DEVELOPMENT OF IDENTIFICATION METHOD FOR A DRAFT VERSION OF PHARMACOPOEIA MONOGRAPH "FRANGULA BARK"

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Russian State Pharmacopoeia XIth edition suggests to use just test tube reactions for identification of Frangula bark. But according to the modern requirements for normative documentation on herbal medicinal products, it is more reasonable to use TLC method. The purpose of our study was to develop and validate a new TLC method for Frangula bark identification. The study object was Frangula bark; TLC was chosen as an analytical method for identification of phenolic compounds profile in the samples. In order to harmonize with leading Pharmacopoeias mobile phase ethyl acetate — 96% ethanol — water (100:17:13) was used (European Pharmacopoeia 7.104/2011:0025). The chromatographic analysis was carried out in the plates TLC silica gel 60 (aluminium sheets, Merck, Germany). Solutions of barbaloin and quercetin were used as reference solutions. 5% alcoholic potassium hydroxide solution was used for detection. The following zones were present 365 nm in the chromatograms obtained with the reference solutions: the zone with brown-yellow, green-yellow or yellow color (R_f approx. 0.3–0.4 that was accepted as $R_s = 1.0$, due to barbaloin) and the zone with light blue color (R_s approx. 0.6–0.7, due to quercetin). The following zones of phenolic compounds were present 365 nm in the chromatograms obtained with the test solution: 2 zones with orange-red or orange color (R_s (by barbaloin) approx. 0.5–0.7 and 1.7–1.9); others zones could be present with the exception of the zone with yellow or red-orange color (R_s approx. 1.0, due to reduced forms of anthracene derivatives). Method validation included evaluation of chromatographic system specificity and suitability. The method specificity was evaluated by coincidence of the different batches chromatographic profiles by the main zones and by compliance of abovementioned profiles with the test solution chromatogram description. The method validation was performed on 4 industrial batches. Chromatographic profiles of the different batches have coincided and been compliant with the test solution chromatogram description. The resolution between zones of quercetin (R_s approx. 0.6–0.7) and barbaloin (R_s approx. 1.0) in the chromatogram obtained with the reference solution has been chosen as a chromatographic system suitability index. The resolution value between the chosen zones must not be less than 1.5. The resolution values that had been calculated by the chromatograms obtained from different batches satisfied the method requirements. The suggested method can be used for identification of Frangula bark in routine analyses. This method has been included into the draft version of Pharmacopoeia monograph "Frangula bark" for XIIth edition of the Russian State Pharmacopoeia.

CHIMERIC ESTERS DERIVED FROM PLANT PHENOLIC ACIDS AND BORNEOL MODULATE HUMAN ENDOTHELIAL CELL ACTIVITIES *IN VITRO* — A NOVEL STRATEGY IN DRUG DISCOVERY

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Angiogenesis is a major pathological component of diseases such as cancer and coronary heart disease. Over the last decade, researchers have discovered pro- and anti-angiogenic compounds from herbs used in traditional Chinese medicine. For example, we revealed that ginsenosides Rgi and Rb! from American, Chinese, Korean, and Sanqi ginseng produce opposing activities on the vascular system (1), through activation of glucocorticoid (2) and oestrogen (3) receptors and distinct signalling pathways. We also showed the natural compound n-butylidene phthalide derived from the volatile oil of Radix *Angelica sinensis* to inhibit angiogenesis *in vitro* and *in vivo* (4). More evidence-based research and chemical optimisation of these compounds could further

enhance the effectiveness of these plant-based medicines in angiotherapy (5). Dantonic® is an oral formulation for the treatment of angina, currently undergoing global Phase **III** clinical trial. It consists of extracts from dried roots of *Salvia miltiorrhiza* (Danshen) and *Panax notoginseng* (Sanqi), plus borneol. Previously, a novel phenolic ester isopropyl 3- (3, 4-dihydroxyphenyl)-2 -hydroxypropanoate (IDHP) derived from danshensu was found to be a major metabolite of Dantonic® in human plasma and rabbit hearts (6). It produced a concentration-dependent (0.0001–30 μM) relaxation of norepinephrine-induced contraction in endothelium-intact and endothelium-free mesenteric arterial rings, mainly by causing the relaxation of smooth muscles through its actions on calcium-activated potassium channels (7). We hypothesise that chimeric esters of S. *miltiorrhiza* phenolic acids and borneol may improve the pharmacodynamic and pharmacokinetic profiles of the parent phenolic acids. To test their potential in therapeutic angiogenesis, IDHP and six chimeric esters were tested on human umbilical vein endothelial cells (HUVECs) for their ability to modulate migration, proliferation and tube formation *in vitro.* Some chimeric esters (1.0 nM-10 μM) stimulated HUVEC proliferation and migration, but had no significant effect on tube formation. Preliminary studies indicate that these chimerics stimulate HUVECs by inhibiting p38 mitogen-activated protein kinase. IDHP did not affect HUVEC proliferation but was cytotoxic at >50 μM, and its effect on HUVEC migration and tube formation are currently under investigation. Overall, this series of studies highlights a new platform for drug discovery based on the holistic principle of traditional Chinese medicine and synergistic interactions between materia medica, and introduces several novel drug candidates for angiogenesis modulation.

References: (1) Sengupta S *et al.* (2004) *Circulation* 110: 1219–1225. (2) Leung KW *et al.* (2006) *FEBS Lett* 580: 3211–3216. (3) Leung KW *et al.* (2007) *Br J Pharmacol* 152: 207–215. (4) Yen JC *et at.* (2011) *Angiogenesis* 14: 187–197. (5) Fan TP *et at.* (2006) *Trends Pharmacol Sci.* 27: 297–309. (6) Zheng XH *et al.* (2007) J. *Sep. Sci.* 30: 851–857. (7) Wang SP *et at.* (2008) *Eur J Pharmacol* 579: 283–288. Supported by The Ministry of Science and Technology of the People's Republic of China.

IDENTIFICATION OF THASPINE AS NOVEL TOPOISOMERASE INHIBITOR, IN A SPHEROIDS BASED SCREENING

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Aim of work — identification of effective compound (s) against solid tumors. The NCI Natural Products Set (221 compounds) was screened for apoptosis induction on HCT116 colon carcinoma human tumor cell line. The cell line was grown three dimensionally as spheroids in the 96-well plate. Apoptosis was detected using M30-CytoDeath ELISA assay. A preliminary indication for the mechanism of action of identified hits was determined through CMAP experiments, which was confirmed by a specific in vitro assay. SCID mice injected with HCT116 and FaDu cell lines were used for in vivo evaluation of identified hits. The screen led to the identification of thaspine, an alkaloid from the South American tree *Croton lechleri*, as a proapoptotic compound in HCT116 spheroids. Analysis of the gene expression signature of thaspine-treated cells, using the connectivity map (CMAP) technique, suggested that thaspine is a topoisomerase inhibitor. Thaspine inhibited both topoisomerase I and II enzymes in vitro. Finally, the compound induced apoptosis in two xenograft mouse models (FaDu & HCT116), and significant, though transient, tumor size reduction in FaDu model. Statistical significance was calculated using Student's t-test. The alkaloid thaspine, is a novel dual topoisomerase inhibitor, effective on human colon carcinoma spheroids with significant anticancer activity in vivo.