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Research Article

Acetone-Based Comparative Phytochemical Profiling of Indonesian Ethnomedicinal Plants: Effects of Maceration and Soxhlet Extraction

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A B S T R A C T

Acetone has rarely been applied as a single solvent in comparative phytochemical studies of Indonesian medicinal plants, and systematic comparisons of extraction techniques remain limited. This study aimed to evaluate the qualitative phytochemical profiles and potential bioactivity of acetone extracts obtained using maceration and Soxhlet extraction from selected ethnomedicinal plants. The samples included the leaves and fruits of Pereskia bleo, leaves and stems of Gnetum gnemon, leaves of Bouea macrophylla, leaves of Psidium guajava, and the banana inflorescence of Musa paradisiaca. Phytochemical screening revealed that phenolics, steroids, and terpenoids were consistently detected in all samples. Strong flavonoid presence (+++) was observed in several plant parts, particularly P. bleo fruit and G. gnemon. Alkaloids and glycosides were detected only in maceration extracts, reflecting their thermolabile nature. These results demonstrate that acetone effectively profiles secondary metabolites, while extraction methods significantly influence the detection of thermosensitive compounds.

1. INTRODUCTION

Indonesia's tropical biodiversity is rich, with an estimated 9,600 plant species used in herbal medicine. The development of modern phytopharmaceuticals is a significant resource for the country [1]. However, this potential can only be realized through proper standardization of the extraction process, considering that the extraction method is a critical independent variable that directly determines the profile and quantity of secondary metabolites extracted from plant samples [2]. In pharmacognosy research, the two most commonly used conventional extraction methods are maceration and Soxhlet extraction. Maceration, a cold extraction technique (performed at room temperature, 24–72 hours), retains thermolabile compounds through a diffusion mechanism based on the Fickian concentration gradient, without exposure to excessive heat [2]. Soxhletation is a technique that uses a hot solvent reflux cycle, which thermodynamically produces higher extraction efficiency. However, it has the potential to degrade heat-resistant compounds through a thermal hydrolysis mechanism [3], [4]. It is essential to understand the advantages and limitations of each method. This knowledge allows for designing an extraction protocol appropriate for the target bioactive compound.

Acetone (2-propanone; CAS 67-64-1; boiling point 56.05°C; log P = −0.24; ε = 20.7; P' = 5.1) is an intermediate polar organic solvent that acts as a hydrogen-bond acceptor

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through its carbonyl group (C=O) but not a hydrogen-bond donor. In this study, acetone was chosen to optimize the extraction of bioactive flavonoids. An intermediate polarity solvent, such as acetone, demonstrates greater effectiveness than highly polar alternatives like ethanol, methanol, and water. This aligns with previous research on dragon fruit extraction, which reported that the substantial solubility of flavonoids in intermediate polarity solvents significantly enhances extraction efficiency [5], [7] . This combination of properties allows acetone to extract compounds in a wide range of polarities, from polar phenolics and flavonoids to semi-polar terpenoids and steroids in a single extraction step without problematic co-extraction of chlorophyll and lipids [5]. [6]. However, the use of acetone as an exclusive solvent in comparative studies of maceration vs. Soxhletation on Indonesian tropical medicinal plants is still very rarely reported.

The five species selected in this study include *Pereskia bleo* (Cactaceae), *Gnetum gnemon* (Gnetaceae), *Bouea macrophylla* (Anacardiaceae), *Psidium guajava* (Myrtaceae), and *Musa paradisiaca* (Musaceae). These five species are ethnomedicinal plants with a long track record of traditional use in Indonesia, but have never been studied simultaneously in a single acetone-based comparative framework. The novelty of this study lies in: (1) the use of acetone as the sole solvent, allowing for a pure comparison of the effects of extraction methods without confounding solvent polarity; (2) the integration of primary qualitative phytochemical data from nine plant materials in one study; and (3) the comparative analysis between organs in two species (*P. bleo* leaves vs. fruits; *G. gnemon* leaves vs. stems).

This study aims to: (1) document the qualitative phytochemical profiles of acetone extracts from nine plant samples from five species using two extraction methods; (2) compare the detection patterns of eight compound groups between the maceration and soxhletation methods; (3) analyze intra-species variations based on organs; and (4) discuss the implications of phytochemical profiles on the potential bioactivity of each species as a basis for developing phytopharmaceuticals based on local Indonesian medicinal plants.

2. METHODOLOGY

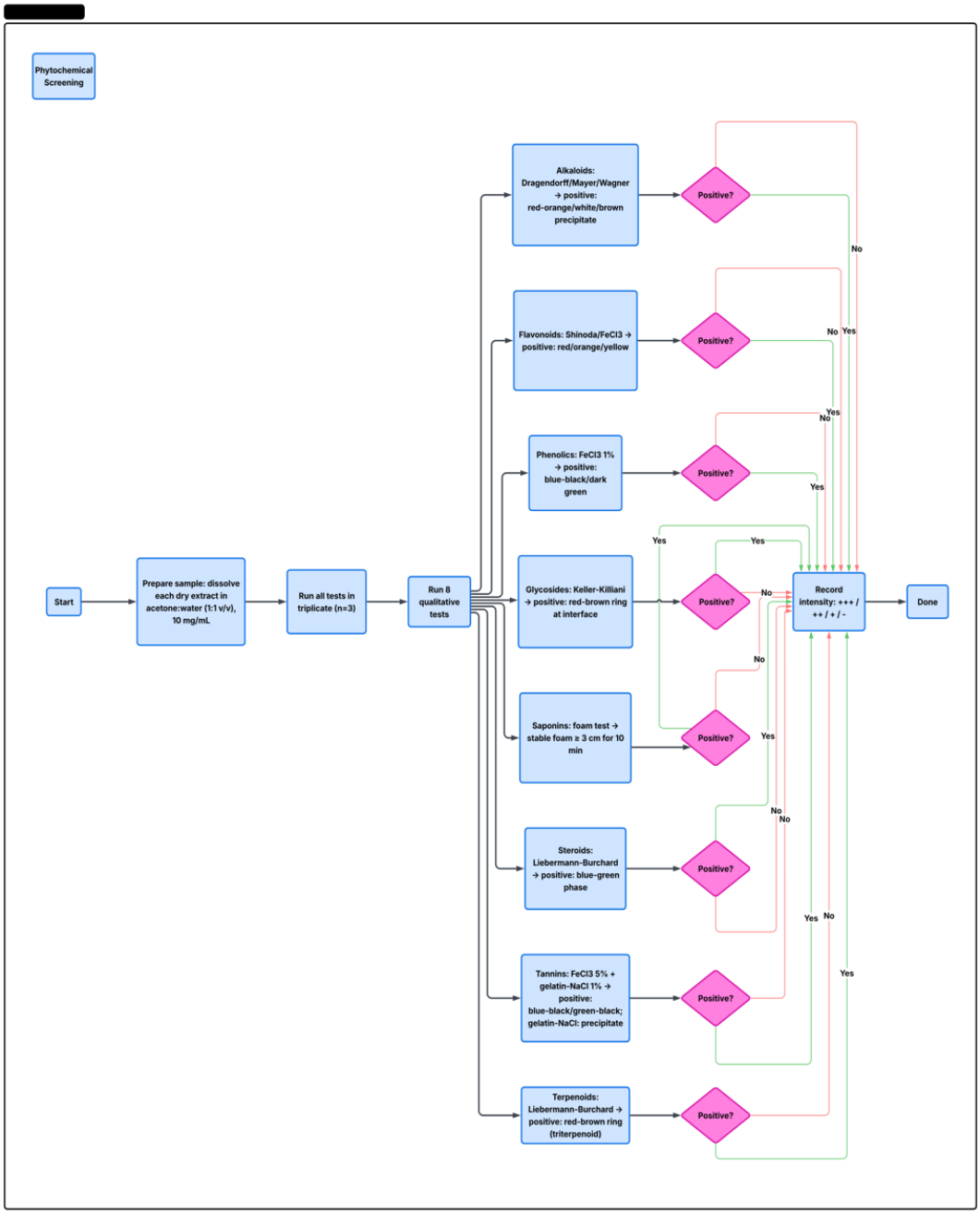
The methodology includes plant materials and preparation of simplicia, an acetone extraction protocol, and qualitative phytochemical screening. The plants used as samples include *P. bleo*, *G. gnemon*, *B. macrophylla*, *P. guajava*, and *M. paradisiaca*. The collected plant tissues were mature leaves and young-ripe fruits of P. bleo; mature leaves and young stems (1–2 cm in diameter) of *G. gnemon*; mature leaves (3rd to 5th from the shoot) of *B. macrophylla* and *P. guajava*; banana blossom of *M. paradisiaca* (8–10 cm from the tip, outermost bractea removed). Fresh materials were washed, dried (45°C air circulation oven, 48–72 hours), ground (dry blender), and sieved (mesh 40, ≤0.425 mm).

An acetone extraction protocol was carried out in two methods, maceration and acetone soxhletation. Maceration using acetone includes (leaves & fruits of *P. bleo*; leaves & stems of *G. gnemon*; heart of *M. paradisiaca*). A total of 200 g of each simplicia powder was weighed (analytical balance Mettler Toledo ME204, accuracy 0.1 mg) and soaked in 1000 mL acetone p.a. (Merck ≥99.5%; ratio 1:10 w/v) in a tightly closed 1000 mL Erlenmeyer flask. The mixture was stirred and then left for 72 hours protected from light, with periodic stirring of 15 minutes every 24 hours. After being filtered (Whatman No. 1 filter paper), the residue was remacerated twice with 1000 mL of acetone each (24 hours/cycle). The filtrates were combined, then dried (vacuum oven 40°C, 24 hours, 50 mbar) until constant weight. The yield was calculated as % w/w of the crude drug.

Next, acetone Soxhletation was performed on *B. macrophylla* and *P. guajava* leaves. A total of 50 g of powdered crude drug was wrapped in a cellulose thimble (43×123 mm, Whatman) and extracted using 350 mL of acetone p.a. in a 500 mL Soxhlet extractor (Pyrex). The heating mantle was set to produce a stable reflux of 56±1°C for 6 hours (~8–10 cycles/hour). Evaporation and drying were performed identically to the maceration procedure. The yield was calculated identically.

The next method is qualitative phytochemical screening based on the standard methods of Harborne (1998), Trease & Evans (2002), and Sofowora (1993) as follows: Each dry extract was dissolved in acetone, distilled water (1:1 v/v, 10 mg/mL) before testing. [7], [8], [9]. Eight groups of compounds were tested: Phenolic identification using the main reagent, FeCl3, 1%. The short procedure is 1 mL of extract + 3 drops of 1% $FeCl_3$ in ethanol; the positive response is blackish blue/dark green. Flavonoids are the main reagents of Shinoda / $FeCl_3$. namely Mg powder + concentrated HCl; observe the color change with the positive response being intense red/orange/yellow. Tannins, the main reagent of $FeCl_3$ 5% + gelatin-NaCl. $FeCl_3$ 5%: color; gelatin-NaCl 1%: sediment. The positive response is blue-black (error); green-black (condensation). Steroids with Liebermann-Burchard, namely acetic anhydride + concentrated H_2SO_4 → blue-green phase. The positive response is blue-green after H_2SO_4. Terpenoids with Liebermann-Burchard Identical steroids → red-brown phase at the interface, the positive response is a red-brown ring (triterpenoid). Saponins with the forth fuoam test, namely 5 mL + 10 mL of distilled water, shake for 30 seconds → observe the foam, stable foam ≥ 3 cm for 10 minutes. Alkaloids with Dragendorff / Mayer / Wagner,

namely 2 mL + 2% HCl; add the reagents separately. The result is a red-orange/white/brown precipitate. Glycosides with Keller-Killiani, namely $FeCl_3$ / as. glacial acetate + concentrated H_2SO_4, the result is a red-brown ring at the interface. All tests were carried out in triplicate (n = 3), intensity: +++ (strong), ++ (moderate), + (weak), – (negative).



3. RESULTS AND DISCUSSION

3.1 *Results: Qualitative Primary Phytochemical Data of Acetone Extract*

Table 1 presents the complete qualitative phytochemical profile, which is the primary data of this study, obtained directly from laboratory screening of eight groups of compounds in nine samples of acetone extracts from five Indonesian ethnomedicinal plants.

Table 1.

Phytochemical Profile of Acetone Extract

X	Y	Z	A	B	C	D	E	F	G	H
AA	R	M	+	+	+	+	+	+	+	+
AA	T	M	+	+++	-	+	+	+	+	+
BB	R	M	+	+++	+	+	+	+	-	-
BB	U	M	+	+++	+	+	+	+	-	-
CC	R	S	+	+++	-	+	+	+	-	-
DD	R	S	+	+++	-	+	+	+	-	-
EE	P	M	+	+++	-	+	+	+	+	+

Description:

X: Species	Y: Plant tissue
R: Leaves	Z: Method
T: Fruit	M: Maceration
U: Stem	S: Soxhletation
P: Heart	
	AA: *P.Bleo*
A: Phenolic	**BB: *G.gnemon***
B: Flavonoid	**CC: *B.macrophylla***
C: Tannin	**DD: *P.guajava***
D: Steroid	**EE : *M.paradisiaca***
E: Terpenoid	
F: Saponin	
G: Alkaloid	
H: Glycoside	

+++ strong (very clear intensity); ++ moderate; + weak; − negative.

3.2 Discussion

3.2.1 Secondary Metabolites

Based on the primary data results as per Table 3.1, the common compounds in each sample were phenolics, steroids, and terpenoids. The positive test results for phenolics, steroids, and terpenoids in all seven samples confirmed two things at once: (1) these three groups of compounds are characteristic of Indonesian tropical medicinal plants, and (2) the ability of acetone to extract them effectively under both temperature conditions. Phenolics are the most widely distributed compounds in the plant kingdom, acting as antioxidants, UV protectors, and natural antimicrobials [6]. Furthermore, the terpenoid compound group, especially triterpenoids and diterpenoids, is a semi-polar part that is very compatible with the solubility parameters of acetone ($\delta T = 20.0$ MPa½), so that it is extracted consistently in both methods [5], [7]. Meanwhile, the group of steroid compounds, especially *β-sitosterol* and *stigmasterol*, are structural components of cell membranes that are commonly found in all vascular plants.

Interestingly, the biomarker compounds of these five species are the secondary metabolite group flavonoids. The test results were very strong (+++), detected in five of the seven samples: *P. bleo* fruit (M), *G. gnemon* leaves (M), *G. gnemon* stems (M), *B. macrophylla* leaves (S), *P. guajava* leaves (S), and *M. paradisiaca* banana blossom (M). Only *P. bleo* leaves showed weak flavonoids (+), consistent with the report of Sim et al. (2010) and Zareisedehizadeh et al. (2014), who reported a much higher distribution of flavonoids in the fruit than in the leaves of *P. bleo* [10], [11]. The strong flavonoid patterns of these species are not an artifact of the methods found in either maceration or soxhletation; rather, the high flavonoid content is an intrinsic property of the species that is insensitive to differences in extraction temperature within the tested range. This can be explained by the fact that the main flavonoid aglycones (quercetin, kaempferol, myricetin) dominant in these five species are semi-polar with relatively good thermal stability up to 60 °C. [7]. However, flavonoid glycosides are also thermolabile, so the difference between maceration and Soxhletation is more visible in the specific glycoside test (Keller-Killiani) than in the total flavonoid test.

Meanwhile, other secondary metabolite groups, namely tannins, are distributed in a limited number of specific genera. Tannins were only detected positively in the leaves of *P. bleo* (R) and both organs of *G. gnemon* (R, U, leaves and stems). All Soxhlet samples (*B. macrophylla*, *P. guajava*) showed negative tannins in the $FeCl_3$ and NaCl gelatin tests, although the literature reports the presence of *gallotannins* and *ellagitannins* in these two species [12], [13]. These results are consistent with two complementary explanations: first, the high molecular weight *gallotannins* and *ellagitannins* (MW > 1000 Da) dominant in *B. macrophylla* and *P. guajava* may be partially hydrolyzed at the soxhletization temperature (56°C), resulting in fragments that do not sufficiently precipitate proteins in the gelatin test; second, the sensitivity of the qualitative tube test for condensed and hydrolyzed tannins with high MW is lower than that of the quantitative spectrophotometric test (Folin-Ciocalteu reagent, vanilla-HCl). Fu'adah (2022) confirmed that tannins in *B. macrophylla* are predominantly hydrolyzed (*gallotannins*), rather than condensed, resulting in different detection limits. For *G. gnemon* and *P. bleo*, the more thermally stable condensed tannins are well preserved under maceration conditions [14].

Alkaloids and glycosides provide strong evidence of thermolability effects. This dataset pattern is informative in the distribution of alkaloids and glycosides exclusively in macerated extracts. Alkaloids were detected in: *P. bleo* leaves (+), *P. bleo* fruit (+ weak), and *M. paradisiaca* banana blossom (+). Glycosides were detected in: *P. bleo* leaves (+), *P. bleo* fruit (+), and *M. paradisiaca* banana blossom (+). No alkaloids or glycosides were detected in Soxhletized samples (*B. macrophylla*, *P. guajava*) or macerated samples of *G. gnemon* (leaf and stem). The absence of alkaloids and glycosides in *G. gnemon* samples (macerated) despite using the cold method indicates that this is not simply a method effect, but rather a reflection of the actual content of the species: Huong et al. (2025) reported no significant alkaloids in *G. gnemon* leaves, while flavonoid glycosides were distributed very sparingly compared to their stilbenoid aglycones [15]. Meanwhile, in the Soxhlet samples, the absence of alkaloids and

glycosides is consistent with the following mechanisms: (a) volatile *phenethylamine* alkaloids, as reported by Zareisedehizadeh et al. (2014) in *P. bleo*, are degraded at 56°C; (b) *O-glycosidic* bonds undergo partial thermal hydrolysis at temperatures above 45°C [11]. This finding is the strongest empirical evidence in this study regarding the importance of selecting a target-based compound extraction method.

3.2.2 Intra-Species Variation: Organs and Ecological Functions

The most dominant difference between plant tissue organs was observed in *P. bleo*, where the fruit showed strong flavonoids (+++) while the leaves only showed (+), with significant differences also in tannins (leaves (+); fruits (−)). This pattern reflects different ecological strategies occurring in leaves that accumulate tannins as herbivore deterrents through protein precipitation mechanisms, while fruits accumulate flavonoids (especially anthocyanins) to attract seed-dispersing fauna through visually conspicuous color signals [7], [10].

The *G. gnemon* species showed high phytochemical consistency between leaves and stems. Both gave identical profiles (6 out of 8 positive groups with similar intensity). This consistency differs from *P. bleo* and indicates that the main secondary metabolites of melinjo (especially stilbenoids) are evenly distributed throughout the vegetative organs, not concentrated in certain tissues [16], [17].

M. paradisiaca banana blossom showed the most complete profile (7 out of 8 positive), including alkaloids and glycosides not found in other species (except *P. bleo*). Gadelha (2024) confirmed steroidal alkaloids and glycosylated flavonoids (*rutin, vitexin*) as the constituents of banana blossom bracts optimally extracted by cold maceration [18]. The completeness of this profile makes *M. paradisiaca* banana blossom the material with the broadest multitarget bioactivity potential of all the samples studied.

In general, the absence of detected tannins, alkaloids, and glycosides in both guava (*Psidium guajava*) and gandaria (*Bouea macrophylla*) leaves in this study presents a notable deviation from standard phytochemical profiles. This outcome can be structurally interpreted through the framework of intra-species variation, specifically examining the role of plant organs and their shifting ecological functions. Although leaves biologically serve as the primary site for the synthesis of defensive secondary metabolites [2], . the absolute chemical expression within these tissues is highly dynamic and heavily influenced by the plant's continuous interaction with the environment.

For the guava (*Psidium guajava*) leaf samples, the negative result for tannins, alkaloids, and glycosides is likely associated with the developmental stage of the leaves at the time of harvest and low environmental stress. Ecologically, the accumulation of tannins and alkaloids acts as a chemical deterrent against predators and microbial infections [20]. If the guava plants were cultivated in highly optimal conditions with minimal pest pressures, the trees might have prioritised metabolic energy toward primary growth rather than synthesizing defense mechanisms. Furthermore, if the collected samples predominantly consisted of either very young or senescent leaves, natural physiological fluctuations could cause the concentrations of these compounds to drop significantly [21] .

Similarly, the negative screening result for the gandaria (*Bouea macrophylla*) leaf samples suggests a strong influence of local microclimates and soil nutrient availability on their biosynthetic pathways. Phenolic compounds, flavonoids, and saponins in Gandaria leaves are typically synthesized in high amounts as a physiological adaptation to mitigate oxidative stress caused by intense solar radiation in tropical habitats [15]. The inability to detect these target compound groups indicates that the weather or soil conditions leading up to harvest did not trigger significant oxidative stress, resulting in secondary metabolite accumulation remaining below standard thresholds.

From a laboratory methodology standpoint, the lack of detection in both plant matrices can also be attributed to the technical limitations of qualitative screening reagents. Although the extraction protocol utilized polar solvents, which are generally efficient at isolating hydrophilic glycosides and polyphenols [2] , the actual concentration of these metabolites within the crude extract might have fallen below the limit of detection (LOD). Additionally, post-harvest handling factors, such as uncontrolled drying temperatures, may have degraded the glycoside linkages or specific alkaloid structures. This degradation prevents the compounds from reacting or forming visible precipitates with standard colorimetric reagents, ultimately yielding false-negative results [22].

3.2.3 Effectiveness of Acetone as a Solvent for Tropical Phytochemical Screening

Positive test results for several groups of secondary metabolite compounds, such as phenolics, steroids, and terpenoids, in all samples. However, the absence of detectable alkaloids and glycosides in certain samples can be attributed to two primary factors: inherent biological variations in the plant material and technical limitations during the extraction and screening processes. Biologically, the accumulation of secondary metabolites, including alkaloids, glycosides, and flavonoids, is highly dynamic. Their concentration often varies significantly across

different samples due to environmental and physiological influences, such as soil nutrient availability, climatic conditions, the plant's developmental stage at harvest, and post-harvest handling practices [20] . Methodologically, the choice of extraction solvent is critical; utilizing a solvent that does not align with the polarity of the target compounds can lead to incomplete extraction[21]. Consequently, this can yield false-negative results, where the compounds remain undetected despite being present in the plant matrix [22]. Flavonoids are strong in the majority of samples, confirming that acetone is an effective and efficient solvent for preliminary phytochemical screening of tropical medicinal plants. Acetone was selected due to its intermediate polarity, which is highly compatible with the aromatic hydroxyl groups in the chemical of the flavonoid structure of flavonoids [20]. Unlike alcohol-based solvents, acetone displays a specific capacity to denature and cleave protein-flavonoid complexes within the plant cell matrix [21] . Furthermore, acetone can inactive polyphenol oxidase, an enzyme that potentially degrades target compounds during the maceration process [22]. This efficacy is consistent with prior research proving that acetone is far more efficient in extracting polyphenols from litchi flowers than methanol, water, or ethanol [23]. The Hansen solubility parameter of acetone (δT = 20.0 MPa½), which is in the optimum range for polyphenolic and terpenoid compounds, has been empirically proven to be able to extract secondary metabolites from all tested species [5].

The identified weakness is that acetone is less than optimal for saponins, which tend to have high polarity. All saponins responded only (+), none achieved (+++). Saponins, especially those with long polar sugar chains, require solvents with higher hydrogen-bond donor capacity, such as ethanol or methanol, for optimal solubilization [6]. These limitations need to be considered in the study design if saponins are the primary target compounds.

3.2.4 Review of Bioactivity Potential Based on Phytochemical Profile

Strong flavonoids (+++) detected in *P. bleo* fruit, both organs of *G. gnemon*, *B. macrophylla* leaves, *P. guajava* leaves, and *M. paradisiaca* banana blossom provide a strong pharmacological basis for antioxidant activity. *Quercetin* and *kaempferol* are known to be dominant flavonoid groups in *P. guajava* and *B. macrophylla*, which were confirmed by Mazumder, Md Anisur Rahman, et al (2023) [20] that α-glucosidase has an IC_{50} DPPH < 5 µg/mL in pure form through hydrogen atom transfer (HAT) and metal chelation mechanisms [12]. *Rutin* compounds from banana blossoms and *vitexin* compounds from *P. bleo* increase antioxidant contributions through the electron transfer (SET) mechanism. *Gnetin C* and *oligomeric resveratrol* from *G. gnemon* have ORAC values exceeding vitamin E [16].

Alkaloids from *P. bleo* maceration, including 3,4-*dimethoxy-β-phenethylamine*, *tyramine*, and *mescaline* (Zareisedehizadeh et al., 2014), can disrupt bacterial cell membranes and be active against *Staphylococcus aureus* and *Candida albicans* [19]. Flavonoids and phenolics from *B. macrophylla* showed inhibition zones against *Bacillus cereus*, *Listeria monocytogenes*, and *Vibrio cholerae* [19]. Tannins from *P. bleo* and *G. gnemon* leaves work through membrane protein precipitation and bacterial adhesin inhibition [20]. The combination of alkaloids, Tannins and flavonoids in *P. bleo* leaves makes it a multitarget antimicrobial candidate.

Potent flavonoids from *B. macrophylla*, especially *mearsetin*, *kaempferol*, *quercetin*, and their derivatives, identified by Mazumder, Md Anisur Rahman et al (2023) α showed α-glucosidase inhibition with IC_{50} = 9.2–266 µM. Glycosides from banana blossom of *M. paradisiaca* (*rutin, quercetin-3-O-glucoside*) competitively inhibited α-glucosidase and α-amylase, slowing postprandial glucose absorption [21]. *Gnetin C* from *G. gnemon* inhibits the formation of advanced glycation end-products (AGEs) and improves insulin sensitivity [15]. *Ursolic acid* from *P. guajava* terpenoids activates the PPAR-γ pathway as an additional antidiabetic mechanism [12].

Alkaloids produced from *P. bleo* induce apoptosis of MCF-7 (IC_{50} = 15.3 µg/mL) and HeLa (IC_{50} = 22.7 µg/mL) cells through caspase-3/7 activation [22]. Terpenoids from *B. macrophylla* stems, in the form of isolated *β-sitosterol* and triterpenoids, show cytotoxicity against MCF-7, A549, and MDA-MB-231 cells (Ibrahim et al., 2021). *Gnetin C* from *G. gnemon* species induces autophagy and inhibits the proliferation of K562 leukemia cells [23]. Flavonoids quercetin and kaempferol from *P. guajava* and *B. macrophylla* induce G2/M cell cycle arrest through CDK1/cyclin B inhibition [24].

4. CONCLUSION

The conclusion of this study presents the first primary qualitative phytochemical profiles of nine plant materials derived from five Indonesian ethnomedicinal species using acetone solvent exclusively through two different methods within an integrated comparative methodological framework.

Acetone proved to be a versatile solvent that effectively extracted phenolics, steroids, and terpenoids from all samples (100% positive detection), confirming its suitability for preliminary phytochemical screening across polarities of tropical medicinal plants.

Strong flavonoids (+++) were a consistent bioactive marker in five of the seven samples (*P. bleo* fruit, both organs of *G. gnemon*, *B. macrophylla* leaves, *P. guajava* leaves, *M. paradisiaca* heart), independent of the extraction method, indicating it is an intrinsic property of the species. Alkaloids and glycosides were detected exclusively in macerated extracts (*P. bleo* leaves and fruit; *M. paradisiaca* heart), consistent with their thermolabile nature at Soxhlet extraction temperature (56°C). This is the strongest empirical evidence that the choice of extraction method should be tailored to the target compound: maceration for alkaloids/glycosides, Soxhletation for terpenoids/steroids.

Tannins showed a species-specific distribution detected only in *P. bleo* leaves and *G. gnemon* (leaves and stems), consistent with the absence of significant condensed tannins in *B. macrophylla* and *P. guajava* under qualitative test tube conditions, although hydrolyzable *gallotannins* were documented quantitatively.

Significant intra-species variation was observed in *P. bleo* (fruit (flavonoid +++), (tannin −); leaf (flavonoid +) and (tannin +). This reflects an organ-specific metabolite allocation strategy according to ecological function. *G. gnemon* showed high-profile consistency between leaves and stems.

Further research recommendations include (a) compound quantification via HPLC-DAD and LC-MS/MS; (b) validated in vitro bioactivity assays (DPPH, MIC, MTT assay); (c) bioactivity-guided fractionation; (d) OECD 423 acute toxicity testing before formulation development.

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