Proposal of Qualitative Determination of Taxus baccata L. Homeopathic Tincture by applying Rapid Horizontal TLC and LC/MS

Proposal of Qualitative Determination of *Taxus baccata* L. Homeopathic Tincture by applying Rapid Horizontal TLC and LC/MS Procedures

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Abstract. The homeopathic tincture of *Taxus baccata* L. is monographed in the current German Homeopathic Pharmacopoeia (HAB 2009). Continuing our work on optimization of TLC-analytical investigations for improved Homeopathic Pharmacopoeia monographs we propose a rapid and distinct qualitative TLC determination as well as a distinct qualitative LC/MS procedure. LC/MS procedures are not yet applied for identification in the HAB monograph. The purpose of the following work has been the quali-quantitative analysis by HPLC/DAD/MS of a Taxus extract in order to make characterization of polyphenols compounds. We have found four flavonoids, in particular we identified quercetin 3-O-rutinoside (0.284 mg/ml) and kaempferol 3-O-rutinoside (0.05 mg/ml) as the main flavonols. We have also found three cinnamic acid derivatives and 10-deacetylbaccatin III. Optimising TLC-analytical investigations for an improved Homeopathic Pharmacopoeia monograph of *Taxus baccata* L. we propose a TLC determination using different sorbens materials and mobile phase mixtures and applying a horizontal chamber. Pre-treatment of the homeopathic tincture by SPE lead to more distinct TLC-bands. By using further reference substances the tested homeopathic tincture could be more peculiarly identified. It was been also measured the antioxidant capacity by DPPH test.
Thus, the analyses lead to a proposal of an updated and optimized identification test of *Taxus baccata* L. homeopathic tincture.

Introduction. Yew, *Taxus baccata* L., a sacred plant favoured by the Druids in ancient times, is an evergreen bush or tree up to 17 m high having a trunk with a diameter up to 1 m. The species is native to Europe as far as Sicily and Minor Asia. Ethanolic tinctures of yew leaves, the needles of *Taxus bacata* L. are used in homeopathy in the treatment of mal digestion and skin pustules (1, 2). The homeopathic tincture of *Taxus baccata* L. is monographed in the current German Homeopathic Pharmacopoeia (HAB 2009). However, the described method for identification test is a common comparative TLC procedure (3) that might be updated. LS/MS procedures are not describes in the HAB monograph.

Materials and methods. TLC was performed by using different sorbens materials and mobile phase mixtures and applying a horizontal chamber (plates 5 x 5 cm, circa 2 ml of mobile phase, 10-20 µL of homeopathic tincture, 2-6 µL of sample solutions, 3-4 min). Pre-treatment of the homeopathic tincture by SPE lead to more recognisable TLC-bands. By using further reference substances (baccatin III, 10-deacetylbaccatin III, cephalomannine; kaempferol, quercetin, myricetin) the tested homeopathic tincture could be more clearly described.

Analyses foTLC

chamber	: DESAGA Horizontal separating chamber (4, 5)
optimum sorbens	: Silica gel 60 ADAMANT® UV254 0.25 mm, 5 cm x 5 cm
application amount of samples	: 2 - 6 µL
application amount of references	: 10 - 20 µL
	solutions (0.5 - 2 mg / mL) of references of chromatographic purity from different purchasers
optimum solvent system	: ethyl acetate:methanol:water (70+20+10) and (75+20+10)
migration distance	: 3.5 cm
separation time	: 3 - 4 min
detection	: UV 254 nm / 366 nm

DESAGA H-chamber

Analyses for polyphenols were carried out by HPLC/DAD/MS using a 250 × 4.6 mm, 5 μm, Lichrosorb column (Phenomenex, USA). Free radical scavenging activity was evaluated with the DPPH• assay. The antiradical capacity of the sample extracts was estimated according to the procedure reported by Brand-Williams et al. (6) slightly modified.

Results and discussion.

Applying the described TLC conditions references and sample substances gave distinct bands in the R_f range of 0.2 to 0.4 for quercetin, myricetin, kaempferol, and 0.55 to 0.9 for baccatin III, 10-deacetylbaccatin III, cephalomannine and, thus, allow to provide TL chromatograms showing a better zone distribution compared to the HAB conditions.

The chromatographic profile of Taxus tincture is presented in Figure 1. We have found four flavonoids (see figure 1), in particular we identified quercetin 3-O-rutinoside (0.378 mg/ml) and kaempferol 3-O-rutinoside (0.05 mg/ml), as previously reported by Krauze-Baranowska (7). We have also identified 10-deacetylbaccatin III (0.075mg/ml) as reported by Das et al. (8) and we found four cinnamic acid derivatives. Baccatin III, 10-deacetylbaccatin III and flavonol aglycons were not found because under HPLC detection limits.

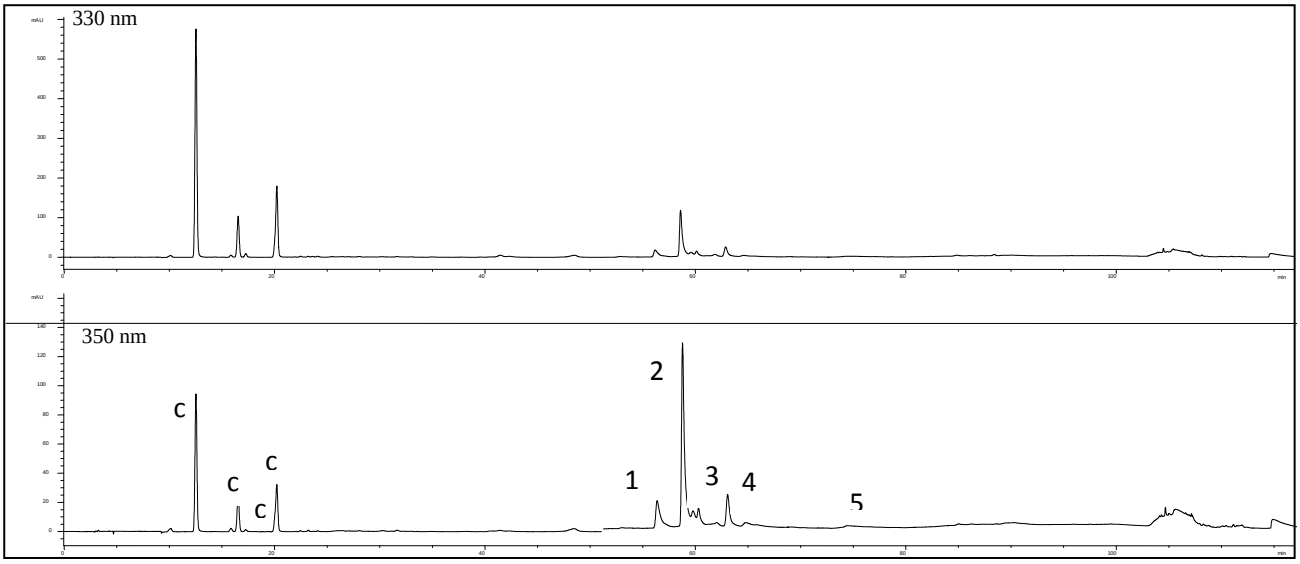

Figure 1. Chromatographic profile acquired at 330 nm and 350 nm, of Taxus tincture. Identified compounds:
1. Quercetin xylosil glucoside, 2. quercetin 3-O-rutinoside, 3. Kaempferol xylosil glucoside,
4. kaempferol 3-O-rutinoside, 5. 10-deacetylbaccatin III, c. cinnamic acid derivatives

Free radical scavenging activity was evaluated with the DPPH• assay, and the tincture have shown an A.R% value of 83.8%

The rapid horizontal TLC analyses (H-chamber, plates 5x5 cm) take little time (3-4 min), require only small amounts of material, and can easily be applied as a routine analytical method.

Hence the described analyses lead to the suggestion of an updated and optimized identification test of *Taxus baccata* L. homeopathic tincture.

[1] Brendler Th., Grünwald J., Jänicke C. (Eds.) **2003** Herbal Remedies, medpharm, Scientific Publishers, Stuttgart
[2] Hagers Handbuch der Pharmazeutischen Praxis **2006** Springer, Heidelberg
[3] Deutsches Homöopathisches Arzneibuch HAB **2009**, Monograph Taxus baccata (Edition 2000)
[4] Kraus Lj.**1993** Concise Practical Book of Thin-Layer Chromatography, DESAGA-Sarstedt Gruppe, Wiesloch
[5] WHO Quality Control Methods for Medicinal Plant Material **1998** TLC-Horizontal Technique, WHO, Geneva
[6 Brand-Williams W., Cuvelier M.E., Berset, C. *Lebensmitt. Wiss. Technol. Int.* **1995**, 28, 25-30.
[7] Krauze-Baranowska M, *Z. Naturforsch*. **2004**, 59c, 43-47
[8] B. Das S. Padma Rao, Srinivas K. V. N. S., Yadav J. S. *Phytochemistry* **1995**, 38, 715-717