess structurally diverse hydroxylated aromatic frameworks associated with antioxidant and other biological activities, although the nature and magnitude of these effects vary substantially among

individual compounds (Kumar & Pandey, 2013; Panche et al., 2016). The comparable qualitative intensity observed across the three extracts should not be interpreted as evidence of equivalent total flavonoid concentrations because the screening procedure did not include a calibrated quantitative determination or chromatographic characterization.

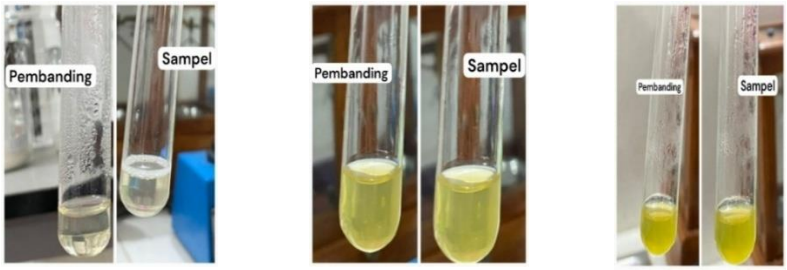


(a) Jamaican cherry (b) basil (c) red gedi

Figure 2. Flavonoid test results: (a) Jamaican cherry leaves, (b) basil leaves, and (c) red gedi leaves.

Source: Authors' documentation (2026).

Saponin screening revealed a distinct qualitative difference among the three botanical extracts, with Jamaican cherry demonstrating a moderately positive (++) response while basil and red gedi produced negative reactions under the applied testing conditions. The positive result was identified through persistent foam formation following sample agitation, a conventional preliminary indication of compounds possessing surface-active characteristics. Saponins contain hydrophilic and hydrophobic structural components that contribute to their amphiphilic behavior, while their biological actions depend on molecular composition, concentration, and interactions with surrounding chemical or biological systems (Francis et al., 2002; Moghimipour & Handali, 2015). The absence of positive foam formation in basil and red gedi should be interpreted as nondetection under the specified assay conditions rather than definitive evidence that every saponin compound is absent from these botanical materials.



(a) Jamaican cherry (b) basil (c) red gedi

Figure 3. Saponin test results: (a) Jamaican cherry leaves, (b) basil leaves, and (c) red gedi leaves.

Source: Authors' documentation (2026).

The comparative findings from the five phytochemical screening procedures are summarized in Table 2, which presents the documented qualitative reaction intensities for each extract. The table distinguishes metabolite classes detected across all three botanical materials from saponins, which exhibited a positive response only in Jamaican cherry. This distinction is analytically relevant because differences in metabolite-class detection may provide a basis for formulating hypotheses about variations in subsequent chemical assay responses, without demonstrating causal relationships between particular constituents and cholesterol-associated activity (Anisa & Najib, 2022; Sari &

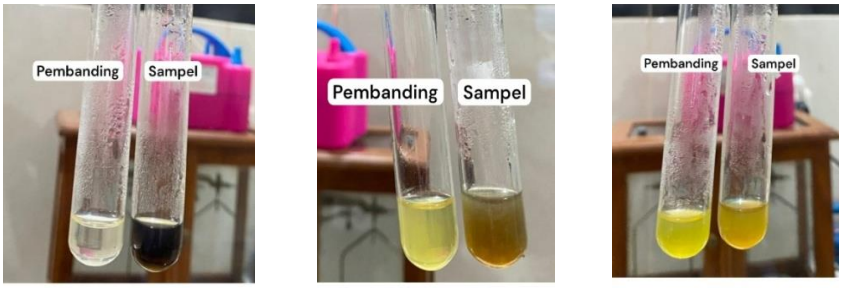
Parbuntari, 2025). The qualitative symbols represent the recorded intensity categories of the screening reactions and should not be interpreted as calibrated concentration measurements.

Table 2. Qualitative Phytochemical Screening Results of Jamaican Cherry, Basil, and Red Gedi Leaf Extracts

Secondary metabolite	Jamaican cherry	Basil	Red gedi	Positive reaction indicator
Alkaloids	+	+	+	Precipitate formation
Flavonoids	+++	+++	+++	Characteristic color change
Saponins	++	−	−	Persistent foam formation
Tannins	+++	+	+	Brown or dark greenish-black coloration
Steroids	++	++	++	Bluish-green color reaction

Source: Primary research data (2026). Note: (−) Not detected; (+) weak positive reaction; (++) moderate positive reaction; (+++) strong positive reaction. Reaction intensity represents qualitative observations rather than quantitatively validated metabolite concentrations.

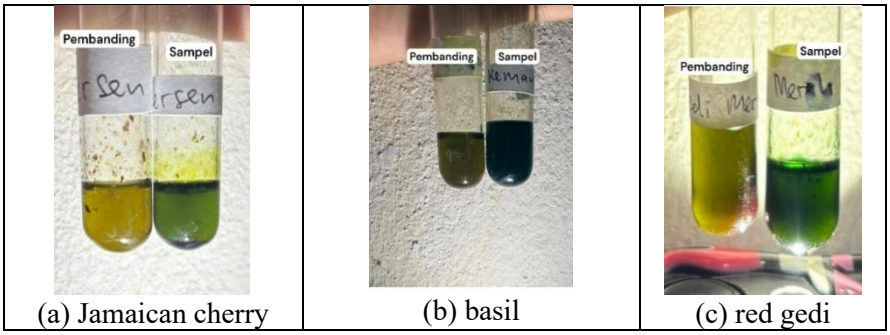
The data presented in Table 2 demonstrate that alkaloids, flavonoids, tannins, and steroids were detected in each extract, whereas the saponin reaction differentiated Jamaican cherry from the other two species. Jamaican cherry also exhibited a strongly positive (+++) tannin reaction, compared with weakly positive (+) reactions in basil and red gedi, suggesting differences in the qualitative responses of phenolic constituents to the screening reagent. The tannin examination involved ferric chloride, which can produce colored complexes through interactions between iron ions and suitable phenolic functional groups, although the reaction is not exclusively specific to tannins (Espina et al., 2022). Consequently, the stronger coloration observed in Jamaican cherry may reflect differences in reactive phenolic constituents, but it cannot independently establish a greater tannin concentration without appropriate quantitative analysis.



(a) Jamaican cherry (b) basil (c) red gedi

Figure 4. Tannin test results: (a) Jamaican cherry leaves, (b) basil leaves, and (c) red gedi leaves.
Source: Authors' documentation (2026).

The steroid screening procedure yielded moderately positive (++) reactions in Jamaican cherry, basil, and red gedi extracts following treatment with chloroform and concentrated sulfuric acid. These observations indicate that the three extracts contained constituents capable of producing positive responses in the applied steroid-oriented color reaction, although the assay did not distinguish among individual steroidal compounds or establish their molecular identities. The Liebermann–Burchard reaction itself involves acid-mediated chemical transformations of cholesterol, demonstrating why color reactions involving sterol structures must be interpreted with attention to reagent chemistry and analytical specificity (Xiong et al., 2007). Research on phytosterols has identified substantial structural and biological diversity within plant-derived sterol compounds, making it inappropriate to infer a specific pharmacological effect solely from a positive qualitative steroid reaction (Shahzad et al., 2017).



(a) Jamaican cherry (b) basil (c) red gedi

Figure 5. Steroid test results: (a) Jamaican cherry leaves, (b) basil leaves, and (c) red gedi leaves.

Source: Authors' documentation (2026).

Differences in phytochemical screening responses were consistent with the broader literature documenting variable secondary metabolite profiles among the investigated botanical species. Previous studies of Jamaican cherry have reported phenolic and flavonoid constituents, while investigations of basil have identified multiple phytochemical classes and demonstrated that botanical preparations possess chemically complex profiles (Anisa & Najib, 2022; Sumarni et al., 2022; Nadeem et al., 2022). Research involving red gedi has identified selected plant constituents and provided preliminary evidence of secondary metabolites in ethanolic leaf extracts, although the available investigations do not establish that its chemical composition is equivalent to that of Jamaican cherry or basil (Mamahit & Soekamto, 2010; Sari & Parbuntari, 2025). The present observations contribute a directly comparable qualitative dataset obtained from three extracts prepared and screened within the same experimental framework, while variations in plant material, extraction conditions, and reagent responses remain relevant when comparing these findings with separately conducted studies.

The relationship between the detected secondary metabolites and cholesterol-associated activity requires careful interpretation because reagent-based phytochemical identification does not establish the functional contribution of individual compounds. Saponins may exhibit sterol-related interactions associated with their amphiphilic properties, while flavonoids, alkaloids, and other plant constituents can participate in biological or physicochemical processes through substantially different mechanisms (Francis et al., 2002; Wang & Zidichouski, 2018; Testai & Calderone, 2017). The particularly strong tannin reaction and positive saponin reaction observed in Jamaican cherry provide a plausible chemical basis for further investigation, but neither observation proves that these metabolite classes determine its subsequent Liebermann–Burchard assay response. The absence of compound isolation, quantitative metabolite measurement, and interference-specific analytical testing restricts interpretation to associations between preliminary phytochemical profiles and the experimental characteristics of the crude extracts.

The phytochemical screening results establish a comparative chemical foundation for evaluating the concentration-dependent analytical responses of the three ethanolic leaf extracts in the subsequent cholesterol-associated assay. Jamaican cherry displayed a distinctive combination of positive saponin detection and strong tannin-associated coloration, whereas basil and red gedi shared broadly similar qualitative reaction categories across the five metabolite classes examined. Established phytochemical screening principles indicate that such findings are appropriate for preliminary classification and selection of materials for further characterization, but not for confirming molecular structures, estimating absolute constituent concentrations, or demonstrating pharmacological mechanisms (Shaikh & Patil, 2020; Ngibad, 2019). Subsequent interpretation of percentage inhibition and IC_{50} measurements must therefore consider the phytochemical observations as supporting descriptive evidence while recognizing that crude-extract absorbance responses may also reflect interactions between botanical constituents and the colorimetric reaction system.

Assessment of In Vitro Cholesterol-Lowering Activity Using the Liebermann–Burchard Method

The in vitro assessment of cholesterol-associated activity was conducted using the Liebermann–Burchard colorimetric method to compare the analytical responses of ethanolic Jamaican

cherry (Muntingia calabura L.), basil (Ocimum basilicum L.), and red gedi (Abelmoschus manihot L. Medik) leaf extracts. The experimental procedure involved three extract concentrations of 50, 100, and 150 ppm, with simvastatin serving as the positive reference at concentrations of 5, 7.5, and 10 ppm. The reaction depends on the interaction of cholesterol with acetic anhydride and concentrated sulfuric acid, producing colored derivatives whose absorbance can be evaluated spectrophotometrically (Xiong et al., 2007). Interpretation of the resulting absorbance changes was restricted to the chemical reaction system because decreased color intensity does not independently establish inhibition of cholesterol biosynthesis, intestinal absorption, or circulating cholesterol concentrations.

Spectrophotometric analysis was performed at 420 nm following preliminary wavelength assessment, while the recorded operating-time evaluation identified 22 minutes as the reference measurement time. The negative control produced an absorbance value of 0.857, which served as the denominator for calculating percentage inhibition across the experimental preparations. UV–Vis spectrophotometry permits quantitative comparison of optical responses under defined analytical conditions, although measurement reliability depends on appropriate wavelength selection, reference correction, and control of interfering sample constituents (Mettler-Toledo, 2015). The use of a common negative control supported descriptive comparison among the extracts, while the absence of reported replicate measurements and precision estimates prevented assessment of analytical variability or statistical significance.

The simvastatin reference exhibited progressively lower absorbance values as concentration increased, with recorded measurements of 0.680, 0.572, and 0.557 at 5, 7.5, and 10 ppm, respectively. These measurements corresponded to inhibition percentages of 20.65%, 33.25%, and 35.01%, indicating a concentration-associated reduction in the Liebermann–Burchard color response within the tested reference range. Simvastatin is pharmacologically recognized as an inhibitor of hepatic HMG-CoA reductase, but this enzymatic mechanism differs fundamentally from the reagent-mediated cholesterol transformations assessed in the present experimental system (Endo & Beppu, 2010; Talreja & Cassagnol, 2023). The reference measurements consequently provided an internal analytical comparison rather than direct evidence that the method reproduced the established pharmacological mechanism of statin therapy.

Jamaican cherry leaf extract exhibited a pronounced concentration-associated reduction in absorbance, declining from 0.560 at 50 ppm to 0.404 at 100 ppm and 0.014 at 150 ppm. The corresponding inhibition percentages increased from 34.66% to 52.86% and subsequently reached 98.36%, representing the largest positive response observed among the three botanical preparations. Previous research involving ethanolic Jamaican cherry leaf extract reported cholesterol-related activity in male mice, providing biological evidence that supports continued investigation of this species without establishing equivalence between animal responses and the present chemical assay (Natasha et al., 2024). The marked decline in absorbance at the highest tested concentration is analytically relevant, although its interpretation requires consideration of potential interactions between crude-extract constituents and the reagents responsible for color formation.

The concentration-dependent absorbance measurements, calculated inhibition percentages, and reported IC_{50} estimates are presented in Table 3 to establish a common quantitative basis for comparing the botanical extracts and the simvastatin reference. The tabulated values retain the experimental measurements documented in the original research records, including negative inhibition percentages observed when sample absorbance exceeded the negative-control absorbance. Percentage inhibition was calculated relative to the negative control, whereas IC_{50} values were estimated using linear concentration–response regression, an approach whose interpretability depends on the measured concentration range and the adequacy of the fitted relationship (Sebaugh, 2011). The resulting values describe analytical responses within the Liebermann–Burchard system and should not be interpreted as validated pharmacological potency estimates.

Table 3. In Vitro Cholesterol-Associated Activity of Ethanolic Leaf Extracts and Simvastatin Reference

Sample	Concentration (ppm)	Control absorbance	Sample absorbance	Inhibition (%)	Reported IC_{50} (ppm)
Basil	50	0.857	1.278	−49.12	
	100	0.857	1.014	−18.31	219.592
	150	0.857	0.780	8.98	
Jamaican cherry	50	0.857	0.560	34.66	
	100	0.857	0.404	52.86	81.220
	150	0.857	0.014	98.36	
Red gedi	50	0.857	1.612	−88.09	
	100	0.857	1.106	−29.05	173.377
	150	0.857	0.661	22.87	
Simvastatin	5	0.857	0.680	20.65	
	7.5	0.857	0.572	33.25	14.590
	10	0.857	0.557	35.01	

Source: Primary research data (2026). IC_{50} values are reproduced from the original research calculations. Negative inhibition indicates sample absorbance exceeding the negative control and does not establish an increase in cholesterol concentration.

The comparative data in Table 3 demonstrate that Jamaican cherry generated positive inhibition values at every tested concentration, whereas basil and red gedi produced negative values at 50 and 100 ppm. At 150 ppm, Jamaican cherry reached 98.36% inhibition, compared with 22.87% for red gedi and 8.98% for basil, indicating substantial differences in the observed colorimetric responses under identical nominal extract concentrations. Negative inhibition reflects an increase in measured absorbance relative to the negative control and may result from intrinsic extract coloration, reagent interactions, or other matrix-dependent optical effects rather than an actual increase in cholesterol content. Studies employing the Liebermann–Burchard method for botanical screening demonstrate its applicability to preliminary cholesterol-associated measurements, but the reaction chemistry requires caution when interpreting crude-extract absorbance differences as biological activity (Chicade et al., 2025; Darojat et al., 2026; Xiong et al., 2007).

The reported IC_{50} values placed Jamaican cherry at 81.220 ppm, red gedi at 173.377 ppm, and basil at 219.592 ppm, suggesting the strongest apparent activity for Jamaican cherry within the original regression-based comparison. Nevertheless, the interpretation of these estimates is constrained by their relationship to the observed responses, particularly because basil and red gedi did not reach 50% inhibition at the highest experimentally tested concentration of 150 ppm. Their IC_{50} values therefore represent extrapolated estimates beyond the investigated concentration range, whereas the Jamaican cherry estimate lies within the tested interval and is supported by measured inhibition values on both sides of the 50% response. Sebaugh (2011) emphasized the importance of appropriate concentration–response data and model selection for reliable IC_{50} estimation, indicating that extrapolated values should not be treated as experimentally confirmed inhibitory concentrations.

An additional interpretive limitation concerns the reported simvastatin IC_{50} of 14.590 ppm, which exceeds the maximum tested reference concentration of 10 ppm despite the highest observed inhibition being only 35.01%. This estimate is extrapolative rather than directly supported by an observed 50% response, and its numerical precision should not be interpreted as evidence of validated concentration–response performance. The original research record also contains a discrepancy between the designated 200 ppm cholesterol solution and the documented preparation of 2 mg cholesterol in 20 mL solvent, which mathematically corresponds to 100 ppm and introduces uncertainty into the stated reaction concentration. Accurate analytical interpretation requires consistency between solution preparation, concentration designation, and measured response, particularly when chemical interactions are evaluated through spectrophotometric methods (Mettler-Toledo, 2015; Sebaugh, 2011).

The phytochemical profiles provide a preliminary basis for interpreting differences among the three extracts, particularly the positive saponin reaction and stronger tannin-associated coloration recorded for Jamaican cherry. Saponins possess amphiphilic structures capable of interacting with sterol-containing systems, while flavonoids and other phenolic constituents exhibit diverse chemical properties that may influence interactions within biological or reagent-based environments (Francis et al., 2002; Moghimipour & Handali, 2015; Kumar & Pandey, 2013). Nevertheless, the available data do not establish that saponins or tannins caused the greater absorbance reduction observed for Jamaican cherry, since isolated-compound testing, metabolite quantification, and interference-specific controls were not documented. Research on plant-derived compounds has demonstrated that chemical identity and molecular structure are critical to interpreting biological activity, preventing the attribution of crude-extract responses to an entire metabolite class without additional experimental evidence (Muharni et al., 2021; Wang & Zidichouski, 2018).

The experimental comparison identifies Jamaican cherry as the extract producing the strongest positive Liebermann–Burchard response within the investigated concentration range, while basil and red gedi exhibited substantially weaker responses and negative inhibition values at lower concentrations. The interpretation remains limited by the preliminary nature of the colorimetric method, the absence of documented replication and statistical uncertainty, the extrapolation involved in several reported IC_{50} estimates, and the unresolved cholesterol-stock concentration discrepancy. Comparable in vitro studies of botanical extracts have supported the preliminary identification of potentially active materials, but confirmation of cholesterol-lowering mechanisms requires experiments capable of distinguishing chemical assay interference from genuine effects on cholesterol metabolism or availability (Andriani & Anggraini, 2023; Parbuntari & Yuliandari, 2023; Pramesty et al., 2023). These findings provide a defensible basis for prioritizing Jamaican cherry for further compound characterization, analytical validation, and biological investigation without establishing therapeutic effectiveness or equivalence to clinically validated lipid-lowering agents.

CONCLUSION

The findings demonstrate that ethanolic leaf extracts of Jamaican cherry (Muntingia calabura L.), basil (Ocimum basilicum L.), and red gedi (Abelmoschus manihot L. Medik) contain alkaloids, flavonoids, tannins, and steroids, whereas saponins were detected only in Jamaican cherry leaf extract under the applied phytochemical screening conditions. Jamaican cherry also exhibited a stronger qualitative tannin reaction than the other two extracts, indicating differences in reagent responses among the investigated botanical materials. The Liebermann–Burchard assay demonstrated that Jamaican cherry produced the greatest reduction in absorbance, reaching 98.36% inhibition at 150 ppm, compared with 22.87% for red gedi and 8.98% for basil at the same concentration. The reported IC_{50} values were 81.220 ppm for Jamaican cherry, 173.377 ppm for red gedi, and 219.592 ppm for basil, although the latter two estimates were extrapolated beyond the experimentally tested concentration range. These results identify Jamaican cherry as the strongest-performing extract within the investigated colorimetric assay, without establishing its superiority in biological cholesterol reduction or clinical lipid-lowering effectiveness. The comparative findings also demonstrate that extraction yield and qualitative metabolite detection cannot independently predict the magnitude of cholesterol-associated absorbance responses. The study contributes preliminary experimental evidence integrating phytochemical characterization with standardized comparative screening of three botanical leaf extracts.

The scientific implications of these findings concern the identification of promising botanical materials for further pharmaceutical and phytochemical investigation, particularly Jamaican cherry leaf extract, which exhibited the most pronounced concentration-dependent analytical response. The observed differences among extracts support the value of conducting direct comparisons under common experimental conditions rather than inferring relative activity from independently published phytochemical or pharmacological studies. Interpretation remains constrained by the absence of documented experimental replication and statistical uncertainty, the potential influence of crude-extract constituents on colorimetric measurements, and the extrapolation required for several reported IC_{50} estimates. The discrepancy between the stated cholesterol concentration and its documented stock-solution preparation also requires resolution before the analytical procedure can be considered sufficiently standardized for reproducible quantitative assessment. Further investigations should

establish solution-preparation accuracy, evaluate analytical interference, incorporate replicate measurements, and examine additional concentrations that adequately characterize the relevant concentration–response relationships. Subsequent compound isolation and biological testing are necessary to determine which constituents contribute to the observed responses and whether the preliminary analytical activity corresponds to genuine effects on cholesterol metabolism. The findings provide a basis for prioritizing Jamaican cherry in future investigations while maintaining a clear distinction between in vitro colorimetric activity and validated therapeutic efficacy.

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