# Development and Validation of a High-Performance Liquid Chromatography Method for Standardization of the Bioactive Ethyl Acetate Fraction of *Alstonia scholaris* (Linn.) R. Br. Growing in Egypt

Hesham I. El-Askary[a,*], Mahmoud M. El-Olemy[b], Maha M. Salama[a], and Mahetab H. Amer[b]

[a] Department of Pharmacognosy, Faculty of Pharmacy, Cairo University, Cairo 11562, Egypt. E-mail: helaskary@hotmail.com
[b] Department of Pharmaceutical Biology, Faculty of Pharmacy & Biotechnology, German University in Cairo, Cairo 11835, Egypt

* Author for correspondence and reprint requests

Z. Naturforsch. **68 c**, 376–383 (2013); received July 28, 2012/July 22, 2013

Bio-guided fractionation of the ethanolic extract of the leaves of *Alstonia scholaris* (Apocynaceae) growing in Egypt was carried out to evaluate its antihyperglycemic activity in alloxan-induced diabetic rats and its hepatoprotective activity against $CCl_4$-induced hepatotoxicity in rats. The ethyl acetate fraction of the ethanolic extract showed the highest antihyperglycemic [(133.6 ± 4.2) mg/mL, relative to metformin with (92.3 ± 2.7) mg/mL] and hepatoprotective [(37.9 ± 1.4) U/L, relative to silymarin with (29.7 ± 0.8) U/L] activities. Four compounds were isolated from this fraction, and identified by spectroscopic techniques and by comparison with reported data: caffeic acid and isoquercitrin for the first time from this plant, in addition to quercetin 3-*O*-*b*-D-xylopyranosyl (1‴⇓ 2″)-*b*-D-galactopyranoside (major compound) and chlorogenic acid. A validated reversed phase-high-performance liquid chromatography (RP-HPLC) method was developed for the standardization of the bioactive ethyl acetate fraction. The calibration curve showed good linearity ($r^2 > 0.999$) within tested ranges. The relative standard deviation of the method was less than 3% for intra- (0.4–2.0%) and inter-day (1.9–2.8%) assays. Mean recovery of the method was within the range of 98.5–102.5%. The minimum detectable concentration of the analyte (LOD) was found to be 0.04 $\mu$g/mL. This developed HPLC method was shown to be simple, rapid, precise, reproducible, robust, specific, and accurate for quality assessment of the bioactive fraction.

*Key words:* Validated RP-HPLC Method, Quercetin 3-*O*-*b*-D-xylopyranosyl (1‴⇓ 2″)-*b*-D-galactopyranoside, *Alstonia scholaris*