ACE Inhibitory Activity and Functional Group Analysis of Solvent-Partitioned Fractions of *Eleusine indica*

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ABSTRACT

Eleusine indica Linn. Gaerth or Indian goose grass has been widely used as an alternative natural anti-hypertensive agent. However, the rationale behind this mechanism is still unknown. The purpose of this study is to confirm the presence of functional groups that may be responsible for its ACE inhibitory activity and its possible synergism. Each solvent-portioned fraction was analyzed by FTIR, individual ACE inhibitory activity, and combination of fractions. FTIR results of solvent-partitioned fractions revealed that O-H and C-H stretches were present in all fractions. In addition to this, C-O and C=O groups were also present in all fractions except in ethyl acetate. S=O groups were also present in chloroform, ethyl acetate, and n-butanol fractions. Other function groups such as N-O and C=C groups were present in ethyl acetate and n-butanol fractions, respectively. The highest % inhibition obtained among the individual fraction is n-butanol at $96.031\% \pm 0.004$ at 500 ug/ml. The lowest % inhibition obtained among the individual fraction is chloroform at $32.544\% \pm 0.011$ at 500 ug/ml. The highest % inhibition among the combined fractions is ethyl acetate:butanol at $95.727\% \pm 20.847$, while the lowest % inhibition in combination is hexane:chloroform at $89.626\% \pm 19.577$. The fraction with the lowest IC_{50} is hexane at 15.357, while the highest IC_{50} is chloroform at 168.207 ug/ml. Functional group present may be associated with the antihypertensive activity of the *E. indica* extracts. The more polar solvent-partitioned fractions of *E. indica* has a higher ACE inhibitory activity compared to its less polar solvent-partitioned fractions. The higher ACE inhibitory activity may be associated with the secondary metabolites present in the more polar fractions. There is also possible synergism present in the combined solvent-partitioned fractions as their % inhibition is higher than their individual effects.

Keywords: *Eleusine indica*; *paragis*; *ace inhibition*; *FTIR*; *ace kit-wst*

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INTRODUCTION

Eleusine indica or commonly known in the Philippines as *paragis*, is a terrestrial plant with a wide distribution across the world including Asia, Africa, North America, and parts of Europe due to its wide tolerance to various environmental conditions. (Cha, et al., 2014). Aside from this, *E. indica* is a native plant in tropical and subtropical regions like the Philippines. Traditionally, its decoction from the leaves are used to treat asthma, muscle pains, febrifuge, diarrhea, and dysentery. In addition, *E. indica* is used to treat bladder disorders, convulsions and childbirth aid in other countries. The whole plant is usually used as a diuretic, laxative, and depurgative making it useful for the treatment of influenza, oliguria, and hypertension (Ettebong et al., 2012).

Hypertension is a major risk factor for cardiovascular diseases that commonly affects adults. Since high blood pressure is usually asymptomatic in its initial stages, it has been labelled as a silent killer. It can cause diseases such as heart failure, kidney failure, and stroke. (Amin

et al, 2013). According to the World Health Organization (WHO) (2017), the rampant increase of hypertension cases is associated with population growth, aging and behavioral risk factors. The Renin-Angiotensin-Aldosterone-System (RAAS), a powerful arterial blood pressure regulator, has become a focus on the hunt for targets in the elimination of hypertension. (Bangalore et al., 2016). A component of the RAAS is the Angiotensin-Converting Enzyme (ACE) which in high levels leads to increased concentration of angiotensin II causing to hypertension.

In the study of Hernandez and Tutor (2018), hexane and ethyl acetate sequential fractions of *E. indica* showed lower ACE inhibitory activity compared to the crude methanolic extract. The researchers attribute the result to a possible synergism, an activity in which activity is lost in purified fractions.

In this study, the researchers confirm the presence of functional groups that may be responsible for its ACE inhibitory activity.

METHODS

Chemicals

The ACE kit-WST by Dojindo Molecular Technologies, Inc. was purchased from Integrated Biolabs, Taiwan.

Plant Sample Preparation

E. indica was collected from the wide grasslands of San Fernando, Romblon, Philippines as the grass is abundant and grows all year round. The plant samples were collected in October 2018. The plant samples were cleaned with distilled water and were air-dried in shade for 3 days. It was grinded with a Wiley-mill grinder and stored in a wide mouthed amber bottle. The samples were identified and authenticated by Philippine Bureau of Plant Industry (BPI) and a voucher specimen was made.

Crude Extraction

The extraction was adapted from the study of Hernandez and Tutor (2018) with a slight modification. Dried powdered sample of *E. indica* was soaked in methanol for 3 days. The sample was collected using a percolator and was evaporated in a digital water bath at 40°C. The collected methanolic extract was oven-dried and weighed. The % yield was calculated using the formula:

$$\% \text{ Yield} = (\text{weight of crude extract} / \text{total weight of sample}) \times 100$$

Solvent Partition

The solvent partition method was adapted from the methods of Kupchan (2016) with slight modifications by adding chloroform into the process. Five g of the crude methanolic extract was partitioned using hexane, chloroform, ethyl acetate, and n-butanol to obtain their fractions. The fractions obtained were evaporated in a digital hot bath at 50°C. and were dried in an oven at 40°C and weighed.

Fourier Transform Infrared (FTIR) Analysis

The spectra measurement of the crude methanolic extract and the fractions collected was performed using the PerkinElmer Spectrum Two FT-IR. A pinch amount of the dried samples was placed in the universal attenuated total reflectance (ATR) (Rohman & Che, 2012).

ACE Inhibitory Activity Determination

ACE inhibition assay kit was used to determine the angiotensin converting enzyme inhibitory activity from the obtained crude methanolic extract, solvent-partitioned fractions of hexane, chloroform, ethyl acetate, and n-butanol, and its chosen combination.

The working solution was prepared as specified in the manual included in the kit. A solution containing Enzyme B was prepared by dissolving the solution with 2 ml of deionized water. Then, 1.5 ml of the solution was added

to Enzyme A to prepare the Enzyme sample. An indicator working solution was also prepared by dissolving a solution containing Enzyme C and coenzyme with 3 ml each of deionized water. Afterwards, 2.8 mL of the solution containing Enzyme C was added to 2.8 ml of coenzyme solution for the preparation of the indicator working solution. The sample solution was prepared into several portions by diluting it with deionized water by two-fold dilution. Starting concentration was 500 µg/mL of plant extract.

Twenty µl of sample solution was placed in a well and a separate two 20 µl of deionized water for blank 1 and blank 2. Twenty µl of the substrate buffer was placed on all wells. Twenty µl of deionized water was added to the well of blank 2. Subsequently, 20 µl of enzyme solution was added to each sample well and blank 1 well. The plate was incubated at 37°C for 1 hour. Afterwards, 200 µl of indicator working solution was added to all wells. In the final step, the solution was incubated at room temperature for 10 minutes before the absorbance was read at 450 nm using a microplate reader (Dojindo Laboratories, 2016).

ACE inhibitory activity was calculated by the following equation:

$$\text{ACE inhibitory activity (inhibition rate\%)} = [(A_{\text{blank1}} - A_{\text{sample}}) / (A_{\text{blank1}} - A_{\text{blank2}})] \times 100$$

IC₅₀

IC₅₀ was determined using Quest Graph™ IC₅₀ Calculator.

The formula used is as follows:

$$Y = \text{Min} + \frac{\text{Max} - \text{Min}}{1 + \left(\frac{X}{\text{IC}_{50}}\right)^{\text{Hill coefficient}}}$$

RESULTS AND DISCUSSION

FTIR

The FTIR analysis was performed to identify the functional groups present in the crude methanolic extract and various solvent-partitioned fractions of *E. indica*. The FTIR spectroscopic analysis showed various functional groups present in the plant and exists both or copiously on almost all solvent-partitioned fractions.

The crude methanolic extract (Figure 1) FTIR results revealed that the O-H (3200-2700 cm⁻¹ and 1420-1330 cm⁻¹), and C-H (3000-2840 cm⁻¹ and 700 ± 20 cm⁻¹) groups have been detected. In addition, C-O and (1210-1163 cm⁻¹) and C=O (1710-1680 cm⁻¹) groups were also detected.

Figure 1. FTIR spectra of crude methanolic extract

Figure 2. FTIR spectra of hexane-partitioned fraction

The hexane-partitioned fraction (Figure 2) FTIR results revealed that the O-H (3200-2700 cm⁻¹ and 1420-1330 cm⁻¹), and C-H (3000-2840 cm⁻¹ and 700 cm⁻¹) groups have been detected. In addition, C-O and (1210-1163 cm⁻¹) and C=O (1710-1680 cm⁻¹) groups were also detected.

The chloroform-partitioned fraction (Figure 3) FTIR results revealed that the O-H (3200-2700 cm⁻¹ and 1420-1330 cm⁻¹), and C-H (3000-2840 cm⁻¹ and 700 cm⁻¹) groups have been detected. In addition, C-O and (1210-1163 cm⁻¹) and C=O (1710-1680 cm⁻¹) groups, and notably, an S=O functional group, were all present.

The ethyl acetate-partitioned fraction (Figure 4) FTIR results revealed that the O-H (3200-2700 cm⁻¹ and 1420-1330 cm⁻¹), and C-H (3000-2840 cm⁻¹ and 700 cm⁻¹) groups have been detected. Notably, S=O (1372-1335 cm⁻¹ and 1070-1030 cm⁻¹) and N-O (1650-1580 cm⁻¹) functional groups were detected.

The n-butanol-partitioned fraction (Figure 5) FTIR results revealed that the O-H (3200-2700 cm⁻¹ and 1420-1330 cm⁻¹), and C-H (3000-2840 cm⁻¹ and 700

cm⁻¹) groups were detected. In addition, C-O and (1210-1163 cm⁻¹) and C=O (1710-1680 cm⁻¹) groups were also detected. Notably, S=O (1372-1335 cm⁻¹ and 1070-1030 cm⁻¹) and C=C (1678-1668 cm⁻¹) group were detected as well.

The overlaid spectra (Figure 6) showed all individual solvent-portioned fractions to have the O-H (3200-2700 cm⁻¹ and 1420-1330 cm⁻¹), and C-H (3000-2840 cm⁻¹ and 700 cm⁻¹) functional groups. In addition, C-O and (1210-1163 cm⁻¹) and C=O (1710-1680 cm⁻¹) groups were also detected in all solvent-partitioned fractions except for ethyl acetate. S=O (1372-1335 cm⁻¹ and 1070-1030 cm⁻¹) groups were detected in chloroform, ethyl acetate, and n-butanol-partitioned fractions, while N-O (1650-1580 cm⁻¹) functional group and C=C (1678-1668 cm⁻¹) were detected in ethyl acetate-solvent partitioned fraction and n-butanol-partitioned fraction, respectively.

The FTIR analysis helps to identify a possible secondary metabolite that may be present in each fraction based on their characteristic functional groups. One of the secondary metabolites that may have exerted ACE

Peak Table Spectrum

Figure 3. FTIR spectra of chloroform-partitioned fraction

Peak Table Spectrum

Figure 4. FTIR spectra of ethyl acetate-partitioned fraction

Peak Table Spectrum

Figure 5. FTIR Spectra of n-Butanol-Partitioned Fractions

inhibitory activity would be the flavonoids. It is shown that various flavonoids can inhibit ACE with varying % inhibition, with luteolin having the highest recorded % inhibition of 95% (Garcia-vallve, et al., 2012). Furthermore, *Eleusine indica* is found to have two flavonoids called vitexin and isovitexin (Yeligar, 2018).

We can validate the presence of the flavonoids, since its characteristic functional groups are O-H, C-O, and C=O which were revealed by the FTIR, and certain extracts of *Eleusine indica* contain flavonoids based on various phytochemical screening conducted in previous studies. Although flavonoids are often related to the

Figure 6. Overlaid FTIR spectra of the solvent-partitioned extracts of *E. indica*

anti-hypertensive effects in literatures, anti-hypertensive effects are not necessarily exclusive to it, and other secondary metabolites may also contribute or exhibit anti-hypertensive properties.

ACE Inhibitory Activity

The % inhibition of crude methanol extract (Figure 7) with varying concentrations of 500 ug/ml, 250 ug/ml, 125 ug/ml, and 62.5ug/ml inhibits ACE at 86.919% $\pm$ 0.006, 80.108% $\pm$ 0.035, 59.179% $\pm$ 0.023, 49.346% $\pm$ 0.048 respectively. The relationship of the % inhibition and concentration is dose dependent.

Figure 7. ACE inhibitory activity of crude methanolic extract of *Eleusine indica*

The % inhibition of hexane-partitioned fraction (Figure 8-A) with varying concentrations of 500 ug/ml, 250 ug/ml, 125 ug/ml, and 62.5ug/ml inhibits ACE at 91.926% $\pm$ 0.003, 74.222% $\pm$ 0.032, 60.352% $\pm$ 0.023, 35.769% $\pm$ 0.011, respectively. The % inhibition of chloroform-partitioned fraction (Figure 8-B) with varying concentrations are similar to the previously mentioned concentrations inhibiting ACE at 90.325% $\pm$ 0.011, 72.779% $\pm$ 0.035, 51.037% $\pm$ 0.112, 32.544% $\pm$ 0.182, respectively. The % inhibition of ethyl acetate-partitioned fraction (Figure 8-C) with varying concentrations are similar to the previously mentioned concentrations inhibiting ACE at 92.309% $\pm$ 0.043, 82.206% $\pm$ 0.027, 51.624% $\pm$ 0.058, 32.815% $\pm$ 0.099, respectively. The % inhibition of n-butanol-partitioned fraction (Figure 8-D) with varying concentrations are similar to the previously mentioned concentrations inhibiting ACE at 96.031% $\pm$ 0.004, 82.251% $\pm$ 0.007, 65.111% $\pm$ 0.005, 43.843% $\pm$ 0.128, respectively. All solvent fractions exhibited a dose-dependent response.

All concentrations of all solvent-partitioned fractions ranging from 125-500 ug/ml have significant % inhibition. N-butanol-partitioned fraction has the highest % inhibition across most concentrations (500 ug/ml, 250 ug/ml, and 125 ug/ml) having a % inhibition of 96.031%, 82.251%, and 65.111%, respectively, with the exemption on the lowest concentration in which methanol crude extract exhibited the highest % inhibition of 43.843%. On the other hand, the chloroform-partitioned fraction has the lowest % inhibition across all concentrations (500 ug/ml, 250 ug/ml, 125 ug/ml, and 62.5ug/ml) having a % inhibition of 90.325%, 74.779%, 51.037%, 32.544%, respectively. We have observed that the polar fractions (ethyl acetate and n-butanol fractions) tend to have

Figure 8 (a-d). ACE inhibitory activity of solvent partitioned-fractions of *Eleusine indica*

a higher % inhibition (Tutor & Chichico-Hernandez, 2017). It is possible that the secondary metabolites such as alkaloids, flavonoids, glycosides, and steroids found on ethyl acetate and n-butanol fractions may have probably contributed to its higher % inhibition (Ajiwe, 2015 and Yeligar, 2018).

ACE Inhibitory Activity of Combined Solvent-Partitioned Fractions

Six combinations of the fractions ethyl acetate: hexane (EA:H), ethyl acetate:chloroform (EA:C), hexane: chloroform (H:C), ethyl acetate:-butanol (EA:B), chloroform:butanol (C:B), and hexane:n-butanol (H:B) at a concentration of 500 ug/ml were tested. Figure 9 shows the % inhibition per fraction combination. EA:H shows a % inhibition of $93.009\% \pm 20.232$, EA:C at $94.204\% \pm 20.604$, H:C at $89.626\% \pm 19.577$, EA:B at $95.737\% \pm 20.847$, C:B at $93.978\% \pm 20.477$, and H:B at $93.978\% \pm 20.604$. It is observed that the combination of the polar fraction EA+B exhibited the highest % inhibition of $95.737\% \pm 20.847$, and that the

combination of the non-polar fraction H+C exhibited the lowest % inhibition of $89.626\% \pm 19.577$. There might be a possible synergism present in the combined fractions, since their % inhibition were greater than their individual effects.

IC₅₀

Figure 10 (a-e) shows the IC₅₀ of the methanol crude extract and each individual solvent-partitioned fraction. The IC₅₀ of the methanol crude extract is at 164.318 ug/ml, n-butanol-partitioned fraction at 37.590 ug/ml, hexane-partitioned fraction at 15.357 ug/ml (the lowest), chloroform-partitioned fraction at 168.207 ug/ml (the highest), and ethyl acetate-partitioned fraction at 153.078 ug/ml.

Both hexane and n-butanol-partitioned fraction exhibited low IC₅₀ compared to other portioned fractions. Both partitioned fractions have also shown some similar peaks in FTIR which refers to functional groups such as C-H, C-O, and C=O.

Figure 9. ACE inhibitory activity of combined solvent-partitioned fractions of *Eleusine indica*

Figure 10 (a-e). IC₅₀ methanolic crude extracts and solvent-partitioned fractions

CONCLUSIONS

Functional group present may be associated with the antihypertensive activity of the *E. indica* extracts. *Eleusine indica* shows significant ACE inhibitory activity across all fractions at concentrations ranging from 500 ug/ml to 125 ug/ml, since % inhibition exceeds 50%. Among the individual fractions, n-butanol shows the highest % inhibition. The more polar solvent-partitioned fractions of *E. indica* has a higher ACE inhibitory activity compared to its less polar solvent-partitioned fractions. The higher ACE inhibitory activity may be associated with the secondary metabolites present in the more polar fractions., while among the combined fractions EA:B showed the highest % inhibition. There is also possible synergism present in the combined solvent-partitioned fractions as their % inhibitions are higher than their individual effects.

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