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Comparison of Antioxidant, Anti-inflammatory, and Antidiabetic, Potential of Hydro-methanolic Extracts Derived from Dried Noni (*Morinda citrifolia* L.) Fruits and Seeds Growing in Sri Lanka

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Abstract: *Morinda citrifolia* L, commonly known as Noni or 'Ahu' in Sri Lanka, has traditionally been used for medicinal and black magic practices. However, Noni also has therapeutic benefits and is used in various products like fresh juice, nutraceuticals, wine, powder, and puree. This study aimed to compare the bioactive compounds, antioxidant, anti-inflammatory, and antidiabetic potential of dried Noni fruit and seeds using spectroscopic methods. Noni seeds exhibit significant antioxidant properties like dried Noni fruit. They also possess antidiabetic and anti-inflammatory potential, making them valuable for food production, suggesting their utilization alongside Noni fruit-based products in Sri Lanka.

Keywords: noni; bioactives; therapeutic properties

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1. Introduction

Commonly known as Noni (Hawaiian) or 'Ahu' in Sri Lanka has two recognized species as Morinda citrifolia (Linn.) and Morinda tinctoria (Roxb.). Morinda citrifolia is the most growing variety and it belongs to genus Morinda in the family Rubiaceae[1]. It is, grown everywhere in Sri Lanka without any climatic difference. Noni fruits are harvested throughout the year, although there are seasonal patterns in flowering and fruit bearing[2]. After planting, the fruits set in 9 months to 1 year. The unripe fruit is dark green in color, and the ripe fruit is lumpy, green to yellowish white in colour and 5 to 10 cm in length in length and 3–6 cm in width and contains up to 260 seeds[3]. Which has an outer surface covered in polygonal-shaped sections. The ripe fruit has a foul odour and taste and, and the pulp has a light dull yellowish white color[4].

Phytoconstituents present in different ripening stages of Noni fruits expressed different ethnobotanic uses. Numerous scientific studies have demonstrated the pharmacological activity of Noni fruit, which has been employed in various types of cancer, including colon, esophageal, breast, and colorectal cancers, as well as cardiovascular diseases, diabetes, arthritis, and hypertension. These findings are supported by preclinical and/or clinical investigations[5]. Noni fruit was reported to be acceptable for human consumption, based on official safety evaluations done by the European Union[6]. Genotoxicity studies, which included an in vitro Ames test, a chromosomal aberration test, and an in

vivo micronucleus test, demonstrate that noni fruits and seeds do not exhibit mutagenic or clastogenic properties. Therefore, noni has the potential to be safely utilized as a therapeutic agent for nutraceutical and pharmaceutical development[3].

Over the past decade, the noni fruit juice industry has experienced significant growth, with numerous producers worldwide. Noni fruit puree is also among the largest agricultural exports to the United States. Concentrated noni fruit juice and puree have furthermore been utilized as innovative food ingredients in a diverse range of food products. Following the industrial production of noni juice, powder, and puree, the residues are predominantly composed of seeds that are typically discarded as waste products. Recent reports indicate that cultivation of M. citrifolia L. on 1 ha of land can yield approximately 35 tons of noni juice. As a result, significant quantities of noni seeds can be obtained at a relatively low production cost [7,8]. Scientific evidence demonstrates that extracts derived from noni seeds contain bioactive compounds that possess a broad range of health-promoting properties, including antioxidant, anti-mutagenic, anti-tumor, anti-inflammatory, anti-allergic, anti-viral, anti-fungal, anti-microbial, and anti-carcinogenic activities. Therefore, noni fruit seed, which is rich in bioactive compounds, has the potential to serve as an excellent source of functional foods[7].

Noni seeds, which used to be considered waste in the noni fruit juice industry, can now be extracted for their oil through a newly developed process, despite the fact that each noni fruit typically contains 200-250 seeds[9]. The annual production of noni seeds exclusively by Polynesians in French Polynesia is more than 150 metric tons [10]. Noni seeds are classified as food by-products and have low economic value; additionally, food industries are typically obligated to expend substantial resources to dispose of these by-products (including drying, storing, and shipping them), which may result in increased costs for fruit products[11]. The assessment of bioactive compounds, identification of potential health benefits, and transformation of food by-products into economically viable and healthy products could effectively decrease the expenses associated with waste management[7]. Despite the numerous pharmacological investigations and chemical composition studies that have been conducted on noni fruits in various countries, only a limited number of studies have been conducted on noni fruits growing in Sri Lanka. It should be noted that the phytochemical composition of noni fruits can vary within the same plant species, depending on factors such as soil nutrient composition, climatic season, plant developmental stage, natural association with other plants, methods of raw material storage and processing, as well as extraction procedures[4]. Therefore, the objective of this research was to assess the physio-chemical parameters of Noni fruits, and evaluate, and compare of proximate composition, bioactives, and functional properties of methanolic extracted ripe Noni fruit and seeds.

2. Methods

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 ℃ until further analysis. Methanolic extraction of fresh Noni fruits was prepared according to the method described in[12] with slight modifications. One gram of fresh fruit samples was weighed and mixed with 10mL 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, Korea) at 40 ℃.

Spectroscopic methods were employed to assess bioactive compounds. The total phenolic content was determined using Folin-Ciocalteau's reagent method[13], with modifications from[12]. Total flavonoids content was determined using a spectrophotometric method explained by[12]. The total anthocyanin content was estimated using the spectrophotometric pH differential method as described by[14]. To estimate β-carotene and

lycopene contents, [15] method was employed with slight modifications. The estimation of ascorbic acid content was conducted following the method described by[16].

In this study, a comprehensive assessment of the antioxidant activity of the prepared extracts was conducted through various methods. The total antioxidant capacity of the extracts was determined by adopting the method of reducing Mo VI to Mo V, as described by[17]. ABTS scavenging activity was measured using the methodology outlined by[18]. To evaluate lipid peroxidation inhibition activity, protocols from[19] were employed. The ability of the extracts to scavenge the stable free radical DPPH was monitored according to the method outlined by[20]. Furthermore, the production of singlet oxygen (O2) induced by sodium hypochlorite and H2O2 was determined using a spectrophotometric method originally described[21], with slight modifications as suggested by [22]. Finally, the antioxidant capacity of the noni extracts was measured using the FRAP assay, following the methodology proposed by[23], with some modifications to suit the specific experimental conditions of this study.

The assessment of anti-diabetic properties encompassed two assays: In vitro α-amylase activity inhibition was determined using the method outlined by[24] with slight modifications. Likewise, the evaluation of α-glucosidase inhibitory activity was conducted in vitro following the approach described by[25] with slight modifications. The investigation of anti-inflammatory potential involved three membrane lysis assays, including heat-induced hemolysis, following the method delineated by[26] with some modifications by[27]. Assessment of the effect on protein denaturation was carried out as per the procedure described by[26] with some modifications introduced by[27]. Proteinase inhibitory activity was determined through a test based on the modified method of[28], with additional modifications suggested by[27]. Furthermore, nitric oxide inhibition activity was assessed according to the protocol established by[29].

3. Results

The present study investigated the bioactive compounds in dried Noni fruit and dried seed extracts. The results revealed significant differences in the contents of various compounds between the two samples. Total phenolics, recognized for their antioxidant properties, were found to be significantly higher in dried Noni seeds (209.52±0.83 μmol gallic acid equivalent per 1 g fresh weight) compared to dried Noni fruit (173.69±0.48 μmol gallic acid equivalent per 1 g fresh weight), indicating that Noni seeds represent a richer source of phenolic compounds compared to the fruit. Similarly, flavonoids were also observed to be significantly more abundant in dried Noni seeds (12.06±0.58 μmol rutin equivalent per 1 g fresh weight) compared to dried Noni fruit (7.18±0.19 μmol rutin equivalent per 1 g fresh weight), suggesting that Noni seeds possess a higher flavonoid content. Ascorbic acid, a potent antioxidant[30], was notably higher in dried Noni seeds (66.22±2.70 μg per 1 g fresh weight) in contrast to dried Noni fruit (29.55±1.18 μg per 1 g fresh weight), indicating that Noni seeds serve as a superior source of ascorbic acid. Monomeric anthocyanins, acknowledged for their anti-inflammatory and antioxidant properties[31], were also significantly more abundant in dried Noni seeds (97.97±0.96 μg per 1 g fresh weight) compared to dried Noni fruit (26.16±1.93 μg per 1 g fresh weight), implying that Noni seeds contain a higher concentration of monomeric anthocyanins. Conversely, β-carotene, a precursor of vitamin A and an antioxidant, was notably higher in dried Noni seeds (0.40±0.03 μg per 1 g fresh weight) in comparison to dried Noni fruit (0.25±0.01 μg per 1 g fresh weight), while lycopene, another antioxidant, also exhibited higher levels in dried Noni seeds (0.29±0.01 μg per 1 g fresh weight) compared to dried Noni fruit (0.16±0.08 μg per 1 g fresh weight).

The results of this study showcased the IC50 values as indicators of the antioxidant potential found in dried noni fruits and noni seeds. These values were determined through colorimetric assays, including Total Antioxidant Capacity (TAC), DPPH Scavenging Activity, ABTS Scavenging Activity, Lipid Peroxidation Inhibition Activity, Singlet O2 Inhibition Activity, and the Ferric Reducing Antioxidant Power Assay (FRAP

assay). Dried noni fruits were found to possess an IC50 TAC value of 38.17 ± 1.23 μg/ml, while noni seeds exhibited an IC50 TAC value of 39.79 ± 0.30 μg/ml. The IC50 values for DPPH Scavenging Activity of dried noni fruits and noni seeds were 50.70 ± 0.20 μg/ml and 44.99 ± 0.41 μg/ml, respectively. Furthermore, dried noni fruits exhibited an IC50 ABTS Scavenging Activity of 32.02 ± 0.31 μg/ml, while noni seeds demonstrated a significantly higher IC50 value of 19.49 ± 0.52 μg/ml. In terms of Lipid Peroxidation Activity, dried noni fruits displayed an IC50 value of 138.98 ± 2.21 μg/ml, in contrast to noni seeds, which exhibited a significantly lower IC50 value of 42.66 ± 1.01 μg/ml. Additionally, dried noni fruits showed an IC50 Singlet O2 Inhibition Activity of 13.73 ± 0.33 μg/ml, while noni seeds displayed a higher IC50 value of 31.51 ± 0.24 μg/ml. Finally, the Ferric Reducing Antioxidant Power Assay (FRAP assay) revealed that dried noni fruits had an IC50 value of 61.24 ± 0.19 μg/ml, while noni seeds exhibited a notably higher IC50 value of 78.26 ± 1.12 μg/ml.

The anti-diabetic properties of dried noni fruits and dried noni seeds were evaluated in terms of their IC50 values. For Alpha-Amylase Inhibitory Activity, dried noni fruits exhibited an IC50 value of 22.62 ± 0.46 μg/ml, while dried noni seeds demonstrated a value of 19.70 ± 0.56 μg/ml. Similarly, in the case of Alpha-Glucosidase Inhibitory Activity, dried noni fruits displayed an IC50 value of 14.46 ± 0.34 μg/ml, and dried noni seeds showed a value of 15.84 ± 0.71 μg/ml.

The anti-inflammatory properties of dried noni fruits and dried noni seeds were assessed in terms of their IC50 values. In the context of Nitric oxide Inhibition Activity, dried noni fruits demonstrated an IC50 value of 144.45 ± 2.22 μg/ml, while dried noni seeds exhibited a significantly lower value of 92.06 ± 1.25 μg/ml. Likewise, for Heat-Induced Hemolysis Inhibition, dried noni fruits displayed an IC50 value of 24.94 ± 0.28 μg/ml, whereas dried noni seeds yielded an IC50 value of 22.98 ± 0.14 μg/ml, which was not statistically significant. In the assessment of Protein Denaturation Inhibition, dried noni fruits showed an IC50 value of 36.90 ± 0.41 μg/ml, while dried noni seeds demonstrated a significantly higher IC50 value of 22.29 ± 0.19 μg/ml. Lastly, in the evaluation of Proteinase Inhibitory Activity, dried noni fruits displayed a significantly higher IC50 value of 26.28 ± 0.22 μg/ml, and dried noni seeds exhibited a value of 19.31 ± 0.21 μg/ml.

4. Conclusion

This research demonstrates that dried Noni seeds contain higher levels of beneficial bioactive compounds, including phenolics, flavonoids, ascorbic acid, and monomeric anthocyanins, compared to dried Noni fruit. However, the effects of these compounds can be influenced by processing, storage, and individual factors. Further research is needed to explore their potential health benefits and applications. Additionally, both dried Noni fruit and seeds exhibit distinct antioxidant and anti-diabetic properties, with varying levels of activity, suggesting their potential as natural sources of antioxidants and anti-diabetic agents.

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