ORIGINAL ARTICLE

# In-vitro ACE-1 Inhibitory Activity of *Coleus scutellarioides* Benth (Mayana) Crude Ethanolic Dehydrated Leaf Extract

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## ABSTRACT

**Objectives.** Herbal plants can be an alternative source of therapy especially against hypertension, which is a prevalent burden in the Philippines. This study investigates the phytochemical composition and angiotensin-converting enzyme 1 (ACE-1) inhibitory activity of Mayana *(Coleus scutellarioides* Benth.*)* leaf extract, a plant ethnobotanically reported to be used for its potential antihypertensive properties and yet still lacking in in-vitro investigations.

**Methods.** Employing a laboratory experimental research design and standard procedures for phytochemical screening and ACE-1 inhibitory assay, the study compares a crude ethanolic dehydrated leaf extract of Mayana with the positive control, Captopril.

**Results.** Phytochemical screening revealed the presence of flavonoids and phenolic compounds. ACE-1 inhibitory activity of Mayana at 10 µg/mL, 25 µg/mL, 50µg/mL, 100 µg/mL, 500 µg/mL, 1000 µg/mL were 0.00% ± 0.0000, 12.40% ± 2.7094, 18.76% ± 0.7232, 27.31% ± 2.2159, 30.44% ± 1.6022, 40.12% ± 2.4385, respectively. Mayana exhibited an $IC_{50}$ value of 55.9154 µg/mL compared to Captopril which was 7.7232 µg/mL, indicating potency disparities.

**Conclusion.** Mayana has been shown to contain flavonoids and phenolic compounds that exhibit preliminary antihypertensive potential through the inhibition of ACE-1. However, the bioactivity of Mayana is lower when compared with a positive control. As such, more research is needed. Despite that, this research contributes to our understanding of Mayana as a medicinal plant and its potential contribution to complementary and alternative healthcare, with implications for patient care, community awareness, farmer livelihood, education, and future research.

*Keywords: ACE-1 inhibition, antihypertensive, Coleus, Mayana, phytochemicals, Davao City, Philippines*

## INTRODUCTION

Hypertension is still considered a national burden in the Philippines. Despite the available medications for this disease, many find that these products have more side effects than herbal counterparts thus resulting to preference of these herbal products.[1] Furthermore, the economic impact of hypertension among Filipinos is projected to double within the next thirty years.[2] As such, herbal products and alternative or traditional medicine may provide cheaper therapies.

Epidemiological data underscore the urgency of improving hypertension management.[3] In a hospital-based population in the Philippines, hypertension is the highest cardiovascular disease among surveyed respondents.[4] Additionally, a study has shown that blood pressure control is still low despite having high treatment and compliance.[5] In older Filipinos, hypertension was found to be prevalent

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in 69.1 percent of the respondents with more than half of them being unaware of their hypertensive condition.[6] Additionally, availability of medications was low in the Philippines despite having tax exemptions for original brands and lowest-priced generics[7] which prompts for scientific inquiry to lean into natural products as alternatives.

Though there is extensive research on many indigenous plants in the country for their antihypertensive effect, Mayana (*Coleus scutellarioides* Benth.) remains underexplored. Mayana is mainly an ornamental plant in the Philippines, but it has been also used traditionally for various medicinal purposes. In fact, it is considered to be a medicinal plant by government authorities. Mayana has been documented to be ethnobotanically used for hypertension.[8,9] Furthermore, the use of Mayana can also be traced to Ayurvedic practices.[10] Despite that, in-vitro studies surrounding Mayana for hypertension is still limited which presents a knowledge and evidence gap.

The use of Mayana for hypertension is not far-fetched. The *Coleus* genus, which Mayana is a member of, have been found to contain rich phytochemicals that can be used in many diseases including cardiac problems, cough, some skin conditions like topical infections and eczema, and more.[11] Furthermore, a systematic review has shown that plants from the *Lamiaceae* family, which includes the *Coleus* genus, not only have antioxidant activity but also antihyperlipidemic, vasorelaxant, antithrombotic, and diuretic activities, which contribute to the management and treatment of cardiovascular diseases like hypertension.[12] The most commonly investigated plant of this genus, *Coleus forskohlii,* has been found to have antihypertensive activity and can be used as a medicinal plant for hypertension.[13]

Despite existing research regarding this genus, investigations and experiments regarding the antihypertensive potential of Mayana is scant, especially in the Philippines. To this end, the objective of this research is to determine the phytochemicals classes present in Mayana crude extract and investigate the angiotensin converting enzyme type 1 (ACE-1) inhibitory effect of Mayana crude extract. Through conducting phytochemical screening and ACE-1 inhibitory assays, the study intends to gather data that would support the use of Mayana in alleviating hypertension. With these objectives, the paper aims to contribute to the current knowledge of a potential herbal remedy and the possible application of Mayana to conventional health care for hypertension in the Philippines and other countries.

## MATERIALS AND METHODS

This research employed an experimental research design to attempt in answering the questions and objectives of this study. The study procedures, from sample collection to the in-vitro assay, were conducted from February to March 2025. The study team followed the following procedures for the experimental design:

### Sample Collection and Identification

The plant was sourced from Herbanext Laboratories, Inc. at Negros Occidental, Philippines. The procured material consisted of Mayana leaves cultivated under full sunlight in an agricultural clearing maintained by the said establishment in Tabunan, Bago, Negros Occidental, Philippines. The plant material obtained was in the form of dried, powdered leaves. To ensure the authenticity of the sample, a certificate of plant identification was provided upon procurement. The powdered plant material was then subjected to organoleptic evaluation.

### Preparation of Plant Extract

One kilo of the powdered leaves of Mayana was used and was macerated with one liter of 95% ethanol. The mixture was stoppered and was placed in a cool dry place for two days with frequent shaking to extract all the dissolvable matter.[14] The menstruum containing the phytochemicals were separated from the marc using gravity filtration using Whatman filter paper number 1. To reduce the bulk volume, the researchers employed a rotary evaporator at a temperature of 40°C and a pressure of 60 torr. The plant extract was next subjected to dehydration using a dehydrator at a temperature of 50°C for six hours. The extracts must be in the form of a solid mass to ensure accuracy in concentration calculation, ensure robust conduct of the in-vitro assay, and convenience in material handling.

Once the dehydration process is complete, the dehydrated product is sealed in airtight containers to prevent moisture reabsorption. The total weight of the dehydrated mass was weighed so that the percent yield is calculated from this, along with the weight of the plant material used for extraction. A portion of the dehydrated material was separated from the rest and was used for ACE-1 inhibitory assay while the remaining material was redissolved in water to make a stock solution that was used for phytochemical screening.

### Phytochemical Screening

Unless otherwise stated, many of the procedures used for the phytochemical screening of Mayana are based on standard procedures as described by Shah and Seth.[14] These tests include qualitative tests for the presence of alkaloids, tannins, saponins, cardiac glycosides, and steroids. Further literature has been used to ensure clear and elaborate procedures of these tests.

#### *Alkaloids*

Many of the tests for alkaloids are done in neutral to slightly acidic environments.[14] The qualitative phytochemical test for alkaloids followed the procedure described by Shaikh and Patil.[15] One milliliter of the reconstituted extract was mixed with two milliliters of 0.1N hydrochloric acid. Then, two drops of Mayer's reagent were introduced along the sides of the test tube. A creamy white precipitate indicates the presence of alkaloids.[15]

### *Flavonoids*

The detection of flavonoids was carried out using the lead acetate test.[15] In this test, one milliliter of the plant extract is exposed to a few drops of 10% lead acetate solution. Flavonoids are considered present when a yellow precipitate is formed.[15]

### *Phenolic compounds*

Ferric chloride test was employed to identify qualitatively the presence of phenolic compounds in the plant sample.[15] Here, one milliliter of the aqueous plant extract is mixed with a few drops of ferric chloride solution having five percent concentration. A dark green or bluish black color solution indicates the presence of the said phytochemical.[15]

### *Terpenoids*

The test for the presence of terpenoids will follow the procedure described by Shaikh and Patil.[15] In a test tube, five milliliters of the plant extract were mixed with two milliliters of chloroform and evaporated to dryness using a water bath. Afterwards, the residue was introduced with three milliliters of concentrated sulfuric acid and boiled in a water bath. A gray-colored solution indicates terpenoids are present.[15]

### *Tannins*

Tannins are generally identified using the gelatin test because such phytochemicals can precipitate proteins in the gelatin.[14] One milliliter of the reconstituted extract was dissolved in five milliliters of distilled water and filtered. The filtrate was then introduced to a solution of one percent gelatin with a few drops of 10% sodium chloride. A white precipitate indicates the presence of tannins.[15]

### *Cardiac glycosides*

Keller-Kiliani's test was employed to determine the presence of cardiac glycosides, specifically digitoxose moiety.[14] Two milliliters of the plant extract were mixed with equal volume of distilled water and 0.5 mL of strong lead acetate solution. The mixture was shaken and filtered. The filtrate was extracted with equal volume of chloroform. After this, the extract was evaporated to dryness and the residue was dissolved in three milliliters of glacial acetic acid. After mixing, a few drops of ferric chloride solution were added. This mixture was then transferred to a test tube that already contained two milliliters of concentrated sulfuric acid. The presence of cardiac glycoside is observed when a reddish-brown layer is formed which then turns to bluish green after standing.[14]

### *Saponins*

Foam test was performed to identify if the plant extract contains saponins.[14] One gram of the lyophilized extract was dissolved in 20 milliliters of water. The mixture was shaken for a few minutes. The formation of froth that persists for one to two minutes is indicative of saponins present.[14]

### *Sterols*

Sterols or steroid-containing plants are generally tested using the Liebermann-Burchard test.[14] In this test, the alcoholic plant extract placed in a test tube was evaporated to dryness in a water bath. Afterwards, one milliliter of chloroform was introduced to extract the phytochemical. After that, two drops of acetic anhydride are added followed by a few drops of concentrated sulfuric acid from the side of the test tube wall. The presence of sterols is indicated by a violet to blue-colored ring at the junction point between the chloroform and aqueous liquids.[14]

## ACE-1 Inhibitory Assay

The angiotensin converting enzyme (ACE) type 1 inhibitory activity of the plant was determined in-vitro using the colorimetric ACE Kit – WST that was purchased from WVN Research and Laboratory Supplies, Davao City. The study team followed the protocols and procedures that were included in the procurement of the kit.

In preparing a 10,000 µg/mL stock solution of dehydrated extract using dimethyl sulfoxide (DMSO) as solvent, the researchers accurately weighed 0.1 g of dehydrated extract. After that, 10 ml of the solvent was used to make a solution of the dehydrated mass. The solution was sealed with a tight-fitting lid. The solution was vigorously shaken until the extract was completely dissolved in the solvent. Once mixed, container was labelled appropriately. The same procedure was used to make a stock solution of the positive control, captopril. Then, working solutions with different concentrations (10, 25, 50, 100, 500, and 1000 µg/mL) were prepared from the stock solutions.

To perform the procedure, 20 µL of the working solutions were taken to each designated sample well, the sample blank well, and positive control inhibitor well. 20 µL of the substrate buffer was introduced into each sample well. Then, 20 µL of the enzyme working solution was added to each sample well. The plate was gently tapped to ensure thorough mixing of all solutions. Since the enzymatic reaction begins immediately upon adding the enzyme working solution, a multi-channel pipette was used to minimize time differences between wells. The plate was incubated at 37°C for 1 hour to allow the enzymatic reaction to proceed. Following this incubation, 200 µL of the indicator working solution was added to each sample well and the plate was incubated at room temperature for 10 minutes.

The absorbance was measured at 450nm using a microplate reader. The procedure was repeated in three independent trials, and the results were reported as mean and standard deviation. The same procedure was applied to the positive control, captopril, and a blank vehicle control. Percent inhibition was calculated using the formula: % inhibition = $((A_o-A_s) / A_o) \times 100$ where $A_o$ is the absorbance of the blank (without inhibition) and $A_s$ is the absorbance of the plant sample or the positive control.

In determining the $IC_{50}$ (half-maximal inhibitory concentration), the study team plotted the concentration and the percent inhibition from the three trials of the plant sample and the positive control in Quest Graph™ $IC_{50}$ Calculator.[16] The $IC_{50}$ was calculated using a three-parameter logistic model based on Hill's equation and the % inhibition data were also normalized. The dose-response relationship was visualized using GraphPad Version 10.3.1. The mean percent inhibition and $IC_{50}$ values of the crude ethanolic dehydrated leaf extract were then descriptively compared with those of the positive control.

### Ethics Statement

This study was submitted to the Research Ethics Committee of the University of the Immaculate Conception and was given a certificate of exemption on February 26, 2024 with the protocol code UG-0059-02-24.

## RESULTS

The powdered Mayana material was dark olive green to brownish, with flecks of lighter tan or yellow from leaf veins and stems that may not have been ground completely. Except for the flecks of leaf veins and stems, the rest of the powder material was generally fine in particle size. The powder had a mildly aromatic, herbaceous scent resembling that of a green tea. The percent yield of the crude ethanolic dehydrated leaf extract of Mayana was found to be 0.702%. The consistency of the dehydrated mass was slightly sticky and had a dark brown color. Table 1 shows the qualitative phytochemical screening conducted as well as the results of these tests. From this table, only flavonoids and phenolic compounds were found to be present in the crude extract. Other phytochemical classes like alkaloids, terpenoids, and glycosides were not present.

The $IC_{50}$ values of the ACE inhibitory action of the crude extract and captopril can be found from Table 2. The $IC_{50}$ of Mayana and captopril was found to be 55.9154 µg/mL and 7.7232 µg/mL, respectively. Figure 1 shows the relationship between the concentration and percent inhibition of Mayana and captopril.

In terms of percent enzyme inhibition, captopril showed a higher inhibitory activity than the crude extract. The percent inhibition of captopril at concentrations of 10, 25, 50, 100, 500, and 1000 µg/mL were 52.33 ± 0.59%, 69.61 ± 2.21%, 78.60 ± 0.64%, 82.28 ± 0.65%, 87.61 ± 0.93%, and 99.05 ± 0.4%, respectively. All the while, the crude ethanolic dehydrated leaf extract of Mayana showed a percent inhibition of 0.00 ± 0.00%, 12.40 ± 2.71%, 18.76 ± 0.72%, 27.31 ± 2.22%, 30.44 ± 1.6%, and 40.12 ± 2.44%, respectively from the same concentrations used.

## DISCUSSION

This study investigated the ACE inhibitory activity of Mayana. Although there is ethnopharmacological evidence showing the plant can be used in certain diseases of the circulatory system like hypertension,[8] the current study provides preliminary experimental data potentially legitimizing ethnic claims of the hypotensive use of Mayana.

The current study shows the percent yield of Mayana when extracted with ethanol and dried using a dehydrator. A dehydrator was used in lieu of a lyophilizer because of maintenance downtime of the currently available instrument and time constraints. The percent yield may be a consequence of the use of a dehydrator. Previous studies may corroborate to this claim showing freeze-drying as a superior method for drying and reconstitution.[17,18]

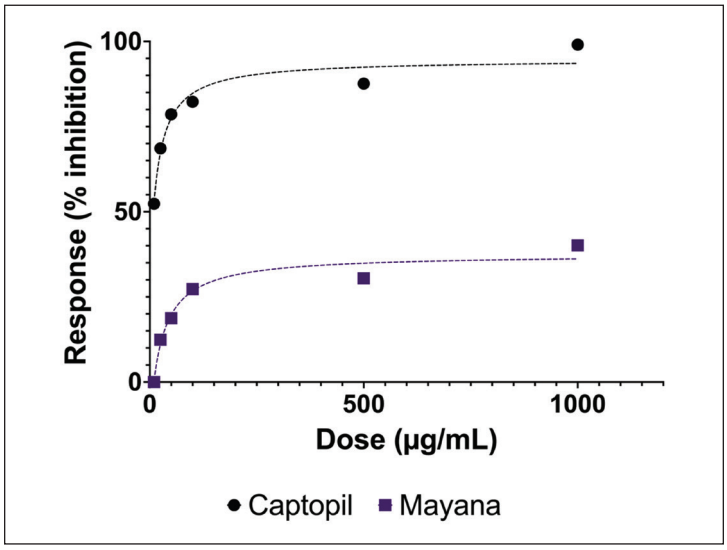


**Figure 1.** Graphical Visualization of Response and Dose Relationship.

**Table 1.** Phytochemical Screening

| Phytochemical | Test | Results |
|---|---|---|
| ***Flavonoids*** | Lead Acetate test | Positive |
| ***Phenolic compounds*** | Ferric Chloride test | Positive |
| ***Alkaloids*** | Mayer's test | Negative |
| ***Terpenoids*** | Salkowski test | Negative |
| ***Tannins*** | Gelatin test | Negative |
| ***Cardiac glycosides*** | Keller-Killiani's test | Negative |
| ***Saponins*** | Froth test | Negative |
| ***Sterols*** | Liebermann-Burchard test | Negative |

**Table 2.** Percent Response Inhibition and $IC_{50}$ of the Plant Sample and Positive Control

| Sample | $IC_{50}$ (µg/mL) |
|---|---|
| ***Captopril*** | 7.7232 |
| ***Mayana*** | 55.9154 |
| ***Blank*** | – |

Phytochemical screening of the extract showed only the presence of flavonoid and phenolic compounds. These findings echo results in previous studies.[19,20] However, the current study shows negative results for the presence of cardiac glycosides which is in contrast to the findings of Casuga and Natividad.[20] A study has shown that drying methods can alter the phytoconstituents present[21,22] indicating that the absence of glycosides in the results of this study may be due in part to the drying choice.

Moving towards the results for bioactivity of this study, we see that the crude ethanolic dehydrated leaf extract of Mayana indeed show inhibitory activity against ACE-1; but from their $IC_{50}$ values, captopril remains to be superior to the crude extract which is expected. The $IC_{50}$ value of Mayana, despite being lower to captopril, is consistent with $IC_{50}$ values of many medicinal plants previously assayed for the same bioactivity.[23] We speculate that several factors may have contributed to the lower percent inhibitory activity observed for the Mayana crude extract, including the type of solvent used and its impact on extraction yield,[24] low flavonoid and phenolic content,[25] and competition among multiple phytochemicals due to the crude nature of the extract[26].

From the data, it can also be inferred that the phytochemicals observed may be responsible for the ACE inhibitory property of the plant. The members of *Lamiaceae* family have been shown to possess vasodilation and vasorelaxation effects.[12] Using in-vitro testing, it has been shown that Mayana is able to inhibit angiotensin converting enzyme type 1. This may indicate a possible phenolic compound or flavonoid compound with anti-hypertensive potential. However, further isolation, purification, and assays are needed to confirm the link between ACE inhibition with the phytochemicals mentioned.

The possible flavonoid component responsible for the positive effect is quercetin and its derivatives since there is evidence showing such flavonoids being identified in Mayana crude leaf extract.[27] Furthermore, rosmarinic acid is the main phenolic compound of Mayana.[28] Rosmarinic acid has been found to not only possess ACE inhibitory effect but can also increase nitric oxide synthesis and endothelin-1 downregulation,[29] all of which are important mechanisms of action for antihypertensive activity. Rosmarinic acid has also been found to be beneficial in diabetes mellitus and its secondary complications including hypertension.[30]

Similar potential antihypertensive activities have also been noted in members of the *Coleus* family. For example, *Coleus forskohlii,* which primarily contains forskolin, reduces blood pressure due to increased cAMP and cAMP-mediated functions.[31] Furthermore, *Coleus amboinicus* Lour powder capsules were also found to lower systolic blood pressure among men with hypercholesterolemia,[32] though limited studies elaborate on the phytochemical responsible and its mechanism of action. Although there is ethnobotanical research surrounding the use of Mayana for hypertension, the current study can be considered one of the few studies that conducted an in-vitro investigation regarding those claims. Despite that, there is still evident need for further investigation as the findings are still in its preliminary stages.

Overall, the studies reviewed in this discussion support the folkloric uses of Mayana and its potential against hypertension. Further research and investigation are warranted to fully understand the pharmacodynamics of Mayana and Mayana-derived compounds in managing hypertension. Additionally, long-term effects are also warranted for investigation to ensure the safety of the people who use Mayana as an herbal remedy. As interest in herbal medicine continues to grow, the integration of plant-based remedies, including Mayana, into healthcare strategies may offer alternative approaches to address cardiovascular health.

## CONCLUSION

The crude ethanolic dehydrated leaf extracts of Mayana (*Coleus scutellarioides* Benth) have demonstrated preliminary antihypertensive potential through inhibition of angiotensin converting enzyme type 1. However, the enzyme inhibitory activity is lower when compared to captopril. The possible phytochemicals for this may be flavonoid and phenolic compounds like quercetin and rosmarinic acid. Additional research is warranted to ascertain the plant's antihypertensive potential whether on its purified form or as an adjunct to conventional medicines.

### Recommendations

The researchers would like to present the following recommendations to address certain limitations encountered in this study:

1. Because the percent yield may have been affected by the drying choice, future research should use lyophilization to ensure product yield and integrity of phytochemicals.
2. The plant sample extracts must undergo further phytochemical analyses including more efficient extraction methods, isolation, purification, and lastly identification to comprehensively characterize the active constituents present in the extracted sample that is responsible for the angiotensin-converting enzyme - I inhibitory effect. It is possible that the bioactive compound may not be extractable with the chosen solvent.
3. There is still a need to standardize the plant material as different factors (like ontogeny, seasonal factors, geographical factors, and preparative factors) may also affect the investigation.
4. To ascertain the bioactivity of Mayana, future research should validate the in-vitro findings with in-vivo experiments as well as determine possible toxicities in animal models.

### Data Availability Statement

Data can be requested from the corresponding author per reasonable request.

## Statement of Authorship

All authors certified fulfillment of ICMJE authorship criteria.

## Author Disclosure

All authors declared no conflicts of interest.

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