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Phytochemical Screening and In Vitro Angiotensin-Converting Enzyme (ACE) Inhibitory Activity of Selected Indonesian Medicinal Plants

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Abstract. *Hypertension remains a major global health challenge, and angiotensin-converting enzyme (ACE) is an important therapeutic target for blood pressure control. Indonesian medicinal plants have long been used traditionally to manage hypertension; however, comparative evaluations of their ACE inhibitory activity remain limited. This study investigated the extraction yield, phytochemical composition, and in vitro ACE inhibitory activity of ten medicinal plant parts traditionally used for hypertension treatment. Plant materials were extracted by maceration with 96% ethanol, followed by phytochemical screening and ACE inhibition testing using the ACE Kit-WST assay at 100 µg/mL. Extraction yields ranged from 9.82% to 45.30%, with Averrhoa bilimbi fruit producing the highest yield. Phytochemical screening revealed the presence of alkaloids and flavonoids in all extracts, whereas saponins, quinones, tannins, and steroids/triterpenoids showed variable distributions. ACE inhibitory activity ranged from 62.61 ± 1.21% to 96.92 ± 3.05%. The highest activities were observed for Morinda citrifolia fruit (96.92 ± 3.05%), Piper betle leaf (96.87 ± 2.05%), and Dracaena angustifolia leaf (95.09 ± 1.27%), with no significant difference from captopril (99.09 ± 0.07%; $p > 0.05$). These findings support the traditional use of selected Indonesian medicinal plants for hypertension management and identify M. citrifolia, P. betle, and D. angustifolia as promising sources of natural ACE inhibitors for further investigation. The scientific validation of these medicinal plant resources may also support the sustainable utilization of biodiversity and the preservation of traditional ethnomedicinal knowledge.*

Keywords: *ACE Inhibition; Antihypertensive; Medicinal Plants; Phytochemical Screening; Piper Betle*

1. Introduction

Blood pressure regulation is largely mediated by the renin–angiotensin–aldosterone system (RAAS), in which angiotensin-converting enzyme (ACE) plays a central regulatory role. ACE catalyzes the conversion of angiotensin I into angiotensin II, a potent vasoconstrictor that promotes vascular constriction and increases systemic blood pressure (1). Owing to its pivotal role in cardiovascular homeostasis, ACE has become one of the most extensively validated therapeutic targets for the treatment of hypertension. Consequently, ACE inhibitors have been a cornerstone of antihypertensive therapy for decades, and the

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discovery of novel ACE inhibitory compounds remains an active area of pharmacological research (2).

The clinical significance of ACE is underscored by the global burden of hypertension. Approximately one in three adults worldwide are affected by this condition, and the number of cases has nearly doubled over the past three decades, increasing from an estimated 650 million in 1990 to 1.3 billion in 2019. Alarmingly, nearly four out of five individuals with hypertension do not receive adequate treatment, reflecting not only limited access to healthcare but also challenges related to medication cost, adverse effects, and long-term treatment adherence. As one of the leading modifiable risk factors for cardiovascular disease, hypertension is strongly associated with stroke, heart failure, and coronary artery disease, highlighting the urgent need for more effective and accessible therapeutic strategies (3, 4).

Although synthetic ACE inhibitors such as captopril are clinically effective, their use is associated with several limitations. Treatment is frequently accompanied by adverse effects including persistent dry cough, hypotension, hyperkalemia, and impaired renal function. In rare cases, bradykinin accumulation may trigger angioedema, a potentially life-threatening swelling that can compromise the airway. These tolerability concerns, together with issues related to cost and accessibility in many regions of the world, have sustained ongoing interest in the discovery and development of alternative ACE inhibitory agents (5, 6).

Plant-derived compounds have emerged as a promising source of novel ACE inhibitors. A structurally diverse range of phytochemicals, including peptides, phenolics, flavonoids, terpenoids, and alkaloids, has been reported to inhibit ACE through mechanisms that resemble those of synthetic inhibitors, while often exhibiting favorable safety profiles in experimental studies. Nevertheless, considerable variability exists within this field. Even within a single class of compounds, such as flavonoids, inhibitory potency may differ substantially depending on subtle structural characteristics. Furthermore, multiple mechanisms of action have been proposed, with some compounds acting as competitive inhibitors at the enzyme's catalytic site, whereas others may interact with regions associated with the catalytic zinc-binding domain. This mechanistic complexity, together with the recognized gap between in vitro activity and demonstrated in vivo or clinical efficacy, highlights that the ACE inhibitory activity of plant extracts should be regarded as a basis for further pharmacological investigation rather than direct evidence of therapeutic effectiveness (7, 8).

Indonesia's rich plant biodiversity represents a valuable source of medicinal plants for the discovery of novel ACE inhibitors. Numerous species are traditionally used to manage hypertension based on knowledge passed down through generations. Among them, *Morinda citrifolia*, *Averrhoa bilimbi*, and *Annona muricata* are widely used, while *Piper betle* is notable for its extensive ethnomedicinal applications, including hypertension (9–11). Despite their longstanding traditional use, few studies have systematically compared these plants using standardized laboratory approaches that relate phytochemical composition to measurable biological activity. As a result, it remains unclear which species possess sufficient ACE inhibitory potential to justify further pharmacological investigation. In addition to their pharmacological relevance, medicinal plants represent important biological resources whose sustainable utilization may contribute to biodiversity conservation and the preservation of traditional knowledge. Scientific validation of ethnomedicinal plants can support evidence-based utilization of local biological resources while promoting their sustainable management for future healthcare and environmental sustainability.

2. Methods

2.1. Plant Material

Plant samples used in this study were obtained from the Indonesian Spice and Medicinal Crops Research Institute (BALITTRO), Bogor. Specimens were subsequently determined at the Center for Plant Conservation and Botanic Gardens – Indonesian Institute of Sciences. The nine plant species used in this study were *Annona muricata* L., *Syzygium polyanthum* Wight., *Sonchus arvensis* L., *Piper betle* L., *Morinda citrifolia* L., *Averrhoa bilimbi* L., *Cucumis sativus* L., *Ziziphus mauritiana* Lam., and *Dracaena angustifolia* Roxb.

2.2. Extraction

Dried plant materials (simplicia) were sorted to remove foreign matter, ground into powder, and sieved through a 20-mesh sieve. A 10 g portion of each powdered sample was extracted by maceration using redistilled 96% ethanol at a solvent-to-sample ratio of 10:1 (v/w). Each maceration cycle was carried out for 24 h with occasional stirring, followed by filtration and replacement of fresh solvent; this cycle was repeated five times until the filtrate became nearly colorless. Combined filtrates were concentrated using a rotary vacuum evaporator (50 °C, 70 rpm) to obtain a viscous extract, which was further dried in an oven (50 °C) to remove residual solvent. The dried extract was weighed, and extraction yield was calculated relative to the initial dry weight of the simplicia.

2.3. Qualitative Phytochemical Screening

Qualitative screening for alkaloids, flavonoids, saponins, tannins, quinones, and steroids/triterpenoids was performed following standard color and precipitation reaction methods (12, 13), as summarized below.

Alkaloids. Powdered simplicia (2 g) was moistened with 30% ammonia solution and extracted with chloroform; the organic phase was collected as solution A. An aliquot of solution A was further partitioned with diluted HCl (1:10) to obtain solution B. Solution A was treated with Dragendorff reagent, while solution B was tested with both Dragendorff and Mayer reagents. Formation of red-orange coloration, a brick-red precipitate, or a white precipitate indicated a positive result.

Flavonoids. Powdered simplicia (2 g) was boiled in distilled water for 5 min and filtered. The filtrate was mixed with magnesium powder, concentrated HCl, and amyl alcohol, followed by vigorous shaking (Shinoda test). Yellow, orange, or reddish coloration in the amyl alcohol layer indicated a positive result.

Saponins. An aliquot of the aqueous filtrate obtained from the flavonoid test was shaken vertically for approximately 10 s and allowed to stand for 10 min. Formation of stable froth persisting after addition of 1% HCl indicated a positive result.

Tannins. Powdered simplicia (2 g) was boiled in distilled water for 15 min and filtered. The filtrate was treated separately with 1% $FeCl_3$ and 1% gelatin solution; bluish-green/dark-blue coloration or a white precipitate indicated a positive result. Additionally, the filtrate was reacted with Stiasny reagent and heated in a water bath: a pink precipitate indicated catechol tannins, while an ink-blue coloration following addition of sodium acetate and 1% $FeCl_3$ indicated gallic tannins.

Quinones. An aliquot of the flavonoid-test filtrate was treated with 1 N NaOH solution; an intense red coloration indicated a positive result.

Steroids/Triterpenoids. Powdered simplicia (1 g) was macerated with ether for 2 h and filtered. The filtrate was evaporated to dryness, and the residue was treated with acetic anhydride and concentrated sulfuric acid (Liebermann–Burchard reagent). Green coloration indicated the presence of steroids, whereas red coloration indicated triterpenoids.

2.4. In Vitro ACE Inhibitory Assay

The ACE inhibitory assay was performed in 96-well microplates using the ACE Kit-WST (Dojindo Laboratories), based on the colorimetric method developed by Lam et al. (14, 15), in which 3-hydroxybutyryl-glycyl-glycyl-glycine (3HB-GGG) served as the substrate. Each sample well contained 20 µL of test solution, 20 µL of substrate buffer, and 20 µL of enzyme solution. For Blank 1 (enzyme control), the test solution was replaced with 20 µL of distilled water, whereas for Blank 2 (reagent blank), the enzyme solution was replaced with distilled water. The reaction mixtures were incubated at 37°C for 60 min, followed by the addition of 200 µL of indicator solution and further incubation at room temperature for 10 min. Absorbance was measured at 450 nm using a microplate reader. To correct for background interference from colored extracts or fractions, sample blanks consisting of 20 µL of test solution and 240 µL of distilled water were prepared, and their absorbance values were used for correction. All measurements were performed in triplicate, and the percentage of ACE inhibition was calculated using the following equation:

$$\text{ACE inhibition (\%)} = \frac{A_{\text{Blank 1}} - A_{\text{Sample}}}{A_{\text{Blank 1}} - A_{\text{Blank 2}}} \times 100$$

Where $A_{\text{Blank 1}}$ is the absorbance of the positive control (without inhibitor), $A_{\text{Blank 2}}$ is the absorbance of the reagent blank, and A_{Sample} is the absorbance of the tested sample.

Captopril was used as the positive control and was tested at the same concentration as the plant extracts (100 µg/mL). The assay was performed at a single concentration as a preliminary screening approach to identify plant extracts with potential ACE inhibitory activity. Therefore, the observed inhibition percentages reflect ACE inhibition only at the tested concentration, and the determination of inhibitory potency through concentration–response analysis and IC_{50} calculation was beyond the scope of the present study.

2.5. Statistical Analysis

Data are presented as mean ± SD of three independent measurements. Statistical analyses were performed using IBM SPSS Statistics version 26. Normality and homogeneity of variance were evaluated using the Shapiro–Wilk and Levene's tests, respectively. Group differences were analyzed by Welch ANOVA followed by Games–Howell multiple-comparison testing. Statistical significance was set at $p < 0.05$.

3. Results and Discussion

3.1. Extraction Yield

The highest extraction yield was obtained from *A. bilimbi* fruit (45.30%), followed by *M. citrifolia* leaf (22.17%) and *A. muricata* leaf (21.77%) (Table 1). The high yields obtained may be attributed to the ability of 96% ethanol to extract a wide range of polar and semi-polar bioactive compounds, including flavonoids, phenolics, and organic acids (16). Ethanol is also recognized for its broad extraction spectrum, ranging from polar to non-polar constituents

(17), and is widely used as a biodegradable, readily available, and relatively low-toxicity bio-solvent compared with other organic solvents (18).

The extraction yield of *A. bilimbi* fruit obtained in the present study was higher than the previously reported yield of 24.18% (19), but considerably lower than the 77% yield reported using a similar ethanol maceration method (20). Variations in extraction yield may be attributed to differences in plant material characteristics, solvent-to-solid ratio, extraction duration, drying conditions, and extraction temperature. These factors can significantly affect the solubility and diffusion of phytochemical constituents into the extraction solvent, thereby influencing extraction efficiency (21).

Table 1. Extraction yield of 96% ethanol extracts from nine plant species (from 10 g of powdered simplicia).

No.	Plant Species	Plant Part	Extract Mass (g)	Yield (%)
1	*Annona muricata*	Leaf	2.17	21.77
2	*Averrhoa bilimbi*	Fruit	4.53	45.3
3	*Cucumis sativus*	Fruit	0.98	9.82
4	*Dracaena angustifolia*	Leaf	1.79	17.96
5	*Morinda citrifolia*	Leaf	2.21	22.17
6	*Morinda citrifolia*	Fruit	1.05	10.5
7	*Piper betle*	Leaf	1.28	12.82
8	*Sonchus arvensis*	Leaf	1.62	16.24
9	*Syzygium polyanthum*	Leaf	1.65	16.53
10	*Ziziphus mauritiana*	Leaf	2.06	20.67

M. citrifolia is known to contain a diverse array of secondary metabolites, including phenolics, flavonoids, anthraquinones, anthrones, iridoids, coumarins, terpenes, carotenoids, and essential oils, which contribute to its pharmacological properties (22). Previous studies have reported considerable variation in the extraction yield of *M. citrifolia* fruit, ranging from 6.87% (23) to 15.78% (24). These differences may be attributed to factors such as geographical origin, environmental growing conditions, and fruit maturity at harvest. In addition, *M. citrifolia* fruit has been reported to reduce hypertension in experimental animal models through ACE inhibitory and antioxidant mechanisms, with phenolic constituents such as scopoletin and rutin proposed as key contributors to these effects (24).

3.2. Phytochemical Screening

Phytochemical screening revealed the presence of several major classes of secondary metabolites in the tested plant extracts (Table 2). Alkaloids and flavonoids were detected in all samples, indicating that these compounds are widely distributed among the selected medicinal plants. Saponins were present in most extracts, except *A. bilimbi* fruit, whereas quinones were absent in *A. muricata* and *C. sativus* but detected in the remaining species. Tannins were identified only in *A. muricata*, *P. betle*, *S. arvensis*, *S. polyanthum*, and *Z. mauritiana*. Positive reactions for steroids/triterpenoids were also observed in several samples.

Table 2. Phytochemical screening of selected medicinal plant extracts

Plant species	Part used	Alkaloids	Flavonoids	Saponins	Quinones	Tannins	Steroids/ Triterpenoids
Annona muricata	Leaf	+	+	+	–	+	+/–
Averrhoa bilimbi	Fruit	+	+	–	+	–	–/+
Cucumis sativus	Fruit	+	+	+	–	–	–/+
Dracaena angustifolia	Leaf	+	+	+	+	–	+/–
Morinda citrifolia	Leaf	+	+	+	+	–	+/+
Morinda citrifolia	Fruit	+	+	+	+	–	+/–
Piper betle	Leaf	+	+	+	+	+	+/+
Sonchus arvensis	Leaf	+	+	+	+	+	+/+
Syzygium polyanthum	Leaf	+	+	+	+	+	+/+
Ziziphus mauritiana	Leaf	+	+	+	+	+	+/–

Note: (+) detected; (–) not detected

Among the tested plants, *P. betle*, *S. arvensis*, *S. polyanthum*, and *Z. mauritiana* exhibited the most diverse phytochemical profiles, containing alkaloids, flavonoids, saponins, quinones, and tannins simultaneously. The broad distribution of flavonoids and alkaloids is particularly noteworthy because both classes have been associated with antihypertensive effects through antioxidant activity, enhancement of endothelial nitric oxide production, and inhibition of angiotensin-converting enzyme (ACE) (25–27). In addition, saponins, tannins, and steroid/triterpenoid compounds may contribute complementary cardiovascular benefits through antioxidant, vasoprotective, and anti-inflammatory mechanisms, which are known to play important roles in the prevention and management of cardiovascular disorders (28). Overall, the occurrence of multiple bioactive metabolite classes provides a phytochemical basis for the traditional use of these plants in hypertension management and supports their further evaluation as potential natural ACE inhibitors.

3.3. In Vitro ACE Inhibitory Activity

At a concentration of 100 µg/mL, ACE inhibitory activity ranged from 62.61 ± 1.21% (*S. polyanthum*) to 96.92 ± 3.05% (*M. citrifolia* fruit), compared with 99.09 ± 0.07% for captopril (Figure 1). Games–Howell post-hoc analysis showed that the ACE inhibitory activities of *M. citrifolia* fruit, *P. betle* leaf, and *D. angustifolia* leaf were not significantly different from that of captopril ($p > 0.05$) at 100 µg/mL under the assay conditions. However, because the evaluation was performed at a single concentration, these findings should be interpreted as preliminary screening results and do not permit direct comparison of inhibitory potency.

The high activity of these extracts may be related to their phytochemical composition. Qualitative phytochemical screening revealed the presence of flavonoids and alkaloids in all three species, together with saponins and quinones. Flavonoids have been widely reported as natural antihypertensive compounds due to their ability to inhibit ACE, improve endothelial function, and reduce oxidative stress, while synergistic interactions among multiple metabolite classes may further contribute to their biological activities (29, 30). In addition, *M. citrifolia* fruit has been reported to contain various bioactive constituents, including iridoids, scopoletin, phenolic acids, and flavonoids, whereas the biological activity of *P. betle* has been associated with its abundant phenolic and flavonoid content (22, 31). However, because the phytochemical screening performed in this study was qualitative, the detected metabolite classes cannot be directly attributed to the observed ACE inhibitory activity. The present

findings indicate only a possible association between phytochemical composition and biological activity.

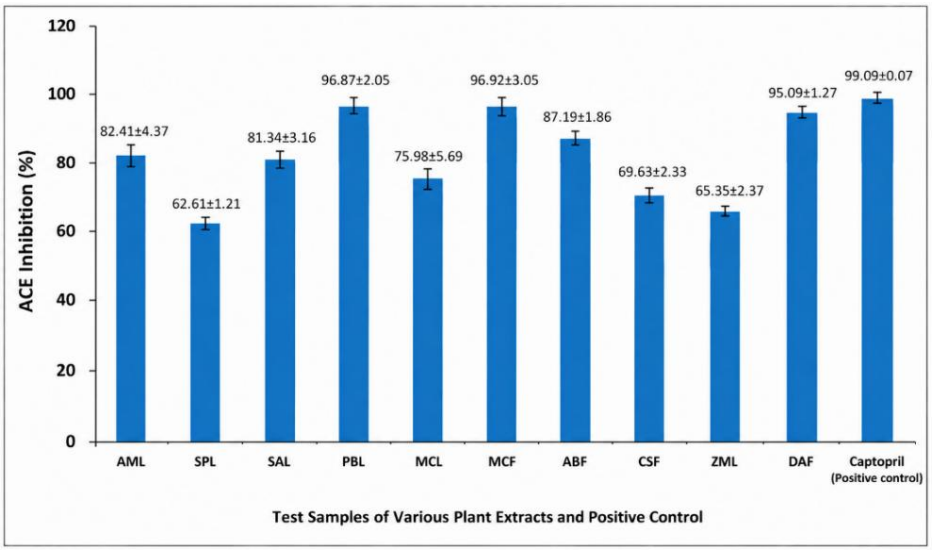

Figure 1. *In vitro* angiotensin-converting enzyme (ACE) inhibitory activity of ethanol extracts from selected medicinal plants and captopril (positive control) at a concentration of 100 µg/mL. Data are presented as mean ± SD (n = 3). Sample codes: AML = *Annona muricata* leaf; ABF = *Averrhoa bilimbi* fruit; CSF = *Cucumis sativus* fruit; DAF = *Dracaena angustifolia* leaf; MCL = *Morinda citrifolia* leaf; MCF = *Morinda citrifolia* fruit; PBL = *Piper betle* leaf; SAL = *Sonchus arvensis* leaf; SPL = *Syzygium polyanthum* leaf; and ZML = *Ziziphus mauritiana* leaf. Error bars represent standard deviation.

In contrast, *S. polyanthum*, *S. arvensis*, and *Z. mauritiana* exhibited lower inhibitory activity despite containing similar phytochemical classes, suggesting that ACE inhibition may depend not only on the presence of particular metabolites but also on their concentration, relative abundance, and structural characteristics. Therefore, quantitative phytochemical analysis, bioactivity-guided fractionation, and compound isolation studies are required to identify the specific constituents responsible for the observed ACE inhibitory activity and to clarify their mechanisms of action. Nevertheless, the present findings provide pharmacological support for the traditional use of these plants in hypertension management. Since the assay was conducted in vitro, further studies are required to determine whether the observed ACE-inhibitory activity translates into antihypertensive effects in vivo.

Conclusions

This study demonstrated that selected Indonesian medicinal plants contain diverse bioactive secondary metabolites that may contribute to their antihypertensive potential. Among the ten medicinal plant parts evaluated, *M. citrifolia* fruit, *P. betle* leaf, and *D. angustifolia* leaf exhibited the highest ACE inhibitory activities at 100 µg/mL and showed no significant difference compared with captopril under the assay conditions. These findings provide pharmacological support for the traditional use of these plants in hypertension management and highlight their potential as sources of natural ACE inhibitors. However, since ACE inhibition was evaluated at a single concentration, further concentration–response studies, including IC_{50} determination, are necessary to establish their relative inhibitory potency. Additionally, further investigations involving bioactive compound isolation,

mechanism-of-action studies, and in vivo validation are warranted to confirm their therapeutic potential and identify the constituents responsible for the observed activity.

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Conflicts of Interest

The authors declare no conflict of interest.

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