

ORTANIQUE PEEL BIOMOLECULES: CHARACTERIZATION AND NANOPARTICULARIZATION FOR POTENTIAL BIOMEDICAL APPLICATION

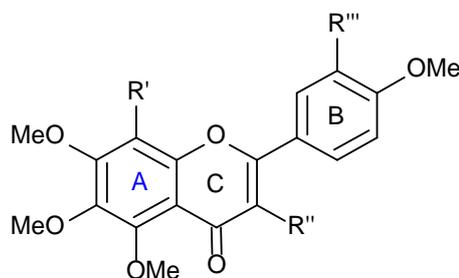
¹Curtis O. Green,^{1,2}Andrew O. Wheatley,¹Lowell L. Dilworth and ^{1,2,3}Helen N. Asemota

¹Department of Basic Medical Sciences (Biochemistry), ²Biotechnology Centre,
University of the West Indies, Mona, Jamaica,

³Dept. of Nat. Sciences & Maths, Nanobiology Division, NNRC, Shaw University, Raleigh, NC., USA.

Introduction

Polymethoxylated flavones (PMFs) are flavonoids that are almost exclusively found in Citrus peel and display potent anti-hypercholesterolemic, anti-cancer, anti-atherosclerotic and anti-diabetic properties [1, 2]. Jamaica is the leading producer of citrus in the Caribbean [3]. A vast majority of the citrus fruits that are harvested are used for citrus juice production which results in large quantities of citrus peel and other citrus by-products that can be used to generate kilogram quantities of PMFs. PMFs supplementation has been shown to result in the reversal of metabolic defects associated with diabetes, including a decrease in insulin level and an improvement in glucose tolerance [4]. Nanoparticles made from retinoic acid coated with CaCO₃ (nanoegg -atRA) were recently developed as a new drug delivery system with possible anti-diabetic application. The nanoparticles stimulated insulin secretion from islets in a glucose-dependent manner in streptozotocin-induced diabetic rats [5]. This study was designed to characterize PMFs from the Jamaican citrus ortanique as well as to fabricate nanobiomaterial from the PMFs for possible biomedical usage. **Experimental**



	R'	R''	R'''
Sinensetin	H	H	OCH ₃
Nobiletin	OCH ₃	H	OCH ₃
Heptamethoxyflavone	OCH ₃	OCH ₃	OCH ₃
Tetramethylscutellarein	H	H	H
Tangeretin	OCH ₃	H	H
Hexamethyl- <i>o</i> -quercetagenin	H	OCH ₃	OCH ₃

Figure 1. Structures of polymethoxylated flavones

Materials

Mature citrus samples of the following Citrus varieties: *Citrus reticulata* (Tangerine and Mandarin), *Citrus aurantium* (Bitter acid Seville orange), *Citrus sinensis* (Valencia), as well as the tangor Ortanique (*C. reticulata* × *C. sinensis*) were obtained from a local farm in Bog Walk, St. Catherine, Jamaica.

Procedures

Citrus samples were sun-dried, milled and 100 grams extracted for 24 hours with reagent grade methanol (500 mL

3) at room temperature with constant stirring. Extracts were filtered via vacuum filtration, filtrates were pooled and evaporated via rotary evaporation. The resulting solids were initially air-dried and further dried at 70 °C. The dried residue was re-dissolved in HPLC grade methanol, filtered through a 0.2 micron syringe filter and used for HPLC analysis. The analyses were made on a Varian Prostar high performance liquid chromatograph having a Prostar 410 autosampler, Varian Prostar 220, 230, 240 pumps and a Varian Prostar 325 UV detector. The separation was achieved using a C-18 5 μm (150 × 4.6-mm) Discovery column (Sigma-Aldrich, USA). Separation was done according to the method of Kurowska and Mantey [6] with slight modifications. The PMFs were eluted with a mobile phase that consisted of water/2% formic acid/acetonitrile (85:5:10 v/v/v; 10 min) initially and increased in linear gradients to (60:5:35, v/v/v) over 20 min then to (45:5:50, v/v/v) for 10 min and to (85:5:10, v/v/v) for 45 minutes at a flow rate of 1 mL/min, the column was at ambient temperature. The PMFs levels were calculated by comparing the integrated peak areas of the individual compounds to that of a standard curve prepared from the corresponding standards. Chromatographic peaks were identified in several ways. Retention times of the unknown peaks were compared with retention times of authentic standards. Sample extracts were fortified with small known amounts of standard material one at a time and re-chromatographed to see if the peak of interest increased in height. Spiked peaks were also checked for peak symmetry to determine if more than a single compound might be present.

Nanoparticles will be designed according to the method of Rolland *et al* [7]. Silicon master templates will be acquired commercially. Replica molds of the master templates will be made and the replica molds will be used to fabricate and harvest individual, monodisperse, nanometer scale particles

in a variety of shapes using the PRINT process. PRINT, the nonwetting nature of fluorinated materials and surfaces confines the liquid precursor inside the features of the mold, allowing for the generation of isolated particles. Polymethoxylated flavones will be injected into the finished mold. These PMFs nanoparticles will be used as a drug delivery system for administering the PMFs at specific target sites.

Results and Discussion

All the samples analysed contained appreciable quantities of the six major PMFs (Figures 2 and 3). Ortanique, a hybrid of *C. reticulata* (Tangerine) and *C. sinensis* (Sweet orange) that is indigenous to Jamaica contains the highest levels of the PMFs (Figure 3). Prior to this study many research findings indicated that tangerine (*C. reticulata*) and sweet oranges (*C. sinensis*) contained the highest levels of PMFs while in this study they contained less than the Ortanique [8]. Jamaica produces relatively large quantities of citrus peels annually which are either ordinarily discarded. These citrus peels and other citrus byproducts could be used to generate PMFs on a commercial scale. From 1000 kg of Ortanique peel extract for example, approximately 33 kg of total PMFs is recoverable, that is 9.4 kg of tangeretin, 7.9 kg of nobiletin, 3.5 kg of sinensitin, 7.5 kg of tetramethylscutellarein, 1.2 kg of heptamethoxyflavone and 3.6 kg hexamethyl-*o*-quercetagenin.

Conclusions

Jamaican citrus varieties contain appreciable quantities of PMFs that could serve as viable sources of obtaining commercial quantities of PMFs for the development of nanoparticles for biomedical applications.

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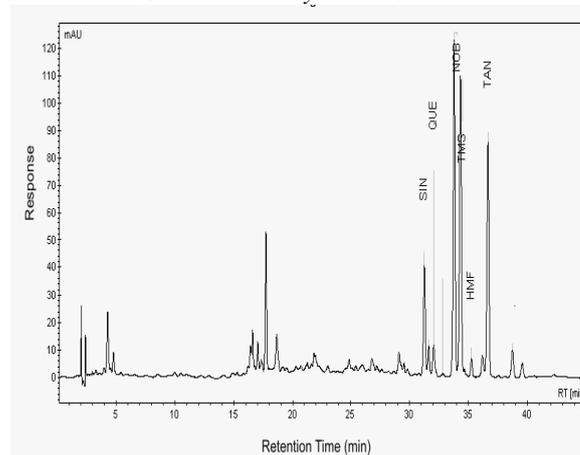


Figure 2. Chromatogram of Ortanique peel extract. Sin – sinensitin, NOB – nobiletin, TAN– tangeretin, TMS – tetramethylscutellarein, HMF – heptamethoxyflavone, QUE – hexamethyl-*o*-quercetagenin.

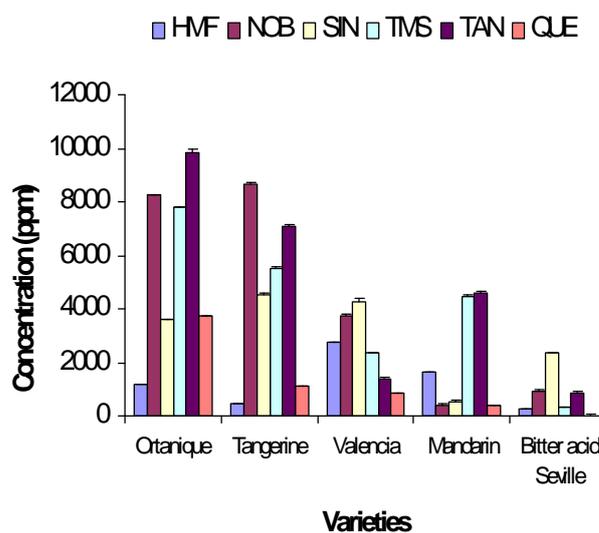


Figure 3. Quantity of PMFs in Jamaican Citrus Varieties. Sin – sinensitin, NOB – nobiletin, TAN– tangeretin, TMS – tetramethylscutellarein, HMF – heptamethoxyflavone, QUE – hexamethyl-*o*-quercetagenin.

