Faculty of Pharmaceutical Sciences, School of Health Sciences, University of Iceland, Reykjavik, Iceland

Investigations on the constituents of SagaPro tablets, a food supplement manufactured from *Angelica archangelica* leaf

N.M. Kowal, R. Eyjolfsson, E.S. Olafsdottir*

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**Corresponding author: Elin Soffia Olafsdottir, Professor, Faculty of Pharmaceutical Sciences, School of Health Sciences, University of Iceland, Hagi, Hofsvallagata 53, IS-107 Reykjavik, Iceland*
elinsol@hi.is

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Saga Pro is a food supplement product manufactured in Iceland and marketed internationally. It is claimed to have anti-nocturia effect and the flavonoid isoquercitrin has been suggested to play a role in this assumed activity. The purpose of this study was to identify and quantify the main flavonoids and furanocoumarins in the SagaPro tablets and to evaluate the importance of their presence. Isoquercitrin was identified as a constituent in an amount of 158 µg/tablet. This is a p.o. dosage highly unlikely to have an effect on nocturia or any other pharmacologically significant effect in humans. The main furanocoumarins, xanthotoxin and imperatorin, were also identified and quantified to 280 and 2 µg/tablet, respectively.

1. Introduction

SagaPro is marketed as a food supplement in the form of tablets claimed to contain 100 mg of a 5:1 aqueous extract of the leaves of *Angelica archangelica*. Excipients are corn starch, dicalcium phosphate dihydrate, glycerol, hypromellose, magnesium stearate, potato starch, povidone, silicon dioxide, talc (www.naturaldatabase.com). Recommended dosage by the manufacturer is 1-2 tablets daily for reduction of frequent urinations due to reduced bladder capacity and/or an overactive bladder. It has also been stated to be useful against frequent urinations owing to benign prostatic hyperplasia. The manufacturer has suggested isoquercitrin (quercetin-3-glucoside) (Fig.), a flavonoid supposed to be present in the plant extract, as a probable candidate responsible for these pharmacological effects (Sigurdsson et al. 2013).

The phytochemistry of *Angelica archangelica* is well known, although isoquercitrin has not been reported from this plant before. More than 20 furanocoumarins have been identified, including angelicin, archangelicin, bergapten, imperatorin, isoimperatorin, xanthotoxin, 2'-angeloyl-3'-isovaleryl vaginate, heraclenol-2'-O-senecioate and heraclenol-2'-O-isovalerate as well as the coumarins osthol and umbelliferone. Volatile oils are present containing α- and β-phellandrene, α- and β-pinene, sabinene, α-thujone, limonene, linalool, borneol and four macrocyclic lactones. Other constituents comprise archangelenone (a flavonoid), palmitic acid, caffeic acid, chlorogenic acid, fructose, glucose, sucrose, umbelliferose (Czygan 1998; Harmala et al. 1992; Hoffmann 2003; Holm et al. 1997; Sun and Jakupovic 1986; Wichtl 2004). None of these compounds have been reported in the scientific literature to have an effect on nocturia in humans.

The manufacturer of SagaPro conducted a clinical study on nocturia in a group of men with at least two nocturnal voids (Sigurdsson et al. 2013). The main overall result showed that there was no statistically significant difference between the effects of SagaPro (dosage 2 tablets daily) and placebo. The authors then extracted a very small subgroup (n=12 in the SagaPro group) for analysis, which resulted in a conceivable beneficial effect of the product on nocturia in individuals with reduced bladder volume. It is important to note that results from this kind of *posthoc* subgroup analyses should be interpreted with extreme care and should not be deemed confirmatory of a true effect (Wang et al. 2007; Lagakos 2006; Bland and Altman 1995). Thus, the claimed beneficial effect of SagaPro on nocturia in this subgroup of men remains unproven.

2. Investigations, results and discussion

In this study, methanolic and aqueous extracts of SagaPro tablets were prepared.

The aqueous extract was analysed by TLC using glucose, fructose and sucrose as reference compounds all of which were present in the extract along with other unidentified saccharides and water soluble materials. These compounds were estimated to represent the vast majority of substances found in the water extract of the SagaPro tablets.

The methanolic extract was investigated by TLC and HPLC using authentic isoquercitrin, imperatorin and xanthotoxin as reference

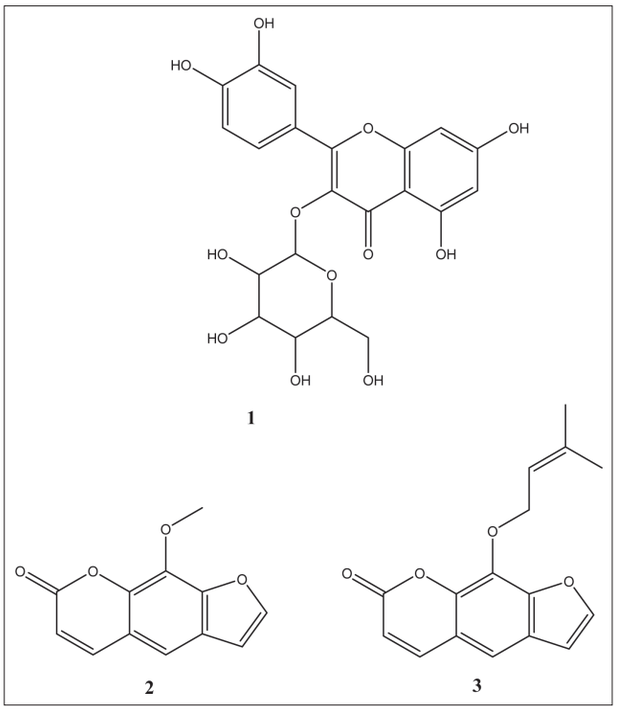

Fig.: Structures of isoquercitrin (**1**), xanthotoxin (**2**) and imperatorin (**3**)

compounds. The results indicated the presence of all these compounds along with a number of other unidentified substances in very low amounts. The HPLC peaks for isoquercitrin and xanthotoxin were not completely resolved and allowed only rough quantification of the compounds. A small peak suggested the presence of imperatorin but it was not resolved well enough for quantification. Subsequently, they were identified and assayed by UPLC/MS together with the authentic compounds as references. Isoquercitrin was found to represent 158 µg per tablet (Table). This gives 158-316 µg of isoquercitrin in the recommended 1-2 tablets daily dosage, or about 2-4 µg/kg body weight in a man weighing 75 kg. This is a dosage much too low to be expected to have an effect on nocturia in humans.

Isoquercitrin in far higher doses (mg/kg) has been reported to produce a number of biological effects in rats and mice (Valentova et al. 2014). This includes diuretic effect in rats when administered in 0.5-4 mg/kg *i.v.* doses (Gasparotto et al. 2012); a concentration of at least three orders of magnitude larger than those found in SagaPro tablets.

The main furanocoumarins identified and quantified by UPLC/MS in the Saga Pro tablets were xanthotoxin and imperatorin (Fig.) giving 280 and 2 µg/tablet, respectively (Table). They have both been reported from *A. archangelica* before and are known to be phototoxic (Sarker and Nahar 2004). However, the dosage from 1-2 tablets of the SagaPro batch investigated would be about 4-8 µg/kg body weight, which is much lower than the minimum furanocoumarin dosage known to cause phototoxicity (140 µg/kg) (Söborg et al. 1996). Therefore their presence in the SagaPro tablets should not lead to safety concerns with regard to phototoxicity, given that their content is carefully monitored for each batch. Another reason to monitor the amount of furanocoumarins is that they are known inhibitors of cytochrome P450 enzymes, also present in the gut, and thus a potential risk for herb-drug interactions (Koenigs and Trager 1998; Ueng et al. 2011).

To conclude, SagaPro tablets contain the flavonoid isoquercitrin. However, the amount is very low and not likely to have an effect on nocturia or any other pharmacologically significant effect in humans. Xanthotoxin and imperatorin were the main furanocoumarins found in the investigated tablets in amounts that are well below the considered levels for phototoxicity.

Table: Quantification of isoquercitrin, xanthotoxin and imperatorin in methanol extracts of SagaPro tablets analysed by UPLC/MS and presented as µg/tablet.

Compd.	Concentration (µg/tablet)
Isoquercitrin	158.3 ± 14.0
Xanthotoxin	280.0 ± 4.0
Imperatorin	2.0 ± 0.02

Each concentration is a mean ± SD of three independent experiments.

3. Experimental

3.1. Preparation of extracts

SagaPro tablets (Lot. Nr. 1347-2) were purchased at a pharmacy in Reykjavik in June 2014. Methanolic and aqueous extracts of the tablets were prepared by finely pulverizing two tablets in a mortar and subjecting 750 mg of the powder (identical to the average weight of the tablets) to sonication with 10.0 ml of methanol or 10.0 ml of water for 15 minutes followed by stirring for 24 hours at room temperature. The extracts were centrifuged and 1.00 ml of the supernatant was subjected to analysis.

3.2. TLC and HPLC

The aqueous extract was analysed by TLC on Merck silica gel plates 60 F_{254} (mobile phase: butanol, acetone, acetic acid and water 35:35:10:20) and the spots were visualized by naphtoresorcinol spray solution using glucose, fructose and sucrose as reference compounds. Methanol extracts were analysed for flavonoids and furanocoumarins using the same kind of TLC plates (mobile phase: ethyl acetate, acetone, dichloromethane, methanol and water 40:30:12:10:8), and the spots were visualized by dipping into aluminium chloride solution and exposing to UV light, using isoquercitrin, xanthotoxin and imperatorin as reference compounds.

The methanol extracts were analysed by HPLC Dionex Ultimate 3000 (mobile phase: water:methanol, 25 min gradient 10-95%, flow 4ml/min, Luna Phenyl-Hexyl 5µ, 10 x 250 mm Phenomenex column, at 254 nm) using isoquercitrin, xanthotoxin and imperatorin purchased from Phytolab GmbH & Co. Germany, as reference compounds. The retention time of isoquercitrin, xanthotoxin and imperatorin was 18.51 min, 22.84 min and 26.34 min respectively.

3.3. Identification and quantification by UPLC/MS spectrometry

The quantification was performed with a Waters Acquity UPLC system, coupled to a Waters Quattro Premier XE triple quadruple mass spectrometer equipped with electrospray ionization (ESI) probe. The analytical column used was an ACQUITY UPLC HSS T3 C18 (2.1 mm x 100 mm i.d.; 1.8 µm) (Waters corp., Milford, MA, USA), maintained at 35 ºC. The gradient system mobile phase consisted of buffer A: 0.1% formic acid B: acetonitrile + 0.1% formic acid, at a flow rate of 0.4 ml/min. A nonlinear gradient was used starting at 98% mobile phase A for 0.8 min up to 95% of mobile phase B in 1.5 min. The gradient was held for 1.2 min before going back to the initial conditions. The total chromatographic run time was 4.0 min. Retention times of isoquercitrin, xanthotoxin and imperatorin were 2.12 min, 1.75 min and 1.95 min, respectively. The sample manager temperature was maintained at 10.0 ºC. The mass spectrometer was optimized for analyzing quantification and qualification ions using multiple reactions monitoring (MRM) in positive electrospray ionization (ESI) mode to monitor parent > daughter ion (m/z) at the quantification transitions m/z 303.11 > 152.93 for isoquercitrin, m/z 216.99 > 201.9 for xanthotoxin and m/z 202.94 > 146.84 for imperatorin. Qualification transitions monitored was m/z 303.11 > 164.77 and m/z 303.11 > 229.00 for isoquercitrin, m/z 216.99 > 160.92 and m/z 216.99 > 188.96 for xanthotoxin and m/z 202.94 > 90.8 and m/z 202.94 > 130.9 for imperatorin. The capillary voltage was 3.5 kV; cone voltage was set individually for each compound during optimization. Data acquisition was carried out using MassLynx 4.1 software.

3.4. Statistics

The concentration of each compound per tablet was calculated in MS Excel and is presented as a mean ± SD of three independent experiments.

The authors have no conflict of interest.

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