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Iron Chelating Activity of Ethanol Extracts From *Annona Muricata* (Guyabano), *Andrographis Paniculata* (Serpentina), and *Basella alba* (Alugbati) Leaves

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ABSTRACT—Limited studies explore the iron chelating activity of ethanol extracts from *A. muricata, A. paniculata,* and *Basella alba* leaves. This study aimed to determine the iron chelating activity of ethanolic extracts from *A. muricata, A. paniculata, and B. alba* leaves. Iron chelating activity was measured spectrophotometrically using a standard assay. Each extract was mixed with $FeSO_4$ and ferrozine, incubated for 10 minutes. The absorbance was recorded at 562 nm. The results of the study showed that the ethanol extracts of *A. muricata, A. paniculata*, and *B. alba* leaves exhibited iron chelating activity at concentrations of 1 mg/mL, 5 mg/mL, and 10 mg/mL when compared to the negative control. The negative control (distilled water) consistently showed no iron chelating activity (*mean* = 0.00, *SD* = 0.00), but their activities were not comparable to the positive control 1 mg/mL (*mean* = 12.62, *SD* = 0.06), 5 mg/mL (*mean* = 12.62, *SD* = 0.12), and 10 mg/mL (*mean* = 12.66, *SD* = 0.20). The 1 mg/mL of *A. paniculata* ethanolic leaf extract had better iron chelating activity when compared to the 1 mg/mL of *A. muricata* and *B. alba* ethanolic leaf extracts. The 5 mg/mL ethanolic extract of *A. paniculata* had better iron chelating activity than *A. muricata* and *B. alba*. In 10 mg/mL, *A. paniculata* had better iron chelating activity when compared to *A. muricata* and *B. alba*. While *A. muricata* had better iron chelating activity when compared to *B. alba*. These plants show potential as iron chelating agents for subjects for further studies.

Keywords: Iron chelating activity, A. muricata, A. paniculata, B. alba

I. INTRODUCTION

Iron overload occurs when excess iron accumulates in the body, leading to harmful effects such as organ damage and conditions like hemochromatosis. This condition is commonly associated with hemoglobinopathies such as thalassemias and structural hemoglobin variants. In the Philippines, hemoglobinopathy was the cause of anemia in 27.5% of anemic individuals, and α-thalassemia was the most common type (Capanzana *et al*., 2018). Sideroblastic anemia could also be one of the diseases related to iron overload. It is a form of anemia caused by improper use of iron in erythropoiesis. Various types of sideroblastic anemia exist, and each type is characterized by ring sideroblasts in the marrow. Ring sideroblasts are erythroid precursors with non-heme iron deposits in their mitochondria, creating a ring-shaped arrangement surrounding the nucleus. Sideroblastic anemia is commonly found in male patients, which can be hereditary or acquired (Ashorobi & Chhabra, 2023). Iron overload can either be hereditary or acquired. Hereditary hemochromatosis is rare among Asians, including Filipinos, while acquired causes, such as frequent blood transfusions, are more common. Research on pregnant Filipino women revealed that single-nucleotide polymorphisms in genes such as TMPRSS6 and TF may act as genetic risk factors for anemia and iron deficiency, highlighting a complex interaction between genetics and iron metabolism (Capanzana & Aguila, 2018).

Chelation can clear away the surplus iron load and keep iron at natural levels. The treatment of patients with iron chelators can decrease the toxic effects of iron overload. (Entezari et al., 2022). Iron overload treatment may also lead to iron toxicity in an individual without proper management. Iron toxicity is categorized as either corrosive or cellular. Consumed iron may lead to direct corrosive damage to the gastrointestinal lining, causing nausea, vomiting, stomach pain, and diarrhea. Substantial fluid and blood loss may result in hypovolemia. Hemorrhagic necrosis of the gastrointestinal mucosa can result in hematemesis, perforation, and peritonitis (Yuen et al., 2023). Certain phytochemicals, natural compounds found in plants, have shown the ability to chelate iron effectively (Wong et al., 2014).

Plants contain a diverse range of bioactive compounds, many of which have demonstrated significant iron-chelating properties. Flavonoids, for example, possess multiple hydroxyl groups that can bind iron ions, reducing their availability and toxicity (Effiong *et al*., 2024). Tannins, known for their astringent properties, form strong complexes with metal ions, including iron (Govindarajan *et al*., 2023). Alkaloids, another class of bioactive compounds, have shown promise as metal chelators in various studies (Arawande *et al*., 2024). These compounds not only help in chelation but also exhibit antioxidant properties, providing additional benefits in conditions associated with oxidative stress caused by iron overload. *A. muricata*, *A. paniculata*, and *B. alba* are medicinal plants known for their therapeutic properties. They contain bioactive compounds such as flavonoids, alkaloids, and tannins, which are potential iron chelators (Dedvisitsakul & Watla-Iad, 2022) (Gavamukulya, Abou-Elella, Wamunyokoli & AEl-Shemy, 2014) (Widjajakusuma *et al*., 2019).

This study aimed to determine the iron chelating properties of *A. muricata*, *A. paniculata*, and *B. alba* leaves ethanolic leaf extract The study provided insight into their potential as natural treatments for iron-related disorders when subjected to more *in vivo* studies

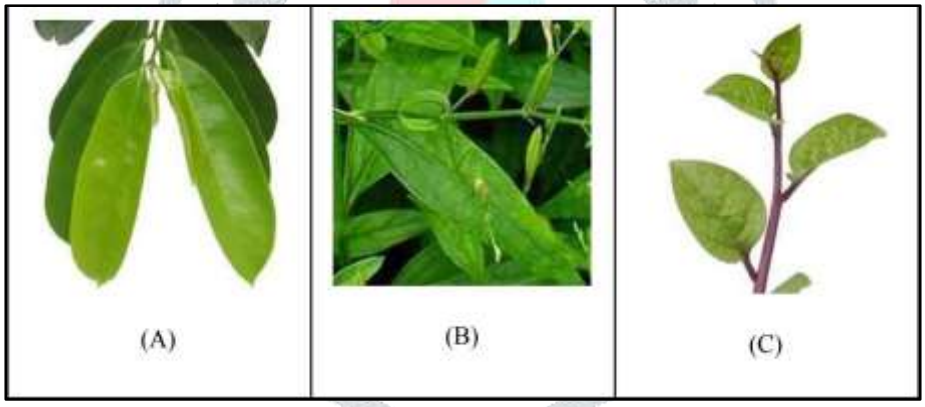
II. MATERIALS AND METHODS

Data Collection Procedures with Parameters to Measure

Plant Identification

A letter for plant identification was submitted to the Department of Agriculture, together with the sample and photographs (Figure 1).

Figure 1.
A. muricata (A), A. paniculata (B), and B. alba (C) plants.



(A) A. muricata is a small tree, 5 to 7 meters in height. The leaves are oblong-obovate, 7 to 20 centimeters in length, pointed at both ends, smooth, shining, and usually with petioles about 5 millimeters long. (Quisumbing, 1951).

A. muricata belongs to the Kingdom Plantae, Subkingdom Tracheobionta, Superdivision Spermatophyta, Division Magnoliophyta, Class Magnoliopsida, Subclass Magnoliidae, Order Magnoliales, Family Anonaceae Juss., and Genus Anona Linnaeus.

(B) A. paniculata, family Acanthaceae, is an annual herb that grows up to 1 meter in height. Leaves are simple, opposite, and exstipulate. The blade is dark green, bitter, glossy, simple, lanceolate, opposite, and 4-8 centimeters by 1.3-2.5 centimeters (Koh et al., 2009).

A. paniculata Kingdom Plantae, Subkingdom Tracheobionta, Superdivision Spermatophyta, Division Magnoliophyta, Class Magnoliopsida, Subclass Asteridae, Order Scrophulariales, Family Acanthaceae Juss., Genus Andrographis Wall. ex Nees.

(C) B. alba, also known as Malabar spinach, is a perennial plant in the Basellaceae family, widely grown in tropical regions for its edible and nutritious leaves. Taxonomically, it belongs to the Caryophyllales order and the eudicots clade. (Eze & Kanu, 2014).

B. alba belongs to the Kingdom Plantae, Subkingdom Tracheobionta, Superdivision Spermatophyta, Division Magnoliophyta, Class Magnoliopsida, Subclass Caryophyllidae, Order Caryophyllales, Family Basellaceae Raf., and Genus Basella L.

Sample Collection

Two kilograms of matured fresh leaves of *A. muricata*, *A. paniculata*, and *B. alba* were collected from the rural areas 100 meters away from the road at around 5:00 AM - 6:00 AM before sunrise, and the samples are free from any insect bites.

Preparation of Plant Extracts

The mature, fresh leaves of *A. muricata*, *A. paniculata*, and *B. alba* were thoroughly washed, rinsed with distilled water, and soaked overnight in distilled water (Klahs *et al*., 2023). Following soaking, excess water was drained, and the leaves were air-dried overnight (Lekjing *et al*., 2024). The air-dried leaves were subsequently blended to produce a fine homogenate.

A 90% ethanol solution was prepared by mixing 937.5 mL of absolute ethanol with 62.5 mL of distilled water. This solution was shaken for two minutes to ensure uniformity. A total of 250 grams of the blended leaf material was homogenized in 625 mL of the prepared 90% ethanol solution, maintaining a 2:5 (w/v) ratio. The mixture was then soaked for 72 hours at a refrigerated temperature.

After soaking, the plant homogenates were filtered using a muslin cloth to remove solid residues. The filtrates were subjected to rotary evaporation at 40°C to remove the ethanol content. Following evaporation, the concentrated extracts were transferred into scintillation vials for storage and subsequently dried using nitrogen gas to yield the crude ethanolic extracts of A. muricata, A. paniculata, and B. alba (Johnson, 2024). These crude extracts were used for the subsequent iron chelating activity assay (Leong-on, 2022).

Preparation of Extract Concentrations

The crude ethanolic extracts were reconstituted to prepare standard concentrations of 1 mg/mL, 5 mg/mL, and 10 mg/mL for each plant species. For *A. muricata*, 0.020 mg, 0.100 mg, and 0.200 mg of the crude extract were each diluted in 20 mL of distilled water to achieve the respective concentrations. For *A. paniculata*, the same amounts of crude extract were each diluted in 20 mL of dimethyl sulfoxide (DMSO). For B. alba, 0.020 mg, 0.100 mg, and 0.200 mg of the crude extract were each diluted in 20 mL of distilled water. Each solution was vortexed thoroughly until a homogeneous solution was achieved.

Preparation of Reagents

The ferrozine reagent was prepared by dissolving 6.155 mg of ferrozine in 50 mL of distilled water to yield a 0.25 mM solution. The ferrous sulfate ($FeSO_4$) reagent was prepared by dissolving 1.04 mg of $FeSO_4$ in 50 mL of distilled water to produce a 0.1M solution.

Preparation of Controls

To prepare the positive control solutions for the iron chelation assay, 50 mg, 250 mg, and 500 mg of EDTA were accurately weighed and dissolved in separate 50 mL volumes of distilled water to obtain 1 mg/mL, 5 mg/mL, and 10 mg/mL solutions, respectively. Complete dissolution was ensured by stirring the mixtures thoroughly. For the control, distilled water was used.

Iron Chelating Activity Assay

To assess iron chelating activity, 200 µL of each plant extract concentration (1 mg/mL, 5 mg/mL, and 10 mg/mL) was mixed with 200 µL of 0.1 mM ferrous sulfate solution and 400 µL of 0.25 mM ferrozine solution. The reaction mixtures were incubated at room temperature for 10 minutes. The absorbance of each solution was then measured using a UV-Visible spectrophotometer at a wavelength of 562 nm, a wavelength specific for measuring the absorbance of ferrozine. The iron chelation activity was determined by comparing the absorbance values against those of the controls. Each concentration was tested in three independent trials, with three replicates per trial, to ensure the reproducibility and reliability of the results.

Determination of Iron Chelating Activity

Chelating activity was calculated using the following formula:

$$\text{Iron chelating activity} = \frac{(A_{control} - A_{sample})}{A_{control}} \times 100$$

Where $A_{control}$ is the absorbance of the control reaction (without plant extract), and A_{sample} is the absorbance in the presence of a plant extract. (Wong et al., 2014). A decrease in absorbance indicates successful chelation, as the plant extract binds free ferrous ions in solution, thus preventing ferrous interactions with ferrozine, forming a vividly colored complex that serves as an indication of the result. Conversely, a higher absorbance indicates weaker or no chelating activity, as more ferrous ions remain available to interact with ferrozine and form the complex (Wong *et al*., 2014; Zenbio, 2022; Ouahhoud *et al*., 2022).

Statistical Analysis

The statistical analysis employed mean, standard deviation, One-Way Analysis of Variance (ANOVA), and the post hoc test, as Tukey's Honestly Significant Difference (HSD) test, $p \leq 0.05$.

Waste Containment

The Standard Operating Procedures (SOPs) of the laboratory and the institution were followed for proper waste disposal in this research. This ensures that all plant residues are disposed of in bio-waste containers that are designated for that purpose, and that chemical waste from the extraction process is collected in hazardous waste containers that are appropriately labeled. In order to dispose of hazardous materials safely and in accordance with institutional and local guidelines, the laboratory works with the environmental health and safety office of the institution.

III. RESULTS AND DISCUSSIONS

Results

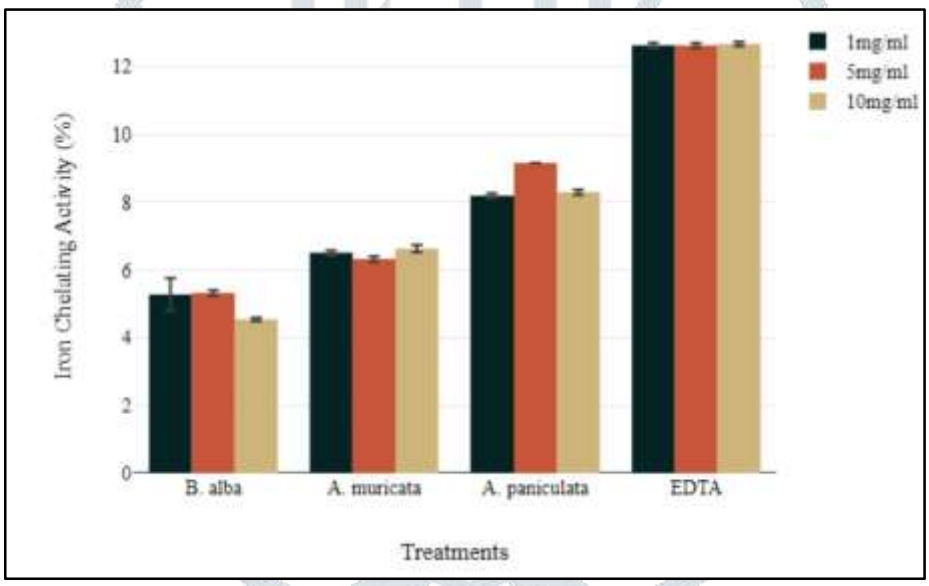
The results of the study showed that the ethanol extracts of *A. muricata, A. paniculata,* and *B. alba* leaves exhibited iron chelating activity at concentrations of 1 mg/mL, 5 mg/mL, and 10 mg/mL when compared to the negative control. The negative control (distilled water) consistently showed no iron chelating activity (*mean* = 0.00, *SD* = 0.00). *A. paniculata* showed the chelating activity at all concentrations: 1 mg/mL (*mean* = 8.20, *SD* = 0.04), 5 mg/mL (*mean* = 9.17, *SD* = 0.00), and 10 mg/mL (*mean* = 8.30, *SD* = 0.00). In determining the best concentration for each plant extract, *A. paniculata* showed the highest iron chelating activity at 5 mg/mL (*mean* = 9.17, *SD* = 0.00). When compared to the positive control (EDTA) (1 mg/mL (mean = 12.62, SD = 0.06), 5 mg/mL (mean = 12.62, SD = 0.12), and 10 mg/mL (*mean* = 12.66, SD = 0.20). The iron chelating activity of all plant extracts at different concentrations was not comparable. *A. muricata* had iron chelating activity at all concentrations, 1 mg/mL (mean = 6.52, SD = 0.00), 5 mg/mL (*mean* = 6.33, *SD* = 0.00), and 10 mg/mL (*mean* = 6.64, *SD* = 0.00) when compared to the negative control but not comparable to the positive control. The best concentration that exhibited iron chelating activity was 10 mg/mL.

B. alba, 1 mg/mL (*mean* = 5.28, *SD* = 0.00), had iron chelating activity together with 5 mg/mL (*mean* = 5.32, *SD* = 0.14). The 10 mg/mL (*mean* = 4.54, *SD* = 0.00) has the highest iron chelating activity among the three concentrations. Its iron chelating activity is dose-dependent but not comparable with the positive control EDTA

The 1 mg/mL of *A. paniculata* ethanolic leaf extract had better iron chelating activity when compared to the 1 mg/mL of A. muricata and B. alba ethanolic leaf extracts. The 5 mg/mL ethanolic extract of *A. paniculata* had better iron chelating activity than *A. muricata* and *B. alba*. In 10 mg/mL, *A. paniculata* had better iron chelating activity when compared to *A. muricata* and *B. alba*. A. *muricata* had better iron chelating activity when compared to *B. alba* (Figure 1).

Figure 1

Iron chelating activity of ethanolic extracts at different concentrations from A. muricata, A. paniculata, and B. alba leaves and EDTA.



Note: Data are reported as mean and SD values

Discussions

The results of the study showed that the ethanol extracts of *A. muricata, A. paniculata,* and *B. alba* leaves exhibited iron chelating activity at concentrations of 1 mg/mL, 5 mg/mL, and 10 mg/mL. Iron overload occurs when the body builds up excessive amounts of iron. In our search for potential iron chelators, three medicinal plants, *A. muricata, A. paniculata*, and *B. alba* leaves, showed promising iron chelating activity; they have the capacity to remove excess iron.

The high iron chelating activity of *A. muricata, A. paniculata,* and *B. alba* may be attributed to its phytochemical compositions, particularly the presence of flavonoids, alkaloids, and tannins. These compounds are known for their iron-chelating properties, which may explain their strong activity as a result of *A. paniculata* (Dedvisitsakul & Watla-Iad, 2022) (Gavamukulya, Abou-Elella, Wamunyokoli & AEl-Shemy, 2014) (Widjajakusuma et al., 2019).

A previous study has shown that plants contain a diverse range of bioactive compounds, many of which have demonstrated significant iron-chelating properties. Certain phytochemicals, natural compounds found in plants, have shown the ability to chelate iron effectively. The iron chelating properties of *A. muricata, A. paniculata,* and *B. alba* leaves are grounded in the plant's iron deposition inhibitory constituents and bioactive compounds, which include flavonoids, alkaloids, and tannins (Dedvisitsakul & Watla-Iad, 2022) (Gavamukulya, Abou-Elella, Wamunyokoli & AEl-Shemy, 2014) (Widjajakusuma *et al*., 2019) (Wong *et al*., 2014). *A. muricata, A. paniculata,* and *B. alba* had iron chelating activity like *C. nutans, H. diffusa, C. formosana,* and *L. cardiaca* (Wong *et al*., 2014).

While this study provides insights into the iron-chelating abilities of crude ethanolic extracts of *A. muricata, A. paniculata,* and *B. alba* leaves, it does not identify the specific bioactive compounds responsible for these activities. Additionally, the results were based on in vitro assays, and further in vivo studies in animals or humans are needed to confirm the medicinal effects. The mature

fresh leaves of *A. muricata, A. paniculata,* and *B. alba* were collected from the rural areas 100 meters away from the road at around 5:00 AM - 6:00 AM before sunrise, and the samples were free from any insect bites.

The iron-chelating activities observed in *A. paniculata, A. muricata,* and *B. alba* suggest that these medicinal plants could be explored as natural alternatives for iron overload diseases. Their potential applications in the pharmaceutical and nutraceutical industries warrant further investigation to develop functional food products or therapeutic agents with iron-chelating benefits.

IV.CONCLUSION

The results of the study showed that the ethanol extracts of A. muricata, A. paniculata, and B. alba leaves exhibited iron chelating activity at concentrations of 1 mg/mL, 5 mg/mL, and 10 mg/mL when compared to the negative control. The 1 mg/mL of *A. paniculata* ethanolic leaf extract had better iron chelating activity when compared to the 1 mg/mL of A. muricata and B. alba ethanolic leaf extracts. The 5 mg/mL ethanolic extract of *A. paniculata* had better iron chelating activity than *A. muricata* and *B. alba*. In 10 mg/mL, *A. paniculata* had better iron chelating activity when compared to *A. muricata* and *B. alba*. While A. muricata had better iron chelating activity when compared to *B. alba*. These plants show potential as natural supplementary agents for managing iron overload, especially in resource-limited settings after conducting their therapeutic and nutraceutical applications.

V.RECOMMENDATIONS

Based on the study's findings, further research is recommended to investigate the therapeutic potential of *A. muricata, A. paniculata,* and *B. alba*. Specifically, *A. paniculata* showed the most promising iron-chelating activity among the tested extracts. Future studies should focus on isolating and identifying the specific bioactive compounds responsible for this activity using phytochemical and chromatographic analyses. Understanding the extract's mechanism of action at the molecular level, particularly its interaction with iron and other metal ions, is essential. *In vivo* studies are also necessary to evaluate the efficacy, bioavailability, and safety of the extracts under physiological conditions, along with toxicological assessments to determine cytotoxicity and biocompatibility.

Additionally, confirmatory testing using techniques such as Atomic Absorption Spectroscopy (AAS) is encouraged to validate the iron-chelating capabilities of *A. muricata, A. paniculata,* and *B. alba*. Finally, future research may explore the development of appropriate dosage forms to support the clinical application of these extracts in pharmaceutical or nutraceutical settings.

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