engineering proceedings

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Proceeding Paper

The Phytochemistry and Anticarcinogenic Activity of Noni Juice †

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† Presented at the 2nd International Electronic Conference on Applied Sciences, 15–31 October 2021; Available online: https://asec2021.sciforum.net/.

Abstract: Noni juice, obtained from the fruit of the noni tree (*Morinda citrifolia* L.), is a popular commodity in the market, particularly in the South Pacific. It is widely used by consumers for the prevention of several lifestyle diseases. Although there is increasing interest in the potential therapeutic use of noni plants, there are no comparative studies on the various commercialized noni fruit juices available to decipher their phytochemical composition and properties against carcinomas. The present study, therefore, aims to fill this research gap and investigate the juice's anecdotal use as complementary alternative medicine to manage cancer. Five commercial brands of noni juice were included in this study, namely, Tahitian Organic Noni (TON), Cook Island Noni (CIN), Dynamic Health Noni (DHN), Fijian Noni (FN), and Life Health Noni (LHN). The juice samples were vacuum-filtered and freeze-dried to obtain crystal products for methanolic extraction. Total phenolic content (TPC) and antioxidant capacity (FRAP—ferric reducing antioxidant power) were determined on the methanolic extracts. The cytotoxicity of the noni juices was also tested on human cervical adenocarcinoma (HeLa cell lines) by dissolving 2 mg of the crystal product in sterile deionized water and diluting to 1000 µg/mL in the media culture. The final concentration of the extracts in the well plate was 500 µg/mL. The MTS cell viability assay was performed after the cells were incubated with the extracts for 48 h at 37 °C with 5% CO_2. The DHN and FN extracts were found to have the highest TPC of 5393 ± 298 and 5060 ± 23 mg gallic acid equivalent /100 g dry weight (DW), respectively, whereas the highest antioxidant capacity was seen in the CIN extract (6389 ± 49 mg Trolox equivalent/100 g DW). The CIN extract also showed the most promising effect with only 63 ± 1% cell viability, whilst the other extracts showed lower cytotoxic effects (76–90% cell viability) on the HeLa cell line. It is possible that greater cytotoxicity could be observed over long exposure times. The noni juice samples contain high levels of TP and antioxidant capacity and appear to show some level of cytotoxic activity, which were statistically different from the negative control. Further work involving more extensive in vitro and in vivo studies are necessary to elucidate its anticarcinogenic activities.

Keywords: noni juice; anti-cancer; total phenolic; antioxidant capacity

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Citation: Mani, J.S.; Johnson, J.B.; Naiker, M. The Phytochemistry and Anticarcinogenic Activity of Noni Juice. *Eng. Proc.* **2021**, *11*, 16. https://doi.org/10.3390/ASEC2021-11154

Academic Editor: Bettina Wolf

Published: 15 October 2021

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1. Introduction

Noni juice, obtained from the fruit of the noni tree (*Morinda citrifolia* L.), is a popular commodity in the herbal supplement market, particularly in the South Pacific. It is widely used by consumers for the prevention of several lifestyle diseases such as diabetes, high blood pressure, cardiopathy, and cerebral apoplexy caused by arteriosclerosis [1]. The noni plant is a small evergreen tree belonging to the Rubiaceae family. The genus *Morinda* consists of 80 species, which are known to grow primarily in coastal tropical regions up to 1330 feet above sea level, and is believed to have originated in Southeast Asia and spread across to Australia, the Pacific Basin, and the Caribbean [2]. As such, apart from "noni", which is the Hawaiian name, it is also known as "nono" in Tahiti, "kura" in the Fiji Islands,

and cheese fruit in Australia [2–4]. The noni fruit has a lumpy surface, appears green when unripe, and turns yellow upon ripening. It is produced all year round and has an unpleasant taste and odor; thus, it is not commonly eaten.

At present, there is an increasing interest in potential anticancer activity of the noni fruit. Noni fruit juice, under the commercial name Tahitian fruit juice, has shown promising activity against breast cancer at the initiation stage of 7,12-dimethylbenz(a)anthracene-induced mammary tumorigenesis in female Sprague–Dawley rats [5]. Several other claims of breast cancer prevention at initiation stage in vitro and in vivo have been reported, and as such, noni fruit has been considered an alternative source of chemo preventive agent for breast cancer [6].

The chemoprotective activity may be attributed to several phenolic compounds, such as ursolic acid, kaempferol, quercetin, and rutin, contained in the noni fruit. Rutin, which is a glucoside of the flavonoid quercetin, exhibits substantial oxygen radical scavenging properties in both in vitro and in vivo [6]. In an in vivo study, the inhibitory effects on 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced inflammation in mice and on the Epstein–Barr virus early antigen (EBV–EA) activation induced by TPA of ten known compounds isolated from methanol noni fruit extracts have been reported. The authors claim it to be only the third such report of anthraquinones in the noni fruits [1].

The role of antioxidant vitamins and some phytochemicals as adjuvants in cancer therapy due to its ability to selectively induce apoptosis in cancer cells and not in normal cells has been shown in experimental studies [7]. However, there are limited comparative studies on the various commercialized noni fruit juices available, which decipher their phytochemical compositions and properties against carcinomas. The present study therefore aims to fill this research gap and investigate its anecdotal use as complementary alternative medicine to manage cancer.

2. Methods

2.1. Noni Juice Samples

Five different commercial brands of noni juice of various origins were procured. The descriptions from the manufacturers' labels are provided in Table 1.

Table 1. Description of the noni juice samples included in this study.

Product Name	Origin (Where Fruits Were Obtained from)	Description
Tahitian Organic Noni (TON)	Tahiti	Never reconstituted—always fresh. No preservatives, coloring agents, or sugars.
Cook Island Noni Juice (CIN)	Cook Island	100% *M. citrifolia* fruit extract. Nonpasturized.
Dynamic Health Noni (DHN)	Tahiti	Contains no added sugar, artificial color, or preservatives. Due to the pure nature ingredients in this product, taste, color, and consistency may vary.
Fijian Noni (FN)	Fiji	Made from pure fruits (wild collection). No additives added. Pasteurized for optimum quality.
Life Health Noni (LHN)	New Zealand	100% Noni fruit juice.

2.2. Sample Preparation

The juice samples were vacuum filtered using 0.45 µm Advantec filter paper, and approximately 30 mL of each sample filtrate was frozen at −80 °C overnight. The samples were then freeze-dried using a FTS System-Flexi dry MP freeze drier operating at −55 °C, 400–500 mTorr for 72 h. Finally, the crystals were weighed and stored in the fridge covered in aluminum foil until required for further analysis.

2.3. Extraction Protocol and Measurement of TPC and FRAP

Methanolic extraction of polar phenolics from each crystal product was performed in duplicates as described previously [8]. The Folin–Ciocalteu assay was used to measure the total phenolic content (TPC), and the antioxidant capacity was determined using the ferric reducing antioxidant power (FRAP) method [8]. The results for the TPC and FRAP assays were reported as gallic acid equivalents (GAE) per 100 g (dry weight basis) and Trolox equivalents (TE) per 100 g (DWB), respectively.

2.4. Anticarcinogenic Activity

The anticancer potential of the extracts will be assessed using the MTS assay against the HeLa cell lines (human cervical carcinoma), using a modified method [9] developed in our laboratory. Briefly, at 80% cell confluency, cell suspension was diluted to obtain a final concentration of 5×10^4 cells/mL, which was inoculated into 96-well plate (100 µL cells/well). The plate was incubated for a period of 24 h at 37 °C with 5% CO_2, prior to the addition of the plant extracts. Subsequently, it was incubated again for a period of 48 h at 37 °C with 5% CO_2. The MTS assay protocol was then followed to assess cell viability [9]. Cisplatin (an anticancer drug) at a concentration of 10 µg/mL was used as the positive control, and the cell culture media DMEM (Dulbecco's Modified Eagle Medium) was used as negative control.

2.5. Data Analysis

Results were expressed as mean ± standard deviation of duplicate experiments. Statistical analyses were carried out using RStudio software.

3. Results and Discussion

3.1. Total Phenolic Content and Antioxidant Activity

The commercial noni juice extracts showed similar TPC (p-value > 0.05) ranging from 4700–5400 mg GAE/100 g DW, as shown in Figure 1. The DHN and FN extracts were found to have the highest TPC of 5393 ± 298 and 5060 ± 23 mg GAE/100 g dry weight (DW), respectively.

The observed results were significantly higher than previously reported value of 284.8 ± 25.9 mg GAE/100 g DW in mature noni fruit sourced from wild noni trees in Guam [9]. Fresh noni juice extracted from fruits sourced from Sri Lanka also showed lower TPC (0.28%) [10] compared to 4.4–5.6 % found in the current study. However, TPC were comparable to values reported in the ethanolic noni plant root extracts (4189 mg GAE/100 g) [11] and somewhat comparable to ethyl acetate fruit extracts (2370 mg GAE/100 g) [12].

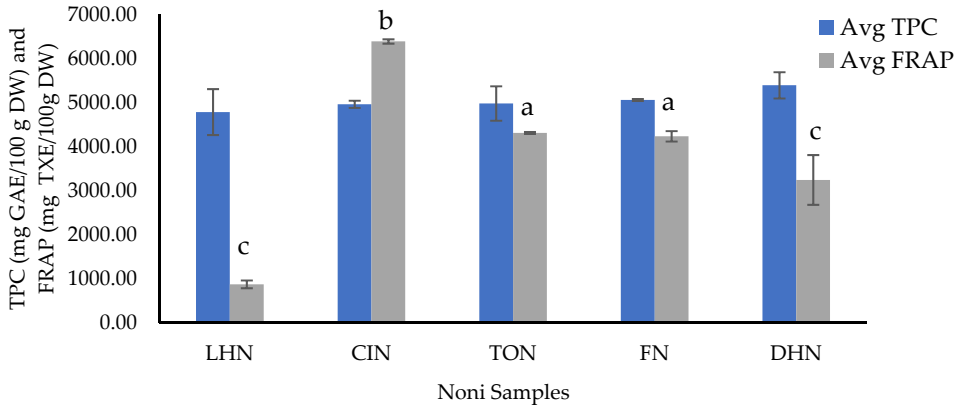

Figure 1. Total phenolic content and FRAP antioxidant capacity of the freeze-dried noni crude extracts. Different letters on the bar indicates significant difference between the tested sources of Noni crude extracts ($p < 0.05$).

The results obtained gave significantly different (p-value < 0.05) ferric reducing abilities for the different brands of noni juice (Figure 1). The antioxidant capacity of CIN was the highest (6389 ± 49 mg TXE/100 g DW), whereas the LHN was the lowest (873 ± 88 mg TXE/100 g DW). The observed results may be due to differences in interaction of polyphenols or the fact that the active compounds that contribute to the antioxidative activity of noni juice were probably non-polar in nature [13]. Moreover, the higher TPC did not always show the higher antioxidant activities, which were similar to trends observed in previous studies [14–17]. It may, however, be inferred that a higher antioxidant capacity would potentially result in high anticarcinogenic activity [18–20].

3.2. Anticarcinogenic Activity

A significantly different (p-value < 0.05) percentage cell viability of HeLa cells were observed upon treatment with the various Noni juice extracts (Figure 2). The CIN extracts showed the highest potency (cell viability of 63 ± 1%), significantly different from the negative control, whereas the FN, LHN, and DHN showed some degree of potency ranging from 76–90%. The effect of TON on the HeLa cells were not significantly different from that of the negative control, hence deeming it non- or the least cytotoxic. It is possible that longer cell exposure times (>48 h) to the Noni juice extracts would have resulted in greater reduction in cell viability.

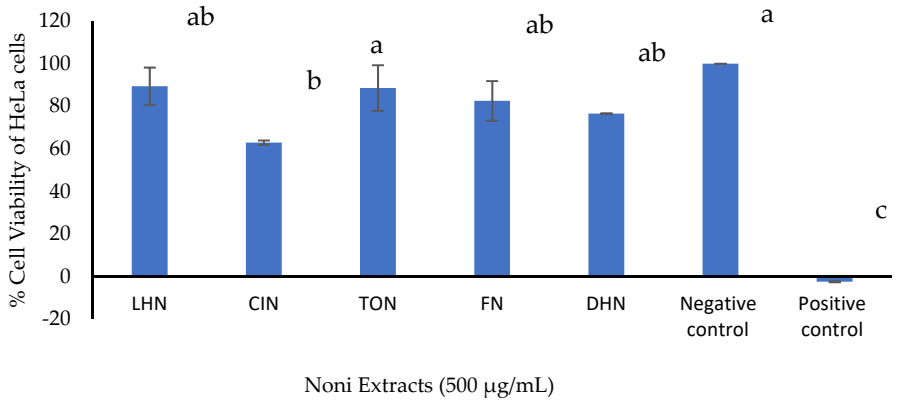

Figure 2. The percentage cell viability of HeLa cells treated with different brands noni juice extracts at 500 µg/mL. Different letters on the bar indicates significant difference between the tested sources of Noni crude extracts (p < 0.05).

Previously, Gupta et al. [21] reported that noni juice and cisplatin, either alone or in combination, were able to induce apoptosis through mitochondrial pathway on HeLa cells. Their findings also reported that cisplatin showed higher cell potency compared to Noni juice, comparative to the results of this study. However, the combination of noni juice and cisplatin showed additive effects, suggesting that it can be used as a chemo adjuvant in the treatment of cervical cancer [21,22]. Another study suggested that, in vitro, a "concentrated component" in noni juice and not the pure noni juice may firstly stimulate the immune system to "possibly" assist the body fight the cancer and then kill a small percentage (0–36%) of cancer depending on the type [2]. This may explain the low potency of the noni juice extracts (0–30%) (Figure 2) obtained in this study. Further fractionation and isolation of noni juice is required to identify the "component" responsible for the anti-carcinogenic activity.

4. Conclusions

From this study, it was found that commercial noni juice samples contain high levels of TP and antioxidant capacity and appear to show some level of cytotoxic activity, which were statistically different from the negative control. Whilst the DHN and FN juice extracts showed the highest TPC values, CIN extracts had the highest antioxidant capacity and, as

such, also showed the greatest cytotoxicity against the HeLa cells. Further work utilizing more extensive in vitro and in vivo studies are necessary to verify its anticarcinogenic activities against various other cancer cell lines. Additionally, isolation and identification of bioactive compounds using high end analytical techniques such as column chromatography, fractionation, and mass spectroscopy is warranted.

Author Contributions: Conceptualization, J.S.M. and M.N.; methodology, J.S.M.; software, J.S.M.; validation, J.S.M.; formal analysis, J.S.M.; investigation, J.S.M. and J.B.J.; resources, J.S.M. and M.N.; data curation, J.S.M.; writing—original draft preparation, J.S.M.; writing—review and editing, J.S.M., J.B.J., and M.N.; visualization, J.S.M.; supervision, M.N.; project administration, M.N. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by a New Staff Grant from CQ University, grant number RSH/5343. One of the authors (J.S.M.) also acknowledges support from the Australian Government in the form of a Research Training Program.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

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