Rapid Isolation of Geraniin from *Nephelium lappaceum* Rind Waste Using the Gilson GX-281 Preparative HPLC Purification System

Application Note PHA 0311

Keywords

Gilson GX-281 Preparative HPLC System, TRILUTION® LC, Geraniin, Alpha glucosidase, Alpha amylase, Aldol reductase, AGE, Nephelium lappaceum L., University of Malaysia, Anti-Hyperglycemic Agent, Hyperglycemia, Purification, Natural Product, HPLC, Diabetes,

Introduction

This application note was performed by Uma D. Palanisamy, Jeffery Cheah School of Medicine and Health Sciences, Monash University Sunway campus, *Malaysia; Lai Teng Ling and Thamilvaani Manaharan, Faculty of Medicine with the Department of Physiology from the University of Malaysia; David Appleton, Faculty of Medicine with the Department of Pharmacology from the University of Malaysia.*

Natural product purification of geraniin (see figure 1) from N. lappaceum (see figure 2) rind waste using the Gilson GX-281 Preparative HPLC Purification System (see figure 3) was performed. Results of this application note strongly support the use of a geraniin-standardised N. lappaceum extract in the management of hyperglycemia.

Nephelium lappaceum L. is native to Southeast Asia , and it is part of the sub-tropical fruit family of Sapindaceae. This fruit is a commercial crop in Asia, where it is often eaten fresh. Dried fruit rind is used in traditional medicine, cooking, and in the manufacture of soap. The roots, bark, and leaves have various uses in medicine and in the production of dyes.

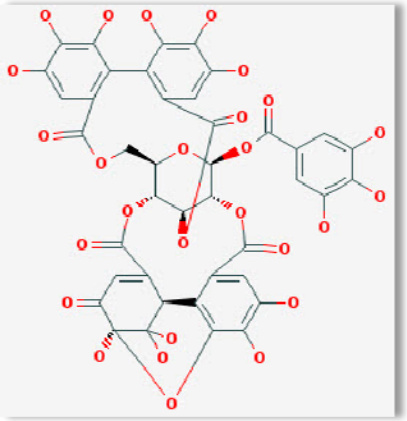

Figure 1. Chemical Structure of Geraniin
(Source: PubChem Public Chemical Database)

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The ability of ethanolic Nephelium lappaceum L. rind extract to act as an anti-hyperglycemic agent has been confirmed. Geraniin, an ellagitannin, was identified as the major bioactive compound isolated from the ethanolic Nephelium lappaceum L. rind extract. In addition to its extremely high anti-oxidant activity and low pro-oxidant capability, geraniin is seen to possess in vitro hypoglycemic activity (alpha-glucosidase inhibition: IC50 = 0.92 lg/ml and alpha-amylase inhibition: IC50 = 0.93 lg/ml), aldol reductase inhibition activity (IC50 = 7 lg/ml) and has the ability to prevent the formation of advanced glycation end-products (AGE). Geraniin was observed to exhibit these properties at more significant levels compared to the positive controls acarbose (carbohydrate hydrolysis inhibitor), quercetin (aldol reductase inhibitor) and green tea (AGE inhibitor). Geraniin therefore, has the potential to be developed into an anti-hyperglycemic agent.

Figure 2. Nephelium lappaceum L. (Source: Encyclopedia of Life (www.eol.org))

Figure 3. Gilson GX-281 Preparative HPLC Purification System

Materials & Methods

Materials

Chemicals and reagents were obtained from various scientific suppliers. All solvents used were HPLC grade or higher. All reagents were ACS grade or better.

Gilson Purification System:

System: GX-281 Preparative HPLC System (GX-281/322/156)

Mobile Phase: 0.1% Formic Acid in Acetonitrile and 0.1% Formic Acid in Ultra Pure Water at a flow rate of 18 mL/min

0–10% Acetonitrile for 3 min

10–40% Acetonitrile for 12 min

100% Acetonitrile for 5 min (column recondition)

Column: Waters Waters Xterra Prep RP18 OBD (19 x 50 mm)

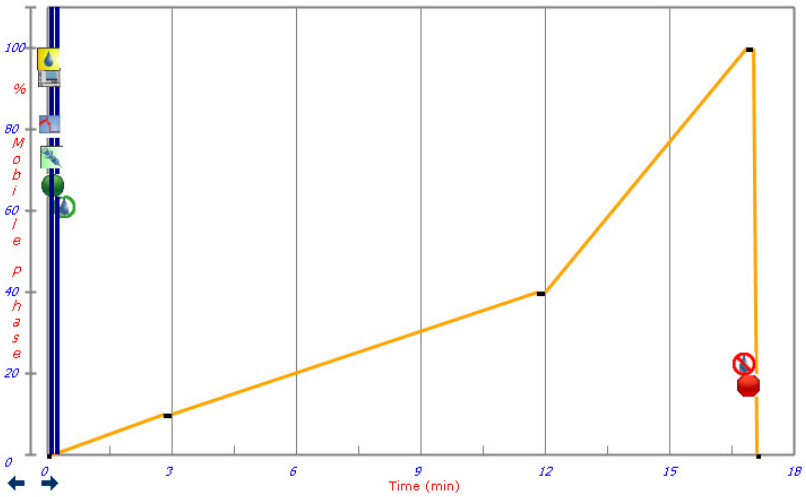

Figure 4. Example Gilson TRILUTION® LC Preparative Gradient Method

HPLC–LCMS/MS Analysis of Geraniin:

System: Shidmazu Prominence UFLC-LCMSIT-TOF (Pos and Neg)

Mobile Phase: 0.1% formic acid in water and 0.1% formic acid in Acetonitrile at 0.5 mL/min

Column: Waters Xterra MS C18 (2.5 x 20 mm, 2.5 um) IS column heated to 40° C

Additional Assays/Tests Performed:

NMR analysis, Pro-oxidant assay, Scavenging activity of geraniin onto Galvinoxyl and ABTS radicals, Advanced glycation endproducts (AGE) formation inhibitory Activity, Anti-hyperglycemic assays, and Aldose reductase (AR) inhibitory activity

Methods – Sample Preparation & Geraniin Isolation Procedure

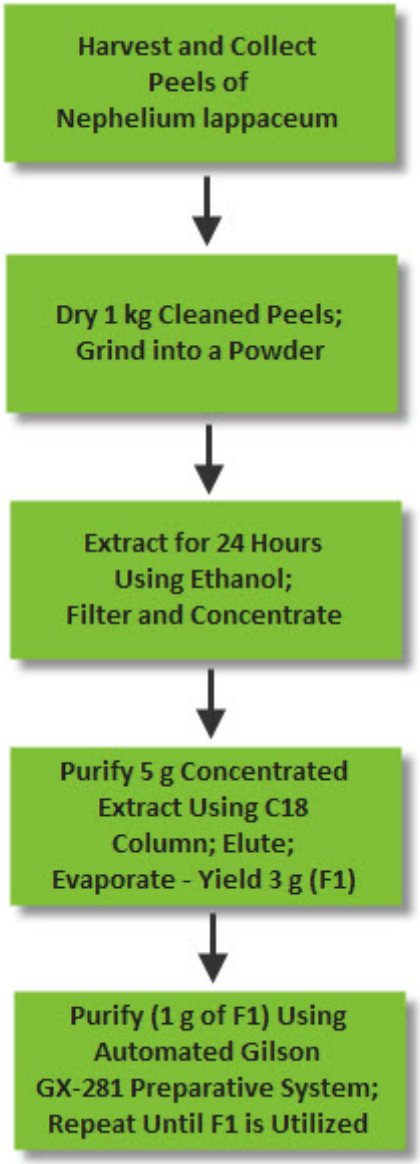

Figure 5. Sample Preparation & Geraniin Isolation Procedure

Results

Table 1. Quantification of Geraniin in the Rapid Purification Method

Sample/Fraction	Extraction Method	Yield (%)	Geraniin in Sample* (%)
N. lappaceum rind	Ethanol Extraction	30.58	3.79
Ethanolic Extract	LiChroprep RP-18	60.00	12.68
F1	Gilson Preparative HPLC	21.15	21.13

***** Calculation of the content of geraniin (%) is based on the assumption that 3 g of F1was in final purification step.

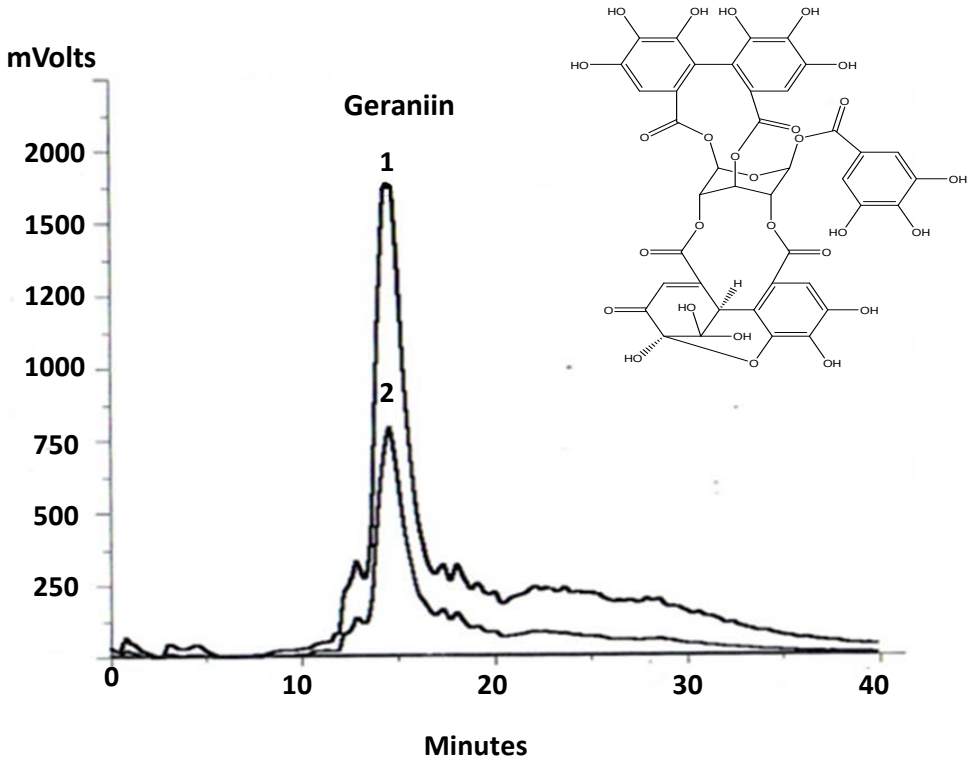

Figure 6. Example GX-281 Purification Geraniin Chromatogram @ 13 minutes – Major Compound in Ethanolic Nephelium lappaceum Rind Extract; Geraniin Peak 1 = 210 nm; Geraniin Peak 2 = 275 nm

Summary

The rind of N. lappaceum, apart from being a highly efficient anti-oxidant, was shown to be effective in inhibiting carbohydrate hydrolysing enzymes and enzymes involved in the polyol pathway. In addition, N. lappaceum was found to prevent the formation of advanced glycation endproducts. Geraniin was found to be the major compound isolated from the rind of an ethanolic extract of N. lappaceum (see table 1). A rapid and mid-scale purification of geraniin was achieved as described in table 1 within this application note.

Geraniin with its ability to reduce free radicals, having a low pro-oxidant capacity, being an excellent inhibitor of carbohydrate hydrolysing enzymes and polyol and AGE formation, makes it an ideal candidate for the management of hyperglycemia in diabetic individuals. In addition, there is additional support for the use of a geraniin-standardised N. lappaceum extract, as an herbal formulation in the management of hyperglycemia.

References

Uma D. Palanisamy, Lai Teng Ling, Thamilvaani Manaharan, David Appleton, Journal of Food Chemistry, 127 (2011) 21-27.