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Original article

Morinda citrifolia L. fruit extracts modulates H_2O_2-induced oxidative stress in human liposarcoma SW872 cells

Z. Ruhomally [a], J. Somanah [b], T. Bahorun [b, **], V.S. Neergheen-Bhujun [a, b, *]

[a] Department of Health Sciences, Faculty of Science, University of Mauritius, Réduit 80837, Republic of Mauritius
[b] ANDI Centre of Excellence for Biomedical and Biomaterials Research (CBBR), MSIRI Building, University of Mauritius, Réduit 80837, Republic of Mauritius

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ABSTRACT

Morinda citrifolia L. commonly known as noni is used by the pharmaceutical and cosmetic industries due to the plethora of pharmacological activities of its metabolites. In Mauritius, the fruits of *M. citrifolia* are used in folk medicine against a number of indications. The present study aimed at evaluating the antioxidant activity of ripe and unripe noni fruit at both biochemical and cellular levels. Using an array of established assay systems, the fruit antioxidant propensity was assessed in terms of its radical scavenging, iron reducing and metal chelating potentials. Ascorbic acid, total phenolic and total flavonoid contents of the fruits were also determined. The ascorbic acid content of ripe noni was 76.24 ± 1.13 mg/100 g while total phenolics of ripe and unripe fruit extracts were 748.40 ± 8.85 μg and 770.34 ± 2.27 μg GAE g^{-1} FW respectively. Both the ripe and unripe extracts of *M. citrifolia* were potent scavengers of nitric oxide, superoxide and hydroxyl radicals. The ferric reducing capacity ranged from 11.26 ± 0.33 to 11.90 ± 0.20 mM Fe^{2+} g^{-1} FW while the IC_{50} values for the iron (II) chelating power were 0.50 ± 0.01 and 1.74 ± 0.01 g FW/mL for the ripe and unripe fruit extracts respectively. Cellular studies additionally demonstrated that noni were able to dose-dependently counteract accumulation of reactive oxygen species (ROS)-induced oxidative stress, a potential obesogenic factor within human liposarcoma SW872 cells as well as significantly restore cell death within the concentration range of 0.106–0.813 g/mL. Results reported herein suggest noni as an interesting source of prophylactic antioxidants modulated by its polyphenol composition.

1. Introduction

Concerns regarding oxidative stress (OS) are primarily due to its involvement in various pathophysiologies ranging from inflammation, reperfusion injury, atherosclerosis, cancer, osteoporosis, aging, fibrosis and cognitive function.[1–3] This has prompted interest in antioxidant phytophenolic rich dietary sources and their putative protective effects on human health.[4] Thus, over the past few decades, herbal and natural products from folk medicines have become increasingly popular globally because of their long standing use, efficacy and reduced toxicity.[5]

Morinda citrifolia L. commonly known as noni, belongs to the Rubiaceae family, and is indigenous to the tropical zones.[6] Its wide array of secondary metabolites including more than 160 phytochemical compounds ranging from phenolic compounds, organic acids and alkaloids, is widely suggested to account for the reported prophylaxis of the plant extracts. Anthraquinones in particular damnacanthal, morindone, morindin, and aucubin, asperuloside and scopoletin have been prominently identified.[7]

These phenolics exhibit their antioxidative activity via several mechanisms of action inter alia: as reducing agents, singlet oxygen quenchers, hydrogen donating antioxidants, free radicals scavengers and metal ions chelators.[8,9] In addition, in view of their pluripharmacological properties, they can exert modulatory actions in cells by interacting with a wide range of cellular and molecular targets.[9,10]

* Corresponding author. Department of Health Sciences, Faculty of Science, University of Mauritius, Réduit 80837, Republic of Mauritius. Tel.: +230 4037412; fax: +230 465 6928.
** Corresponding author. ANDI, CBBR, University of Mauritius, Réduit, Republic of Mauritius. Tel.: +230 4675582.
E-mail addresses: tbahorun@uom.ac.mu (T. Bahorun), v.neergheen@uom.ac.mu (V.S. Neergheen-Bhujun).

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In the last decade, extensive research have credited noni with antioxidant,[11] anti-microbial properties,[12] anti-inflammatory,[13] anticarcinogenic,[14] antidiabetic activity,[15] immune stimulating[16] and analgesic activity.[17] In Mauritius, noni fruits and leaves have ethnomedicinal applications against type 2 diabetes, hypercholesterolemia, hypertension and pain.[18]

In the light of the documented beneficial properties of noni, the evaluation of the phytophenolic richness and antioxidative properties of the locally cultivated noni fruit was carried out using several *in-vitro* assays and on human adipocytes SW872, a dual model of obesity and oxidative stress. Results reported herein support the traditional use of noni as a health enhancer in herbal and complementary medicine.

2. Methodology

2.1. Chemicals

Aluminium chloride was purchased from Surechem Products, United Kingdom, Nitrobluetetrazolium, Nicotinamide-adenine dinucleotide, ferrozine and Dulbecco's modified eagle's medium (DMEM) were bought from HiMedia laboratories, Mumbai (India). Moreover, quercetin was purchased from Sigma–Aldrich, India and deoxyribose from Fluka Analytical Laboratories, Germany. Fetal bovine serum, L-glutamine and penicillin–streptomycin were purchased from Sigma (USA).

2.2. Fruit source

M. citrifolia L. ripe and unripe fruits were collected from Grand-Bel-Air in the South East of Mauritius during the month of October 2013. The fruits were identified and authenticated at the Herbarium of Mauritius, Mauritius Sugar Industry Research Institute.

2.3. Vitamin C determination in whole fruits

Ascorbic acid content in *M. citrifolia* L. fruits was determined according to the AOAC 967.21 official method, using the 2, 6-dichloroindophenol titri-metric method. 50 g of each fruit sample was weighed and blended with 100 mL of distilled water. The mixture was filtered and was made up with distilled water up to 250 mL in a volumetric flask. To 5 mL of metaphosphoric acid solution, 2 mL of sample fruit juice was added and titrated with indophenol dye solution until a light rose – pink color persisted for more than 5 min. Results were expressed as mean mg ascorbic acid 100 g^{-1} fresh fruits of three replicates.

2.4. Phytophenolic analyses

2.4.1. Extraction

Pulps from the ripe and unripe fruit respectively were freeze dried. They were then extracted with 80% methanol (1:3 w/v) and allowed to macerate exhaustively at 4 °C prior to being concentrated *in vacuo* at 37 °C. Finally, the concentrated extract was lyophilized and the resulting powders were subsequently dissolved in deionized water or 80% methanol for further analyses.

2.4.2. Total phenolic content determination

The Folin-Ciocalteu assay assay adapted from Neergheen et al. (2006) was used to estimate the total phenolic content of the fruit extracts of *M. citrifolia* L.[19] The results were expressed in terms of μg gallic acid equivalent (GAE) g^{-1} FW.

2.4.3. Determination of total flavonoid content

Total flavonoid content of fruit extracts were investigated using the spectrophotometric assay adapted from Zhishen et al. (1999).[20] The results were expressed in terms of mg quercetin equivalent (QE) g^{-1} FW.

2.5. Determination of antioxidant capacities

2.5.1. Ferric reducing antioxidant power

The FRAP assay adapted from Benzie and Strain (1996) was modified to evaluate the reducing power of fruit extracts of *M. citrifolia* L.[21] At low pH, ferric tripyridyltriazine complex is reduced to ferrous form, the resulting intense blue color being linearly related to the amount of reductant present. The FRAP reagent consisting of 2,2,6 tripyridyl-5-triazine (TPTZ, 10 mM) in 40 mM HCl and ferric chloride (20 mM) in 200 mL of sodium acetate buffer (pH 3.6, 0.25 M) was freshly prepared and warmed at 37 °C prior to analysis. To 180 μL FRAP reagent, 20 μL of the extracts was added in a 96 – well plate and was left to react for 6 min at ambient temperature. The absorbance was read at 593 nm (Synergy HT, BioTek instruments, USA). A calibration curve of ferrous sulphate was used and results were expressed in terms of mM Fe^{2+} g^{-1} FW.

2.5.2. Iron chelating activity

The method adapted from Neergheen-Bhujun et al. (2014). was used to assess the iron (II) chelating effect of the fruit extracts.[22] The reacting mixture contained 200 μl of varied concentration of the ripe and unripe fruits extract (0.1–2.8 g/mL) and 50 μl $FeCl_2.4H_2O$ (0.5 mM).The reacting mixture was then made up to 1 mL with distilled deionized water and incubated for 5 min at room temperature. After incubation, 50 μl of ferrozine (2.5 mM) was added and the purple coloration formed read at 562 nm. EDTA was used as a positive control. The percentage of chelating activity was calculated and results were expressed as mean IC_{50} (g FW mL^{-1}).

2.5.3. Inhibition of deoxyribose damage

The hydroxyl scavenging activities of the extracts under study was determined using the deoxyribose assay adapted from Halliwell et al., 1987[23] and Aruoma 1994.[24] The reaction mixture for the deoxyribose assay contained in a final volume of 1 mL the following reagents, order of addition indicated: 200 μL KH_2PO_4–KOH (100 mM), 200 μL $FeCl_3$ (500 μM), 100 μL EDTA (1 mM), 100 μL sample of varied concentrations (0.1–2.8 g/mL), 200 μL deoxyribose (15 mM), 100 μL H_2O_2 (10 mM) and 100 μL ascorbic acid (1 mM).The reaction mixture was incubated at 37 °C for 1 h.

After incubation, 1 mL of 1% (w/v) thiobarbituric acid (TBA) was added to each mixture followed by the addition of 1 mL of 2.8% (w/v) trichloroacetic acid (TCA). The solutions were heated on a water bath at 80 °C for 20 min to develop the pink colored malondialdehyde–thiobarbituric acid: MDA–$(TBA)_2$ adduct. The MDA–$(TBA)_2$ chromogen was extracted into 3 mL butan-1-ol and its absorbance measured at 532 nm. Gallic acid was used as positive control and results were expressed as mean IC_{50} (g FW mL^{-1}).

2.5.4. Nitric oxide radical inhibition assay

Nitric oxide was generated from sodium nitroprusside and was measured by the GriessIllosvoy reagent using 0.1% w/v naphthylethylene–diamine–dihydrochloride as described in Mandal et al., 2009.[25] The reaction mixture contained 2 mL sodium nitroprusside, 0.5 mL phosphate saline buffer, pH 7.4 and 0.5 mL of plant extract (variable concentrations, 0.008–0.712 g/mL) and was allowed to incubate for 2½ h at 25 °C. Following incubation, 0.5 mL of the reaction mixture was added to 1 mL sulphanilic acid (0.33% in 20% glacial acetic acid) and the mixture was allowed to stand for 5 min 1 mL of naphthylethylene–diamine–dihydrochloride (0.1%)