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Proceeding Paper

Assessment of Bioactive Compounds, Antioxidant, Anti-Inflammatory, and Antidiabetic Potential of Hydro-Methanolic Extracts Derived from Fresh Noni (*Morinda citrifolia* L.) Fruits Growing in Sri Lanka †

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Abstract: *Morinda citrifolia* L., or Noni, thrives in tropical and sub-tropical regions globally, garnering interest as a bioactive source. Despite Sri Lanka's myths, Noni's potential remains underutilized. The United States commercialized Noni products in the early 1990s, introducing Noni juice as a wellness drink in 1996. This study assessed Sri Lankan Noni fruit's functional properties through methanolic extraction and various assays, revealing notable antioxidant, anti-inflammatory, and anti-diabetic potential. Methanolic-extracted fresh Noni fruits may serve as natural sources of antioxidants and anti-inflammatory agents. Exploring specific bioactive compounds could yield innovative treatments for oxidative stress, inflammation, and diabetes-related conditions.

Keywords: Noni fruit; bioactive properties; Sri Lanka

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1. Introduction

Morinda citrifolia L., locally known as Ahu or Noni in Sri Lanka, has gained global recognition for its significant role in a wide range of therapeutic activities (Ali et al., 2016). Noni fruit has been deemed safe for human consumption by the European Union (European Commission, 2002), and there is no established causal relationship between noni juice consumption and acute hepatitis (European Food Safety Authority, 2006). A wide array of value-added products, encompassing Noni juice, capsules, powder, concentrates, tea, and others, derived from various components of *Morinda citrifolia*, have become commercially available (Kulathunga & LDAM, 2017), while Noni products, predominantly in the form of juices and dietary supplements (Motshakeri & Ghazali, 2015), have achieved widespread global accessibility, and the fruit itself has been increasingly utilized for the production of dietary supplements in recent times (Almeida et al., 2019). Despite extensive global research on Noni fruits, only a limited number of interventions have been conducted in Sri Lanka (Samarasiri et al., 2019), and this study aims to evaluate the functional properties of Noni fruits cultivated in Sri Lanka.

2. Methodology

Ripen fruits were obtained from trees grown in the *Katugathota* area of the Kandy district, Sri Lanka. The fruits selected based color and shape were vacuum packaged in polyethylene bags and stored at −18 °C until further analysis.

For quantitative determination of proximate composition of *Morinda citrifolia* (L.) fruit and seed samples, moisture was determined using hot air oven (Association of Official Analytical Chemists; AOAC 930.04, 1990), dried leaf samples at 105 °C, until constant weight. Crude protein was determined according to the Kjeldahl method (AOAC 978.04, 1990); total nitrogen was multiplied by a protein factor of 6.25. Total fat was determined according to the acid hydrolysis method (AOAC 948.15, 1990), using Soxhlet extractor at 60 °C, until constant weight. Dietary fiber was determined according to the enzymatic gravimetric method (AOAC 930.10, 1990). Ash was determined according to gravimetric method (AOAC 930.05, 1990), incinerated leaf samples at 550 °C, until constant weight. Total carbohydrate was determined according to the difference method by calculation (Rybak-Chmielewska, 2003).

Methanolic extraction of fresh Noni fruits was prepared according to the method described in (Gunathilake, Somathilaka Ranaweera, et al., 2018) with slight modifications. One gram of fresh fruit samples was weighed and mixed with 10 mL of 80% methanol and vortexed at high speed for thirty minutes and then centrifuged (Hettich, EBA 20, Tuttlingen, Germany) for 10 min at 792 g. The extraction was subsequently filtered through a filter paper (Whatman No. 42; Whatman Paper Ltd., Maidstone, UK). The crude extract was desolventizing in a rotary evaporator (HAHNVAPOR, Model HS-2005 V, HAHNSHIN Scientific, Kyonggi-do, Republic of Korea) at 40 °C. The total polyphenol content of the methanolic extraction was estimated by the Folin–Ciocalteu method explained by (Singleton et al., 1999). The total antioxidant capacity of noni extraction was analyzed according to the modified method described by (Gunathilake et al., 2019). A modified thiobarbituric acid reactive substances (TBARS) assay was employed to measure the level of lipid peroxide formed in egg homogenates as lipid-rich media, following the method described by (Ohkawa et al., 1979). The ability of the prepared extracts to scavenge the 'stable' free radical DPPH was monitored according to the modified method (Gunathilake & Ranaweera, 2016). The ABTS free radical scavenging activity was done using the methodology of (Prieto et al., 1999) with some modifications. The antioxidant capacity of noni extracts was assessed using the FRAP assay, following the method of (Gunathilake and Rupasinghe, 2014) with certain modifications. The Singlet Oxygen Scavenging Assay was conducted in accordance with the protocol outlined in (Gunathilake, Somathilaka Ranaweera, et al., 2018).

In vitro anti-inflammatory activity was assessed through three membrane lysis assays, namely the Preparation of Erythrocyte Suspension, Heat-Induced Hemolysis, and the Effect on Protein Denaturation, following the methods elucidated by (Gunathilake, Ranaweera, et al., 2018) with some modifications. Furthermore, Nitric oxide inhibition activity was performed as reported by (Kumari & Gunathilake, 2020).

The anti-diabetic potential was evaluated using two diabetic assays: alpha-amylase inhibitory activity, following the methodology outlined by (Poovitha & Parani, 2016), and alpha-glucosidase inhibitory activity, as assessed through the methodology detailed in (Kim et al., 2004).

3. Results

The proximate chemical composition analysis of Noni fruit was conducted, and the results for the fresh part were revealed. It was observed that the moisture content was found to be approximately 89.20 ± 1.98% per 100 g of fresh weight, indicating a high-water content. In contrast, the fruit had a relatively low crude fat content, which was measured at 0.16 ± 0.01% per 100 g of fresh weight. The protein content was also relatively low, with a measurement of 0.64 ± 0.12%. Noni fruit exhibited moderate levels of crude fiber at 1.97

± 0.31% and crude carbohydrate content at 6.94 ± 1.98% per 100 g of fresh weight. Additionally, the ash content was found to be 1.08 ± 0.06% per 100 g of fresh weight, indicating the mineral composition of the fruit. These findings provided insights into the nutritional composition of fresh Noni fruit.

The significant total phenolics content was determined at 198.60 ± 2.48 μmol Gallic Acid Equivalent per 1 g of fresh weight in the methanolic extract of fresh noni fruits. Furthermore, a series of IC50 values were obtained in various assays, demonstrating the extract's antioxidant potential. The Total Antioxidant Capacity was measured at 33.96 ± 0.30 μg/mL, while the IC50 value for DPPH Scavenging Activity was determined as 35.87 ± 0.48 μg/mL. Additionally, the ABTS Scavenging Activity exhibited an IC50 value of 24.36 ± 0.42 μg/mL. Lipid Peroxidation Inhibition Activity was notably effective, with an IC50 value of 77.42 ± 0.84 μg/mL, and the inhibition of Singlet Oxygen had an IC50 value of 6.91 ± 0.24 μg/mL. In the Ferric Reducing Antioxidant Power Assay (FRAP assay), an IC50 value of 45 ± 0.81 μg/mL was recorded. Significant anti-inflammatory potential was also observed, with IC50 values for Nitric oxide Inhibition Activity measured at 73.40 ± 1.20 μg/mL, Heat-Induced Hemolysis Inhibition at 9.40 ± 0.80 μg/mL, Protein Denaturation Inhibition at 4.81 ± 0.21 μg/mL, and Proteinase Inhibitory Activity at 9.12 ± 0.89 μg/mL. Additionally, anti-diabetic activities were evaluated, with IC50 values of 13.40 ± 0.20 μg/mL for Alpha-Amylase Inhibitory Activity and 6.92 ± 0.34 μg/mL for Alpha-Glucosidase Inhibitory Activity.

4. Conclusions

In summary, this study has confirmed that the extract from fresh noni fruits, obtained using methanol, has impressive health benefits. The extract is shown to be a powerful antioxidant, effectively combatting harmful molecules called free radicals and preventing damage to fats in our bodies. Additionally, it has demonstrated its ability to reduce inflammation, which is linked to various health issues. Furthermore, it exhibits potential in managing diabetes by blocking certain enzymes involved in sugar metabolism. These findings emphasize that methanol-extracted fresh noni fruits can serve as a valuable source of natural antioxidants and anti-inflammatory substances with various health uses. Further research aimed at pinpointing the specific active compounds responsible for these effects could lead to new treatments and healthy food products for conditions related to oxidative stress, inflammation, and diabetes.

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References

1. Ali, M.; Kenganora, M.; Manjula, S.N. Health benefits of morinda citrifolia (Noni): A review. *Pharmacogn. J.* **2016**, *8*, 321–334. https://doi.org/10.5530/pj.2016.4.4.
2. Almeida, É.S.; de Oliveira, D.; Hotza, D. Properties and Applications of Morinda citrifolia (Noni): A Review. *Compr. Rev. Food Sci. Food Saf.* **2019**, *18*, 883–909. https://doi.org/10.1111/1541-4337.12456.
3. Gunathilake, K.D.P.P.; Ranaweera, K.K.D.S. Antioxidative properties of 34 green leafy vegetables. *J. Funct. Foods* **2016**, *26*, 176–186. https://doi.org/10.1016/j.jff.2016.07.015.
4. Gunathilake, K.D.P.P.; Ranaweera, K.K.D.S.; Rupasinghe, H.P.V. In vitro anti-inflammatory properties of selected green leafy vegetables. *Biomedicines* **2018**, *6*, 107. https://doi.org/10.3390/biomedicines6040107.
5. Gunathilake, K.D.P.P.; Ranaweera, K.K.D.S.; Rupasinghe, H.P.V. Response surface optimization for recovery of polyphenols and carotenoids from leaves of Centella asiatica using an ethanol-based solvent system. *Food Sci. Nutr.* **2019**, *7*, 528–536. https://doi.org/10.1002/fsn3.832.

6. Gunathilake, K.D.P.P.; Ranaweera, K.K.D.S.; Rupasinghe, H.P.V. Effect of Different Cooking Methods on Polyphenols, Carotenoids and Antioxidant Activities of Selected Edible Leaves. *Antioxidants* **2018**, *7*, 117. https://doi.org/10.3390/antiox7090117.
7. European Commission. 2002. "Scientific Committee on Food. Opinion of the Scientific Committee on Food on Tahitian Noni ® Juice." SCF/CS/NF/DOS/18 ADD 2 Final (December): 1–68.
8. European Food Safety Authority. Opinion on a Request from the Commission Related to the Safety of Noni Juice (Juice of the Fruits of Morinda Citrifolia). *Eur. Food Saf. Auth. J.* **2006**, *376*, 1–12.
9. Gunathilake, K.D.P.P.; Rupasinghe, H.P.V. Optimization of Water Based-extraction Methods for the Preparation of Bioactive-rich Ginger Extract Using Response Surface Methodology. *Eur. J. Med. Plants* **2014**, *4*, 893–906. https://doi.org/10.9734/ejmp/2014/10322.
10. Kim, Y.M.; Wang, M.H.; Rhee, H.I. A novel α-glucosidase inhibitor from pine bark. *Carbohydr. Res.* **2004**, *339*, 715–717. https://doi.org/10.1016/j.carres.2003.11.005.
11. Kulathunga, S.; Ldam, A. Morinda citrifolia Linn Grown in Sri Lanka: Shelf Life of Fruit Juice. *Am. J. Ethnomed.* **2017**, *4*, 18. https://doi.org/10.21767/2348-9502.1000018.
12. Kumari, G.U.W.U.P.; Gunathilake, K.D.P.P. In vitro bioaccessibility and antioxidant activity of black plum (*Syzygium caryophyllatum*). *J. Food Biochem.* **2020**, *44*, e13499. https://doi.org/10.1111/jfbc.13499.
13. Motshakeri, M.; Ghazali, H.M. Nutritional, phytochemical and commercial quality of Noni fruit: A multi-beneficial gift from nature. *Trends Food Sci. Technol.* **2015**, *45*, 118–129. https://doi.org/10.1016/j.tifs.2015.06.004.
14. Ohkawa, H.; Ohishi, N.; Yagi, K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.* **1979**, *95*, 351–358. https://doi.org/10.1016/0003-2697(79)90738-3.
15. Poovitha, S.; Parani, M. In vitro and in vivo α-amylase and α-glucosidase inhibiting activities of the protein extracts from two varieties of bitter gourd (*Momordica charantia* L.). *BMC Complement. Altern. Med.* **2016**, *16* (Suppl. 1), 185. https://doi.org/10.1186/s12906-016-1085-1.
16. Prieto, P.; Pineda, M.; Aguilar, M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Anal. Biochem.* **1999**, *269*, 337–341. https://doi.org/10.1006/abio.1999.4019. https://doi.org/10.1037/a0037168.
17. Rybak-Chmielewska, H. AOAC (1990) Official Method of Analysis of the Association of Official Analytical Chemists. In *Chemical and Functional Properties of Food Saccharides*; Publisher: Location, 2003; Volume 1. https://doi.org/10.7312/seir17116-004.
18. Samarasiri, M.H.; Chandrasiri, T.A.; Wijesinghe, D.B.; Gunawardena, S.P. Antioxidant Capacity and Total Phenolic Content Variations against *Morinda citrifolia* L. Fruit Juice Production Methods. *ETP Int. J. Food Eng.* **2019**, *5*, 293–299. https://doi.org/10.18178/ijfe.5.4.293-299.
19. Singleton, V.L.; Orthofer, R.; Lamuela-Raventós, R.M. Analysis of Total Phenols and Other Oxidation Substrates and Antioxidants by Means of Folin-Ciocalteu Reagent. *Sci. Hortic.* **1999**, *213*, 281–286. https://doi.org/10.1016/j.scienta.2016.11.004.