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Antihypertensive and Antiobesity Activities of Selected Plants from Albay and Sorsogon Philippines

Lilibeth A. Cajuday[1]*, Ivane Malaluan[2], Phil V. Morano[1], Jocelyn E. Serrano[1], and Jazzlyn T. Imperial[1]

[1] *Biology Department, College of Science Bicol University, Albay 4500 Philippines*
[2] *Chemistry Department, College of Science Bicol University, Albay 4500 Philippines*

*Corresponding author's email: lacajuday@bicol-u.edu.ph

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ABSTRACT

The present study evaluated the antihypertensive and antiobesity activities of twenty-three (23) selected plants from biodiverse sites in Albay and Sorsogon Philippines. *In vitro* antihypertensive and antiobesity activities of the plant extracts were screened using angiotensin-1 converting enzyme (ACE) and porcine pancreatic lipase (PPL) inhibition assays. The safety of the bioactive extracts was also evaluated using organ-specific toxicity assays. A total of five plants were potent ACE inhibitors (>70%) at 100 ppm concentration. The IC50 values of the bioactive extracts (50.77 to 67.94 ug/mL) were comparable to the potent drug Captopril (53.35 ug/mL). Seven plants were active against the PPL enzyme at 100 ppm concentration. Three plants exhibited strong PPL inhibitory activity (>70) with IC50 values of 46.1 to 53.45 ug/mL, less than half the value computed for Orlistat (96.04 ug/mL), indicative of its strong potential as an antiobesity agent. Cytotoxicity results revealed that all the bioactive plant extracts were non-toxic and safe. The study's findings are very promising in the search for potential herbal drugs against hypertension and obesity from natural sources in the region.

Keywords: ACE activity, Pancreatic lipase, CVDs, Herbal drug

INTRODUCTION

Cardiovascular diseases (CVDs) are one of the significant challenges of the 21st century. Globally, the burden of CVD remains high, accounting for 17.9 million deaths, where over three-quarters of the deaths are from low- and middle-income countries [1]. CVDs are estimated to account for approximately 20% of all deaths among Filipinos and Filipino Americans, affecting one in six Filipinos and one in three Filipino Americans [2]. Based on a current report on the incidence of CVDs in the Philippines among the hospital-based population surveyed, of the CVDs, hypertension was the most prevailing, with risk factor prevalence for diabetes at 3.9% and obesity at 4.9% (BMI) [3]. Furthermore, a systematic review by Gutierrez and Sakulbumrungsi [4] found that hypertension, the highest risk factor for CVDs in the country, has a prevalence rate of 28% despite medical intervention. Hypertension remains poorly controlled because approximately two-thirds of hypertension is undetected or inadequately treated.

Obesity, the abnormal accumulation of ≥20% of body fat over the individual's ideal body weight, is rising as a worldwide epidemic. According to WHO, the situation is critical because many diseases that can occur due to obesity are widespread, particularly cardiovascular diseases, the leading cause of mortality worldwide [5]. Moreover, prolonged obesity increases the risk of other non-communicable diseases (NCDs), resulting in more costly interventions [6]. At the national level, overweight and obesity are top nutrition concerns based on the results of the Expanded National Nutrition Survey (ENNS) [7].

Currently, there are already commercially available medicines used to treat hypertension and obesity. However, they remain inaccessible and unaffordable to many who need them, especially in low- and middle-income countries where the prevalence of the diseases is increasing. Moreover, conventional antihypertensive and antiobesity drugs are usually associated with many side effects.

The inhibition of pancreatic lipase, which will delay fatty acid absorption, is the most widely studied mechanism for evaluating natural products as antiobesity agents [8,9,10]. The renin-angiotensin-aldosterone system is a signalling pathway in the regulation of blood pressure. One of the vital elements responsible for the hypertensive

mechanism is the Angiotensin-converting enzyme (ACE), which converts angiotensin-1 to angiotensin-2[11,12]. Therefore, the inhibition of this enzyme can cause an antihypertensive effect. There are several plants from diverse plant families that can inhibit ACE and pancreatic lipase activities.

The present work conducted a comparative *in vitro* screening of the antihypertensive and antiobesity activities of selected plants from two biodiversity areas in the region, namely Mayon Volcano Natural Park (MVNP) [13,14] and Bulusan Volcano Natural Park (BVNP) [15], in Albay and Sorsogon respectively. The plants were selected using an integrated approach: traditional documented use, non-documented use, availability, and exhaustive literature search. Results of the investigation reported 5 and 7 bioactive and non-toxic plant extracts with antihypertensive and antiobesity activities, respectively, out of total plant samples screened from the biodiverse sampling sites.

MATERIAL AND METHODS

Unless specified, all the chemicals used in the study were analytical and purchased from Sigma-Aldrich.

Collection of Plant Materials

Prior to plant collection, Gratuitous Permit (GP R5-12I & R5-133) was secured from the Department of Environment and Natural Resources, Region V in Legazpi City, Albay Philippines. Samples of the selected plants from MVNP and BVNP were collected as identified *in situ* using reference materials [16]. The morphoanatomy of the plants was further characterized and documented in the lab, and samples were validated at Jose Vera Santos Herbarium at the University of the Philippines, Diliman Institute of Biology.

Preparation of Extracts

Leaf samples of the identified plants were obtained by pruning shears, washed, labelled, dried at room temperature, and pulverized. The pulverized samples were soaked in 95% ethanol (1:4 w/v) for 72 h. The ethanol extract was filtered and concentrated at 45 °C using a rotary vacuum evaporator. The plant extract was prepared and diluted accordingly for each inhibition assay to be performed.

In vitro Enzyme-based Assays

The ethanol leaf extracts of the selected plants were screened for primary activity using angiotensin-1 converting enzyme (ACE) and porcine pancreatic lipase (PPL) inhibition assays. All the enzyme-based assays were performed at the Natural Products Laboratory of Bicol University College of Science, Legazpi City, Philippines.

ACE Assay

Inhibition of angiotensin-1 converting enzyme (ACE) was used to screen the antihypertensive activity of the plant extracts. The assay was performed on a 96-well black plate, following the protocol based on Tutor and Hernandez [17] with some modifications. Angiotensin-converting enzyme (ACE) from rabbit lung, Abz-Gly-Phe (NO_2)-Pro, Captopril, and 150 mM Tris Buffer (pH 8.3) were used as enzyme, substrate, positive control, and negative control, respectively. The reaction mixtures consisted of 20 µL of 150 mM Tris Buffer (pH 8.3), either plant extract (30 µL, 1,000 ppm) or positive control, captopril (30 µL, 100 mM). Negative control wells contain Tris Buffer (47 µL, 150 mM, pH 8.3) and 3µl of DMSO. 50 µL, 7.5 µg/mL of angiotensin-1 converting enzyme solution was added to each of these wells. For blank well reaction mixture, it consisted of 70 µL of 150 mM Tris Buffer (pH 8.3), either plant extract (30 µL) or positive control, captopril (30 µL). Tris Buffer (97 µL, 150 mM, pH 8.3) and 3µL of DMSO are for the negative control. Mixtures were incubated for 10 minutes at 37 ºC. The substrate was incubated simultaneously in a separate water bath at 37 ºC. After incubation, 200 µL of 0.45 mM Abz-Gly-Phe (NO2)-Pro was added to all wells. The enzymatic reaction was allowed to proceed for 30 min while maintaining the temperature at 37 ºC during the experiment. The fluorescence excitation wavelength was measured in the 355 to 375 nm range and 400 to 430 nm emission using a FLUOstar® Omega multi-mode microplate reader. The percent inhibition of ACE activity was calculated according to the following equation:

$$\% \text{ Inhibition per trial} = \{1 - [(A - B) / (C - D)]\} * 100 \quad (1)$$

where: A = fluorescence of the test sample or the positive control; B = fluorescence of the test sample or the positive control blank; C = fluorescence of the negative control; and lastly, D = fluorescence of the negative control. The ACE inhibitory activity of the test plants was classified as either active (at least 50% inhibitory

activity and ± SD across all trials is <10) or inactive (less than 50% inhibitory activity and ± SD across all trials is >10**).**

PPL Assay

A lipase inhibition assay was used to determine the antiobesity potential of the plant extracts. Inhibitory activity was determined against the enzyme lipase from the porcine pancreas. 4-Nitrophenyl butyrate (p-NPB) was used as a substrate, and Orlistat, a well-known pancreatic lipase inhibitor, was used as the positive control. The assay procedure was based on the methods of Allanigue et al. [18] with some modifications.

The enzyme-buffer solution was prepared by adding 100 µL of 100 U/mL pancreatic lipase (in 10 mM morpholine propanesulfonic acid (MOPS) and 1mM EDTA, pH 6.8) to 150 µL of 100 mM Tris-HCl Buffer, 5mM $CaCl_2$, pH 7.0 in a 96-well quartz microplate. Either 30 µL of 100 ppm test sample or 30 µL of 100 ppm orlistat was added to the enzyme-buffer mixture. Mixtures were incubated for 5 min at 37 ºC. After incubation, 20 µL of 0.03 mM p-NPB substrate was added. The enzymatic reactions were allowed to proceed for 10 min at 37 ºC. The absorbance was measured at 405 nm and was monitored every 30 seconds using a Thermo Fisher Scientific Multiskan™ Go Spectrophotometer. The observed change in absorption was due to the enzyme activity as the lipase hydrolyzes the p-NPB to p-nitrophenol. The lipase inhibitory activity was calculated according to the following formula:

$$\% \text{ Inhibition per trial} = [(\text{uninhibited} - \text{inhibited}) / \text{uninhibited}] * 100 \quad (2)$$

Inhibitory activities were expressed as % inhibition. The data presentation and analysis were done using the Skanit software (v.3.2). The lipase inhibitory activity of the test plants was classified as either active (at least 50% inhibitory activity and ± SD across all trials is <10) or inactive (less than 50% inhibitory activity and ± SD across all trials is >10).

Cytotoxicity Assay

Organ-specific toxicity assay of the bioactive extracts identified from the primary enzyme-based screening were evaluated for hepatotoxicity and nephrotoxicity using human liver cancer (HepG2) and human normal kidney (HK-2) cell lines at the Disease Molecular Biology and Epigenetics Laboratory, National Institute of Molecular Biology and Biotechnology, University of the Philippines Diliman Quezon.

Half-maximal Inhibitory Concentration (IC50)

The determination of the half-maximal inhibitory concentration (IC50) of the bioactive extracts was computed using the formula [19]:

$$IC50 = (\text{Concentration of test sample} * 50) / \% \text{ Inhibition value} \quad (3)$$

Thus, the IC50 value is a function of the % Inhibition value.

RESULTS AND DISCUSSION

The study screened a total of twenty-three (23) plants from Albay and Sorsogon, Philippines. Thirteen plants were found in Albay, while ten samples were retrieved from Sorsogon from the total plants collected from the sampling locations. Table 1 shows the botanical characteristics and biological activities of the 23 medicinal plants investigated in the study.

The diversity of the plants evaluated in the study was evident. Each plant has a unique family, and there were 19 families documented with one plant member each, except for Lamiaceae, Rubiacea, and Apocenaceae. Three species belong to family Lamiaceae, including *Clerodendrum brachyanthum* Schauer, *Premna nauseos* Blanco, and *Gmelina arborea* Roxb; two species are members of Rubiaceae family, namely, *Borreria hispida* Spruce ex K. Schum, and *Mussaenda philippinensis* Merr.; and two species are from Apocenaceae family, specifically *Cascabela thevetia (L.)* Lippold *and Ervatamia divaricate* (L.) Burkill. Almost 83% of the plants evaluated in the study have folkloric uses, and the leaves were the most commonly utilized part of the plant as a treatment for the identified ailments. Surprisingly, four plants have no reported folkloric use so far, to be specific: *Syzygium albayense* Merr., *Dinochloa luconiae (Munro)* Merr., *Mussaenda philippinensis* Merr., *and Guioa falcata* Radlk. Results of the primary enzyme-based assays, identified a total of 12 bioactive plants out of the 23 screened for *in vitro* antihypertensive and antiobesity activities.

Table 1 The 23 medicinal plants from biodiverse sites in Albay and Sorsogon

Scientific Name	Family	Local Name	Habit	Status	Part Used	Folkloric Use
Clerodendrum brachyanthum Schauer[a]	Lamiaceae	Hamindang	Small Tree	Common	Leaves	Tonic for stomach problems
Borreria hispida Spruce ex K. Schum[a]	Rubiaceae	Landrina	Shrub	Common	Leaves Roots	For diarrhea, pneumonia
Crotalaria incana L.[a]	Fabaceae	Kalog-kalog	Shrub	Common	Leaves Roots	For goiter, burns
Canarium ovatum Engl.[a]	Burseraceae	Pili	Tree	Endemic	Fruit	As laxative
Lygodium circinnatum (Burm. f) Swartz[b]	Schizaeaceae	Nitong puti	Fern	Common	Leaves	As contraceptive
Selaginella plana (Desv. ex Poir.) Hieron[b]	Selaginellaceae	Kamariang gubat	Moss	Common	Leaves	For wounds, respiratory infections, urinary tract infections, rheumatism
Premna nauseosa Blanco[a]	Lamiaceae	Alagau-gubat	Shrub	Common	Leaves Bark	For stomach disorders, headache
Gmelina arborea Roxb[a]	Lamiaceae	Not known	Tree	Common	Bark	For fever
Syzygium albayense Merr.[b]	Myrtaceae	Sambulauan	Tree	Endemic	NA	Undocumented
Asplenium nidus L.[b]	Aspleniaceae	Pakpak lauin	Fern	Common	Leaves Shoots	For cold, cough, sores, ulcers, and labor pains during childbirth
Dendrocnide meyeniana (Walp.) Chew[a]	Urticaceae	Lipang kalabaw	Shrub/ Small Tree	Common	Leaves	For bleeding
Cascabela thevetia (L.) Lippold[a]	Apocynaceae	Campanero	Shrub/ Small Tree	Common	Leaves Bark	For fever
Dinochloa luconiae (Munro) Merr.[b]	Poaceae	Bikal babui	Liana	Native	NA	Undocumented
Raphidophora merrillii Engl.[b]	Araceae	Bakag	Liana	Endemic	Leaves	For rheumatism, fractures
Arcangelisia flava (L.) Merr.[b]	Menispermaceae	Lagtang	Liana	Common	Leaves Stem Roots	For fever, as tonic agent
Mussaenda philippinensis Merr.[a]	Rubiaceae	Aurora	Shrub/ Small Tree	Endemic	NA	Undocumented
Alpinia elegans (C.Presl) K.Schum[a]	Zingiberaceae	Bagombon	Herb	Endemic	Rhizomes, leaves, stems.	For rheumatism
Ervatamia divaricata (L.) Burkill[a]	Apocynaceae	Pandakaking-Tsina	Shrub/ Small tree	Common	Leaves	As tonic
Adiantum caudatum L[b]	Pteridaceae	Alambrillong-gubat	Fern	Common	Fronds	For skin disease, diabetes, cough
Pleocnemia brongniartii (Bory) Holttum[b]	Tectariaceae	Not known	Fern	Endemic	NA	Undocumented
Dicranopteris linearis (Burm. f.) Underw.[b]	Gleicheniaceae	Dilim	Fern	Common	Leaves	For asthma, fever, wounds
Guioa falcata Radlk.[a]	Sapindaceae	Not known	Shrub	Endemic	NA	Undocumented
Semecarpus philippinensis Engl[a]	Anacardiaceae	Ingas	Tree	Endemic	Bark	As teeth blackening agent

Antihypertensive Activity

A total of five plants from Myrtaceae, Tectariaceae, Burseraceae, Gleicheniaceae, and Sapindaceae families were found to be active and strong ACE inhibitors (>70%) comparable to the activity of the control (93.72 ± 6.31). The bioactive plants include *S. albayensi* (98.49 ± 0.23); *P. brongniartii* (87.31 ± 0.11); *C. ovatum* (78.48 ± 7.89); *D. linearis* (87.16 ± 15.32); and *G. falcata* (73.59 ± 1.19). The IC50 value for Captopril was 53.35 ug/mL. In contrast, bioactive extracts with strong ACE inhibitory activity ranged from 50.77 to 67.94 ug/mL, which indicates the comparable antihypertensive effects of the extracts to the control drug, Captopril (Figure 1).

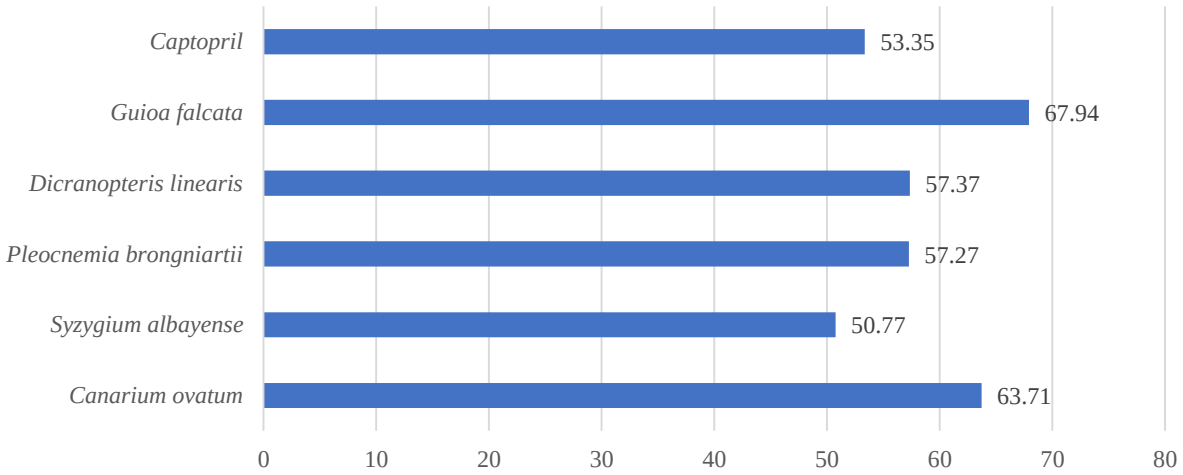

Fig. 1 IC_{50} values of bioactive extracts against ACE with Captopril at 100 ppm concentration (ug/mL)

Angiotensin-converting enzyme (ACE) is one of the crucial components responsible for the hypertensive mechanism, which converts angiotensin-1 to angiotensin-2[11,12]. Therefore, the inhibition of this enzyme can generate an antihypertensive effect. The study's findings suggest that five potent plant extracts reported in this paper are of therapeutic interest for treating hypertension. Previous studies conducted supported the antihypertensive activity of some members of Myrtaceae Family, which include *Eugena uniflora* L. [20], *Eugenia mattosii* D. Legrand [21] and *Syzigium cumini* L. [22], while from Burseraceae Family is *Canarium pimela* KD. Koenig [23] and *Bursera simaruba* L. [24]. Similarly, geraniin extracted from *Nephelium lappaceum* L. from the Sapindaceae has a blood pressure-lowering effect [25]. However, no published data focused on the potential antihypertensive activity of other fern species in Tectariaceae and Gleicheniaceae families which this present paper has documented for *Pleocnemia brongniartii* and *Dicranopteris linearis.* Interestingly, *Allium sativum,* a popular plant for controlling blood pressure, works as a strong ACE inhibitor with an IC_{50} value of 300 ug/mL [26] far below the ACE inhibitory activity of the bioactive plants investigated in this study (IC_{50} ranged from 50.77 to 67.94 ug/mL).

Antiobesity Activity

Seven plants were active against porcine pancreatic enzyme (at least 50% inhibition) at 100 ppm concentration. There were three plants from Tectariaceae, Schizaeaceae, and Lamiaceae families found to exhibit strong PPL inhibitory activity (>70%); *Pleocnemia brongniartii* (Bory) Holttum (108.47 ± 9.68), *Lygodium circinnatum* (Burm. f) Swartz (96.8 ± 3.32), and *Premna nauseosa* Blanco (93.55 ± 3.45). The IC_{50} values of these bioactive extracts (46.1 to 53.45 ug/mL) were below half compared to *Orlistat* (96.04 ug/mL), indicative of its strong potential as an antiobesity agents (Figure 2).

Moderate PPL inhibition (≥50%) was observed in the extracts of four plants from Menispermaceae, Burseraceae, Myrtaceae, and Gleicheniaceae families, including *Arcangelisia flava* (L.) Merr. (66.12 ± 1.78); *Canarium ovatum* Engl. (52.62 ± 4.81); *Syzygium albayense* Merr. (51.72 ± 3.0); and *Dicranopteris linearis* (Burm. f.) Underw (51.38 ± 2.88). Interestingly, the lipase inhibitory activity of these extracts (IC_{50} 75.62 to 97.31 ug/mL) was still comparable to the control (IC_{50} 96.04 ug/mL).

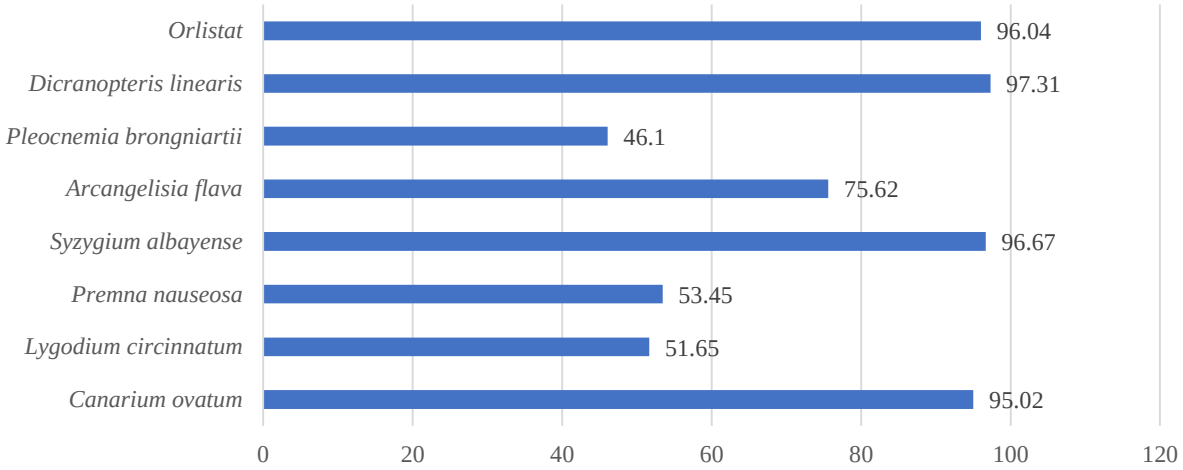

Fig. 2 IC_{50} values of bioactive extracts against PPL with Orlistat at 100 ppm concentration (ug/mL)

Seven plants from biodiverse families exhibited potential antiobesity properties. The most widely studied mechanism for assessing herbal products as remedies for obesity is the inhibition of pancreatic lipase, which will slow down the absorption of fatty acids [10]. Bustanji and co-workers [27] reported active lipase inhibitors from leaf extracts of nine plants, including *Anthemis palestina* (Asteraceae), *Ononis natrix* L. (Fabaceae), *Fagonia arabica L.(*Zygophyllaceae), *Origanum syriacum* L. (Lamiaceae), *Hypericum triquetrifolium* Turra (Hypericaceae), *Malva nicaeensis* (Malvaceae), *Chrysanthemum coronarium* L. (Asteraceae), and *Paronychia argentea* Lam. (Caryophyllaceae), with half-maximal inhibitory concentrations (IC_{50}) ranging from 107.7 to 342.7 g/mL. Likewise, leaf samples of freeze-dried *Canarium sp*. (Burseraceae) were shown to exhibit high anti-lipase activity with an IC_{50} value of 118.66 µg/Ml [28] as well as plant parts of *Clematis vitalba* L. (Ranunculaceae) have strong inhibitory effect on pancreatic lipase (IC_{50}=0.99 mg/ml) [9]. Findings suggest that the bioactive plants evaluated in the study exhibited high potency against porcine pancreatic lipase and thus can be good sources of new candidates in obesity treatment.

Cytotoxicity assay

The bioactive extracts identified from the primary enzyme-based screening were evaluated for hepatotoxicity and nephrotoxicity using human liver cancer (HepG2) and human normal kidney (HK-2) cell lines. All the bioactive plant extracts were non-toxic and safe. A test sample is classified as safe if cytotoxicity is <10% in 3 out of 3 trials or is <10% in 2 out of 3 trials, but ± SEM across all trials is <10%. The cytotoxicity of the extracts ranges from 4.85% to <0% for nephrotoxicity, whereas 2.80% to <0% for hepatotoxicity. All the bioactive plants tested safe in the cytotoxicity tests. One of the practical applications of organ-specific toxicity is the identification of unsafe plants earlier in the developmental process, thus eliminating them from further screening.

CONCLUSION

The activity profiles of angiotensin-1 converting enzyme (ACE) and porcine pancreatic lipase (PPL) in the plants collected from Albay and Sorsogon Philippines were presented in this paper. To our knowledge, the plants screened in the present work have never been evaluated for *in vitro* biological activity and cytotoxicity. The present work scientifically documented five plants (*C. ovatum, S. albayensi*, *P. brongniartii, G. falcata*, and *D. linearis*) with antihypertensive activity and seven plants (*P. brongniartii, L. circinnatum, P. nauseosa, A. flava, C. ovatum, S. albayense*, and *D. linearis*) with potential antiobesity property. Furthermore, the results suggest that the identified bioactive plants were non-toxic. Hence these plants can be safely consumed, manufactured as nutraceuticals or herbal formulations, and used as potential therapies for hypertension and obesity.

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