Research report

on

Effect of *Morinda citrifolia* Linn. fruit and a biomarker scopoletin on upper gastrointestinal motility and inflammation in rats

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ABSTRACT

A solid phase extraction (SPE) procedure on conventional C_{18} sorbents followed by an RP-HPLC assay was successfully developed for rapid and economic analysis of scopoletin in an aqueous and ethanolic *Morinda citrifolia* (noni) fruit extract as used in Thai traditional medicine and commercial product. The extract applied on SPE cartridge was purified by a cleanup procedure consists of 0.01M KH_2PO_4 (pH 7) followed by 100% methanol. Chromatographic separation was achieved on a C_{18} column using 0.01 M sodium acetate (pH 3.0) and acetonitrile (80:20 v/v) as mobile phase and a UV-Vis detector at wavelength of 350 nm. The recovery of scopoletin from SPE cartridge was quite high with 84.52 $\pm$ 3.65% of scopoletin before the chromatography. The established method provided a rapid phase separation with short retention time (5.5 $\pm$ 0.01 min), good sensitivity (LOD = 2.6 ng/ml; LOQ = 7.9 ng/ml), high precision (the RSD's intra- and inter-day precision < 2%), high accuracy (a recovery percentage > 80%), and high reliability (total peak purity > 0.99). The linear correlation coefficient (R^2) was also more than 0.999, within the range of concentration investigated (0.01-100 µg/ml). The scopoletin content in an aqueous fruit extract (AFE) and ethanolic fruit extract (EFE) are between 6.70 to 6.78 and 20.65 to 20.75 µg/ml, respectively.

The pharmacological study was carried out to evaluate the effect of noni fruit extract and its biomarker scopoletin on gastro-esophageal disorder models that related to its claimed pharmacological properties. AFE (0.63-2.50 g/kg) strongly increased gastric emptying rate of phenol red meal and intestinal transit of charcoal meal with a higher potency than cisapride. AFE also significantly inhibited gastric acid secretion and pepsin activity in pylorus ligated rats. Additionally, AFE significantly prevented the formation of acid reflux esophagitis and reduced the formation of ethanol-induced acute gastric lesions in rats with equal potency as those of standard antisecretory agents (ranitidine and lansoprazole). Pure scopoletin, at the

same equivalent dose containing in AFE, possessed slightly less prokinetic and antiulcer acitivities than AFE but exerted the similar potency to AFE in suppression gastric acid secretion and pepsin activity. EFE (0.4 g/kg) which contained the same equivalent dose of scopoletin as that of 1.25 g/kg AFE, exerted the comparable prokinetic, antisecretory and antiulcer efficacy to that of AFE. This may be due to a three time higher yield of scopoletin containing in EFE than in AFE.

In conclusion, the novelty of the established SPE followed by UV detection RP-HPLC method for quantitative and qualitative assessment of scopoletin includes not only a rapid, low cost and easy purification and isolation process of pure scopoletin before the chromatography but also a rapid (short retention and analytical time less than 15 min) economic (low consumption of organic solvents), simple, sensitive and precise method for quantitative analysis of scopoletin in commercial noni juice products and medical preparation. Furthermore, these advantages may be of significant economic values for either routinely quality-control testing of commercial noni fruit juice or large scale production of scopoletin from the plants. Additionally, the pharmacological findings indicated that an aqueous and ethanolic *Morinda citrifolia* (noni) fruit extract as well as its biomarker: scopoletin may be beneficial as a potential preventive and therapeutic agent for gastro-esophageal disorders, mainly through its antisecretory and prokinetic activities. This is also the first report to show that scopoletin is a potent antisecretory and prokinetic agent in animal models. Furthermore, scopoletin might be one of biomarker constituents for quality assessment of noni fruit products used for treatment of upper gastrointestinal disorders.

Keywords: *Morinda citrifolia*; noni, method development; method validation; solid phase xtraction (SPE); HPLC, scopoloetin, prokinetic activity, gastroesophageal disorder, gastric emptying, intestinal transit