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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 qualitative 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 sorbents 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.

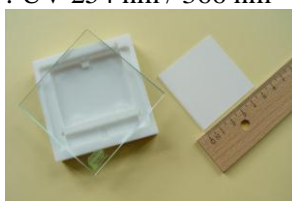
Thus, the analyses lead to a proposal of an updated and optimized identification test of *Taxus baccata* L. homeopathic tincture.

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 baccata* 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 sorbents 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 sorbents	: 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.

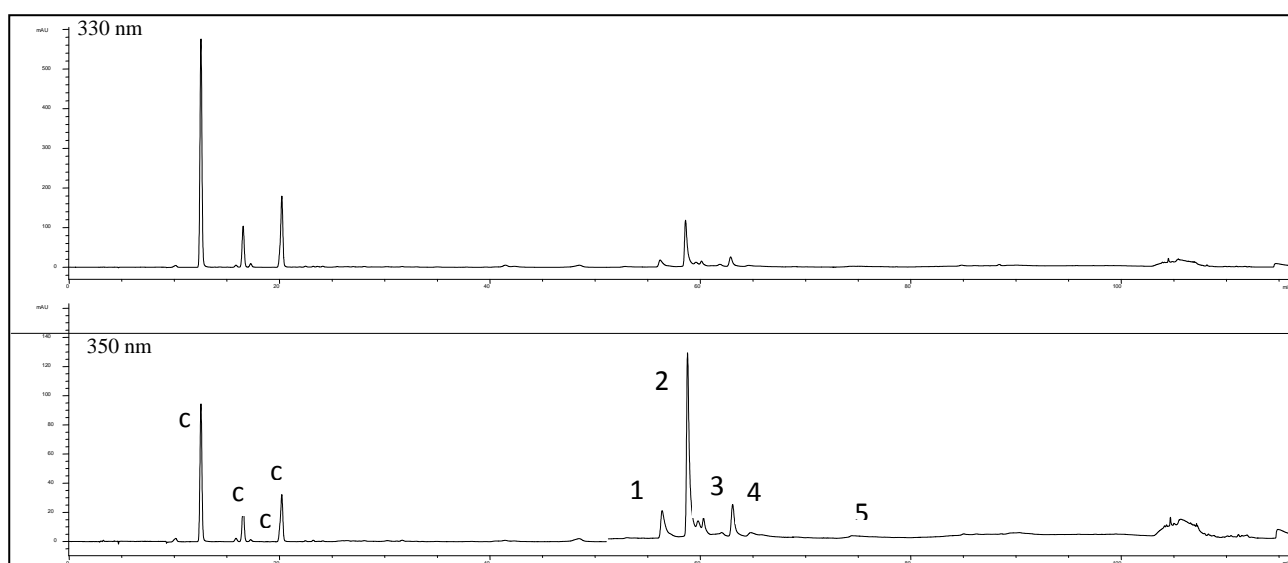


Figure 1. Chromatographic profile acquired at 330 nm and 350 nm, of *Taxus* tincture. Identified compounds:

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.

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