Research Article

Effect of Varying Acetic Acid Concentration in the Total Phenolic and Flavonoid Content and Cytotoxicity of *Ipomoea batatas* Lam. (Convolvulaceae) Leaf Extracts

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ABSTRACT

Introduction: *Ipomoea batatas* Lam. is grown in the Philippines for food. Its young leaves, reported to exhibit medicinal properties, are eaten fresh in salads with vinegar or shrimp paste. **Objectives:** However, the effect of varying acetic acid concentration on the extractability of its phenolics, flavonoid, and cytotoxic compounds using a safer and cheaper solvent such as acetic acid is not yet explored. Thus, the cytotoxicity and the total phenolics and flavonoid content of the aqueous acetic acid extracts of *I. batatas* leaves is evaluated. **Materials and Methods:** Cytotoxicity of the *I. batatas* leaves extracted with 5%, 3%, and 1% aqueous acetic acid were determined through Brine Shrimp Lethality Assay (BSLA) while the total phenolics and flavonoid content were analyzed using Folin-ciocalteu and aluminium chloride method, respectively. **Results and Discussion:** The 5% aqueous acetic acid extract contains significantly higher amount of phenolics and flavonoids as compared to the 3% and 1%. BSLA also showed that the 5% aqueous acetic acid extract (LC_{50} = 520.61 mg/L) exhibited cytotoxic activity while the 3% (LC_{50} >1000 mg/L) and 1% (LC_{50} >1000 mg/L) extracts were non-cytotoxic. **Conclusion:** The concentration of the acetic acid affects the extractability of the phenolics, flavonoids and cytotoxic compounds in the *I. batatas* leaves. The 5% aqueous acetic acid is more efficient in the extraction than the 3% and 1%. The use of acetic acid for the extraction of phenolics, flavonoids, and cytotoxic compounds from *I. batatas* leaves can be a better option than other organic solvents.

Keywords: Acetic acid, Cytotoxicity, Flavonoids, *I. batatas*, *Phenolics*.

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INTRODUCTION

Vegetables, fruits, spices and whole grains contain significant amounts of bioactive phytochemicals, which provide desirable health benefits beyond basic nutrition to reduce the risk of the development of chronic diseases.[1] Phenolics and flavonoids are among the phytochemicals which are explored because of their potent antioxidative properties, abundance in the diet and notable effects in the prevention of various oxidative stress-associated diseases.[2] However, most of the available information regarding the traditional medicinal value of the plants is not provided with credible scientific data. Thus, several researches have been conducted to determine the bioactivity of plants, such as antioxidative, antimicrobial and cytotoxicity among others.

Ipomoea batatas Lam. (Convolvulaceae), commonly known as sweet potato, is an important food crop in many parts of the world including the Philippines. It is grown locally for food where its young leaves and shoots are eaten fresh in salads with vinegar or shrimp paste. Ethno-medicinal information on the leaves of *I. batatas* has shown it as a valuable medicinal plant

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with anti-cancer, antidiabetic, anti-inflammatory,[3] and antimicrobial properties.[4] The tubers or roots, on the other hand, are rich in vitamins, complex carbohydrates, dietary fiber, and other micronutrients, including essential minerals, such as potassium, manganese, iron, and calcium.[5] Despite the numerous studies on *I. batatas* tubers or roots and leaves, cytotoxicity of *I. batatas* leaves and extractability of its phenolics and flavonoids using safer and cheaper solvent such as acetic acid is not yet explored. In this study, the efficiency of acetic acid in extracting phenolics, flavonoids, and cytotoxic compounds from *I. batatas* leaves is investigated. Moreover, the cytotoxicity of the extracts is evaluated *via* brine shrimp lethality assay.

MATERIALS AND METHODS

Sample Collection, Preparation, and Extraction

Healthy and young leaves of *I. batatas* L. were randomly collected from the Agricultural Experiment Station (AES), Central Mindanao University (CMU), Musuan Maramag Bukidnon. Plant samples including the leaves and stem were submitted to the Botany section of the CMU Museum for identification and authentication. The collected leaves were washed, tapped dry with paper towel, and chopped using a ceramic knife. A 100 g of the chopped fresh leaf samples were extracted separately with 5, 3, and 1% aqueous acetic acid for 1.5 h. After extraction, the mixture was filtered through a Buchner funnel. The residue was discarded while the filtrate was set aside for freeze-drying. The weights of the dried extracts were recorded and the extracts were dissolved in distilled water to give a Solution A of concentration C_A.

Total Phenolics Content (TPC)

TPC was determined employing Folin-ciocalteu method.[6] For the samples, a 1.00 mL aliquot of solution A was diluted to 20.00 mL with ethanol to yield sample Solution B. Solution B was assayed for TPC. A 1.00 mL of Solution B was added with 9.00 mL of distilled water, 1.00 mL of 0.5N Folin-ciocalteu reagent, swirled and set aside. After 5 min, the solution was added with 10.00 mL of 7% Na_2CO_3 and incubated for 90 min in the dark at room temperature. The same steps were done for the working standards (gallic acid) and the blank (ethanol). Absorbance were determined against the blank at 750 nm using visible spectrophotometer (Spectronic 20). A calibration curve was constructed through Least Squares Method with the concentration of the working standards (20-140 mg/L) as the abscissa and the absorbance reading as the ordinate. From the calibration curve, the TPC was determined. The TPC, expressed as microgram gallic acid equivalent (GAE) per gram of fresh sample was calculated using Equation 1. Analysis was carried out in three replicates. Result was then reported as mean of the analysis.

TPC (ug GAE/g fresh sample) = A x D/C_A x 1000 ug/mg Equation 1

where: A (mg GAE/mL) is the gallic acid concentration in Solution B; D is the dilution factor (20); and C_A is the concentration of Solution A

Total Flavonoids Content (TFC)

Aluminum chloride Colorimetric Method was used to determine TFC.[7] For the samples, a 1.00 mL aliquot of solution A was diluted to 20.00 mL with ethanol to yield sample Solution B. Solution B was assayed for TFC. A 3.00 mL of Solution B was added with 1.50 mL of 2% (w/v) $AlCl_3$ and 1.50 mL of distilled water. After vigorous shaking, the mixture was incubated for 10 min at room temperature. Absorbance of the samples and working standard solutions (quercetin) were determined against the blank at 425 nm using visible spectrophotometer (Spectronic 20). A calibration curve was constructed through Least Squares Method with the concentration of the working standards (0-30 ug/mL) as the abscissa and the absorbance reading as the ordinate. From the calibration curve, the TFC, expressed as microgram quercetin equivalent (QE) per gram of fresh sample was calculated using Equation 2. Analysis was carried out in three replicates. Result was then reported as mean of the analysis.

TFC (ug QE/g fresh sample) = A × D/C_A Equation 2

where: A (ug QE/mL) is quercetin concentration in Solution B; D is the dilution factor (20); and C_A is the concentration of Solution A

Brine Shrimp Lethality Assay

The brine shrimp lethality assay was carried out with slight modification.[8] An aliquot of Solution A with concentration C_A was diluted with sterilized seawater to give 10,000 mg/L stock solution. From the stock solution, appropriate volumes of 500.0, 250.0, 50.0, 25.0, 5.0 and 2.5 µL of the stock solution were placed into separate test tubes marked up to 5.00 mL to give 1000, 500, 100, 50, 10 and 5 mg/L test solutions, respectively.

Ten brine shrimp nauplii were transferred from the hatching chamber into each test tubes. Sterilized seawater was added up to the 5 mL mark and the test tubes were incubated under illumination for 24 hr. Negative and positive controls were run in parallel with the test solutions using sterilized seawater and

aqueous solution of potassium dichromate, respectively. The number of surviving shrimps were counted and recorded after 24 hr. The percentage mortality of nauplii was then calculated. For sample test solutions giving 0% and 100% mortalities, necessary corrections were done.[9] Using the Probit Table of Finney, the corrected percentage mortality values were transformed to probit values.[10] The best-fitting straight curve was then drawn with the logarithm of the concentration values as the abscissa and the probit values as the ordinate. The $\log_{10}$ concentration value that corresponds to the probit point of 5.00 from the curve was recorded and converted to its antilog value to give the LC_{50}. LC_{50} of less than 1000 mg/L was considered cytotoxic.[11] Analysis was carried out in three replicates. Result was then reported as mean of the analysis.

Statistical Analysis

The results were subjected to one-way Analysis of Variance (ANOVA) at 0.05 level of significance. Significant differences among the means were determined using Tukey's Test.

RESULTS

Total Phenolic Content

Phenolic compounds influence the nutritional quality, biochemical functions, and pharmacological implications of foods.[12] Phenolics were detected in the aqueous acetic acid extracts of *I. batatas* leaves (Figure 1). The detection of phenolics in the *I. batatas* leaves is consistent with the findings of a previous study.[13] The results provide additional scientific basis to support the medicinal value of the *I. batatas* leaves as potential source of phenolic compounds which are known antioxidants.

Total Flavonoid Content

Flavonoids are polyphenolic compounds that exhibit biological activities such as antioxidant, anti-inflammatory, anti-hepatotoxic, and anti-ulcer among others.[14] The presence of flavonoids in the extracts of *I. batatas* leaves would imply that it can be a promising source of antioxidative compounds. The 5% aqueous acetic acid leaf extract gave the highest TFC (Figure 2).

Cytotoxicity

The brine shrimp lethality assay (BSLA) was carried out to evaluate the cytotoxicity of the aqueous acetic acid leaf extracts of *I. batatas*. It is a general bioassay that appears capable of detecting broad spectrum of bioactivity of the plant crude extract. BSLA has been routinely used in the primary screening of plant extracts as well as isolated compounds to provide an indication of possible cytotoxic properties of the test materials.[15] The percent mortality of the brine shrimp nauplii increases with increasing concentration of the test solutions in each extraction solvent (Table 1). Likewise, percent mortality of the nauplii apparently increases with increasing concentration of the extraction solvent in all test solution concentrations. The highest mortality values were consistently recorded for 5% aqueous acetic acid leaf extracts while the 1% gave the lowest values.

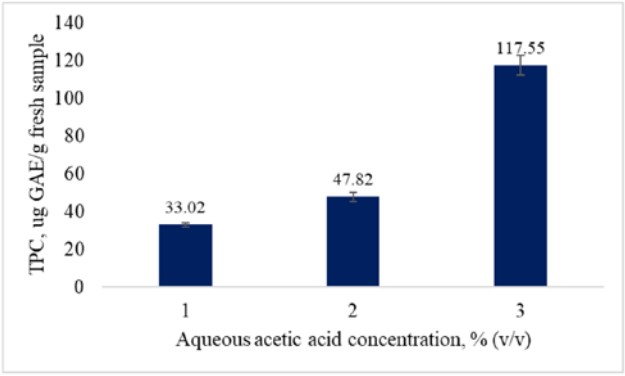

Figure 1: Total Phenolics Content (TPC) of the *I. batatas leaves* extracted with varying concentration of acetic acid. Error bars are standard error of the mean (*n*=9).

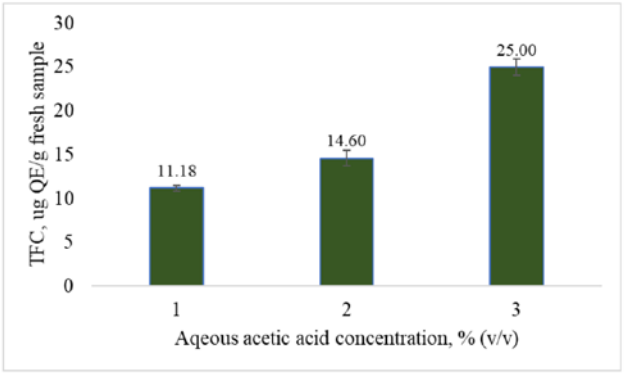

Figure 2: Total Flavonoid Content (TFC) of the I. batatas leaves extracted with varying concentration of acetic acid. Error bars are standard error of the mean (*n*=9).

DISCUSSION

Total Phenolic Content

Among the aqueous acetic acid extracts, the 5% gave the highest phenolic content, followed by the 3%, then the 1%. The results of the ANOVA indicated significant differences in the phenolic content among the *I. batatas* leaf extracts. Subsequent Post hoc Tukey's test revealed that the TPC in 5% aqueous acetic acid extract is significantly higher compared to that of 3% and 1%.

Table 1: LC_{50} value of the leaf extracts and the percent mortality of the brine shrimp nauplii after 24hr exposure to I. batatas aqueous acetic acid leaf extracts.

Acetic Acid Concentration, %	MORTALITY, %						LC_{50}	*Inference
	5 mg/L	10 mg/L	50 mg/L	100 mg/L	500 mg/L	1000 mg/L		
1	0.60	1.20	9.20	12.50	19.20	23.30	>1000	non-cytotoxic
3	0.60	3.33	17.50	26.70	39.00	47.50	>1000	non-cytotoxic
5	3.33	12.50	24.20	36.70	49.20	57.50	520.61	cytotoxic
Positive Control ($K_2Cr_2O_7$)	27.50	45.00	65.00	99.40	99.40	99.40	12.10	cytotoxic

*LC_{50} of less than 1000 mg/L is cytotoxic[11]

The present findings imply that the amounts of phenolic compounds extracted from the leaves of *I. batatas* is dependent on the concentration of aqueous acetic acid. Increasing trend on the extractability of the phenolic compounds with increasing concentration of aqueous acetic acid was observed. A number of studies have reported that solvent polarity plays a key role in enhancing phenolic solubility. Accordingly, the extractability of the phenolic compounds generally increases with increasing polarity of the solvents.[16] In a study conducted on the effects of solvent polarity in the phenolics extraction, it has been shown that higher content of polyphenols is obtained with an increase in the polarity of the solvent.[17]

The use of acetic acid for extraction of antioxidants of phenolic nature is justified by its relatively high polarity compared to other organic solvents. Higher polarity solvents are more useful for extracting phenolics from protein matrices since they appear to degrade the phenolic-protein complexes.[18] Moreover, the addition of water to the extraction system improves the extraction yield of phenolics.[19] The increasing amount of acetic acid in water and the subsequent increase in the polarity of the extraction solvent may account for the improvement in the extractability of phenolic compounds from the leaves of *I. batatas*. Acetic acid, a weak acid, facilitates formation of hydrogen bonds while dissociated. As a consequence, enhanced molecular interaction occurs due to the formation of hydrogen bonds between the acetic acid and the oxygen-containing functional groups in the phenolics.[20] Although comparative studies on the effect of different solvents in the extractability of phenolic compounds have shown that methanol is a good solvent for extraction, practical consideration on safety, economy, and efficiency shows that the use of acetic acid can be a better option than the other organic solvents.

Total Flavonoid Content

ANOVA at 0.05 level of significance showed significant differences in the TFC among the aqueous acetic acid leaf extracts. Post hoc Tukey's test indicates that TFC in 3% and 1% aqueous acetic acid leaf extracts are statistically lower as compared to that of 5%. More flavonoids are extracted with increasing concentration of acetic acid. Extractability of flavonoids is, thus, affected by the difference in the polarity of the extracting solvents.

Interestingly, the same trend of results for TPC and TFC in the order of 5%>3%>1% aqueous acetic acid extract was observed. This may be accounted knowing that flavonoids comprise the largest subgroup of the phenolic compounds.[21]

Cytotoxicity

The exhibited activity among the extracts may be due to the phytochemicals present. However, the relatively high cytotoxicity of 5% aqueous acetic acid leaf extract than the 3% and 1% can be attributed to the increased polarity of the extraction solvent. The extraction of secondary metabolites is affected by solvent polarity. Phytochemicals that exhibit anti-tumor, anticancer, and anti-inflammatory effects such as phenolics and flavonoids,[22] which are polar in nature, are better extracted with polar solvents such as aqueous acetic acid.[23] Thus, the concentration of acetic acid utilized as the extraction solvent affects the extractability of the cytotoxic compounds in the *I. batatas* leaves.

CONCLUSION

The 5% aqueous acetic acid extract (LC_{50} = 520.61 mg/L) of *I. batatas* leaves exhibited cytotoxic activity while the 3% (LC_{50} >1000 mg/L) and 1% (LC_{50} >1000 mg/L) extracts were found non-cytotoxic. The TPC and TFC of 5% aqueous acetic acid leaf extracts were significantly higher than those of 3% and 1%.

Extractability of the phenolics, flavonoids and cytotoxic compounds in the *I. batatas* leaves is affected by the concentration of the acetic acid which is used as an extraction solvent. The 5% aqueous acetic acid showed to be more efficient than the 3% and 1%. Moreover, the use of acetic acid for the extraction of phenolics, flavonoids, and cytotoxic compounds from *I. batatas* leaves can be a better option than other organic solvents.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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