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Data Descriptor

Dataset of Targeted Metabolite Analysis for Five Taxanes of Hellenic *Taxus baccata* L. Populations

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Abstract: Novel primary sources of one of the world's leading anticancer agent, paclitaxel, as well as of other antineoplastic taxanes such as 10-deacetylbaccatin-III, are needed to meet an increasing demand. Among the *Taxus* species the promise of *Taxus baccata* L. (European or English yew) has been documented. In this study, the metabolite analysis of two marginal *T. baccata* populations in Greece (Mt. Cholomon and Mt. Olympus), located at the southeastern edge of the species natural distribution, are being explored. A targeted liquid chromatography – mass spectrometry (LC-MS/MS) analysis was used to determine the content of 10-deacetylbaccatin III, baccatin III, 10-deacetyltaxol, paclitaxel and cephalomannine in the needles of each of the populations from three sampling periods (spring, summer and winter). This is the first survey to generate a taxane targeted metabolite data set, since it derives from Hellenic natural populations that have not been explored before. Furthermore, it has used an extensive sample design in order to evaluate chemodiversity at the population level. The analysis revealed significant levels of chemodiversity within and among the investigated populations and significant seasonal variation that could be exploited for the selection of superior germplasm native to Greece, for yew plantations and further exploitation which is necessary for the production of important taxanes.

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Keywords: *Taxus baccata*; Metabolites; LC-MS/MS; Taxanes; Yew

1. Summary

Taxus L. species (yew), a slow-growing and shade-tolerant dioecious conifer tree, are the source of one of the world's leading anticancer agent, paclitaxel [1,2]. It was first isolated from the bark of *Taxus brevifolia* Nutt [3] and it is now used for the treatment of several forms of breast, lung, blood and gynecological cancers [4]. Nowadays, different plant parts of various *Taxus* species, such as *T. baccata* needles, are being used for the collection of this diterpenoid alkaloid. While the demands of paclitaxel are high, the yield is low (0.001–0.05%). Additionally, for the treatment of one cancer patient, almost eight 60-year-old yew trees are needed [5]. Considering the above, it is of a high interest to explore new sources of taxanes.

Besides paclitaxel (PACL), 10-deacetylbaccatin III (10-DAB), the precursor of PACL, is also of high importance [6,7]. It has also been used extensively for the semi-synthesis of Taxotere, which shows a more efficient anti-tumor activity than taxol, as well as the analogue of PACL, cephalomannine

(CEPH) [6,8]. Other taxanes that are collected from the *Taxus* species are baccatin III (BAC), brevifoliol and 9-dihydro-13-acetylbaccatin III (9-DAB III) [9].

There is a high inter- and intraspecific variability in the taxane content [7,10] and it has been shown that this variability can depend on the season and taxoid type. The concentration of 10-DAB of first- and second-year needles of *T. baccata*, grown in a Polish garden was highest in January, and lowest in April [11], where in a study in Tehran, Iran [6], the highest concentration of yew needles was obtained in August. The bark of *T. baccata* grown in a Czech Republic Forest showed that the highest concentration of PACL and three other taxanes was in October and the lowest in January. [12] found that 17 *Taxus* x media cultivars had large variation in the content of 10-DAB, BAC, CEPH and PACL.

Due to the fact that *T. baccata* populations in Greece are located at the southeastern edge of the species natural distribution we anticipate that there is a considerable possibility that their genetic diversity presents unique characteristics, in line to the rear edge theory expectations [13,14]. Until now, no other studies exist that embody quantitative data from Greek *T. baccata* populations with variation of taxane compounds. This first dataset will: (1) provide an assessment of the variation on taxane compounds which is associated with genotype and seasonal effect, (2) serve as a baseline for the selection of superior germplasm, native to Greece and (3) assist future breeding strategies towards high taxane production.

In this study, two natural yew populations were chosen, both located in Northern Greece, Mt Cholomon and Mt Olympus, in order to estimate the levels of five important taxanes; 10-DAB (Chemical Abstracts Service "CAS": 32981-86-5), 10-deacetyltaxol (10-DTAX; CAS: 78432-77-6), BAC (CAS: 27548-93-2), CEPH (CAS: 71610-00-9) and PACL (CAS: 33069-62-4). An Ultra Performance Liquid Chromatography Mass Spectrometry (UPLC-MS/MS) analysis was adapted from [15] using needle samples that were collected from 27 trees of each population, from north facing and well shaded branches. The sampling was repeated three times: (1) at the beginning of May after the end of the flowering period, (2) in late August at the end of the growing season and (3) in early December at the beginning of winter in order to estimate population form one hand and seasonal variation of taxanes compounds.

Analysis of the current dataset revealed significant levels of chemodiversity within and among the selected populations, and significant seasonal variation.

2. Data Description

Data were organized in tabular form in an MS Excel®spreadsheet, which is available as Supplementary Table S1. Each line in the dataset presents information from the LC – MS/MS needle analysis of one tree per population and for each season.

The first column (A), with the heading "Sample name" displays the entry identification number of each sample into the mass spectrometry. Column "B" (population), indicates the population that each sample belongs to (either Mt Cholomon or Mt Olympus). The third column (C), with the heading "Season" presents the season in which the samples were collected. Columns D, E, F, G, and H with the headings "10-deacetylbaccatin III_Q1", "10-deacetyltaxol_Q1", "baccatin III_Q1", "cephalomannine_Q1" and "paclitaxel_Q1" respectively, display the quantitative data of each of the five taxanes. The concentration of BAC in column (F), in a number of samples was too low (indicated as "N/A"), i.e., lower than the detection or even quantification limit for the LC-MS/MS analysis followed. Statistically this is usually considered as 'zero' and in the Supplementary Table S1 is appropriately presented as 'not identified' - "N/A".

Table 1 shows the multiple reactions monitoring (MRM) parameters (RT: retention time, R2: R-squared, ES: electrospray ion mode, electrospray molecular ion, qualifier and quantifier ions) of the five taxanes. Furthermore, Figure 1 shows the calibration curves of the five targeted compounds that were created with the use of external standards. On each of the calibration curves information of the regression model and the coefficient of determination (R-squared) are given. Ten blanks and

10 standard mix (std_mix) were inserted every 17 samples, in order to optimize the quality and precision of the API 5500 triple-quadrupole mass spectrometer.

Table 1. MRM parameters of the five taxanes of interest.

Compound	RT (min)	r^2	ES	Molecular Ion (m/z)	Quantifier Ion	Qualifier Ions	
					Q1 m/z	Q1 m/z	Q2 m/z
10-DAB	6.10	0.9955	+	545	105	207	171
BAC	6.91	0.9987	+	587	105	121	177
10-DTAX	7.66	0.9964	+	812	286	105	122
PACL	9.81	0.9953	+	854	105	286	77
CEPH	9.57	0.9967	+	832	264	105	83

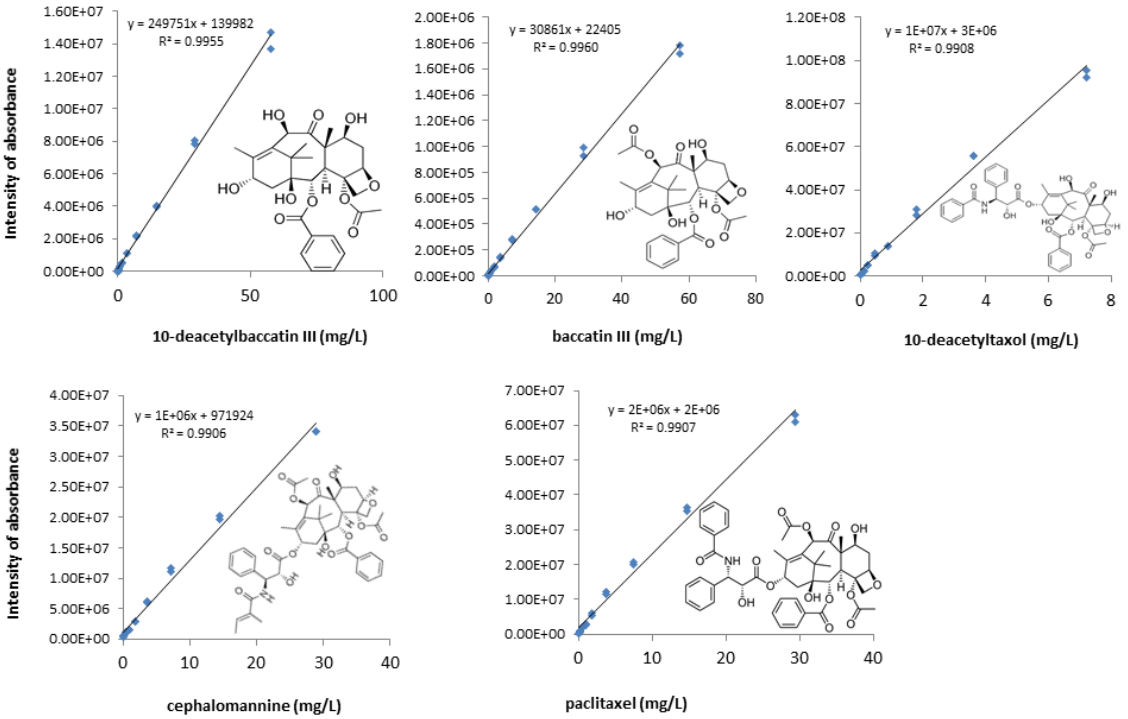

Figure 1. Calibration curves of the five external standards used for the quantification of the taxanes in the *T. baccata* collected needles.

3. Methods

3.1. Experimental Sites and Collection

Needle clippings were collected from 27 healthy trees of two Hellenic natural *T. baccata* L. populations: Mt Cholomon (Taxiarchis; 40° 26.299′ N, 23° 32.374′ E; altitude: 804 m asl) and Mt Olympus (Litochoro; 40° 5.624′ N, 22° 25.797′ E, altitude: 819 m asl). Selected trees were spatially distributed in order to avoid sampling related individuals, while isolated trees were not sampled. Samples were collected from three sampling periods: (1) at the beginning of May after the end of the flowering period, (2) in late August at the end of the growing season and (3) in early December at the beginning of winter.

The climate of Mt Cholomon is classified as subhumid Mediterranean. The mean annual air temperature is 11.1 °C and the annual rainfall is 767 mm [16]. The main rock formation of the area is mica- and talk-schist [17].

In the case for Mt Olympus the climate is characterized as sub-Mediterranean, with a mean annual air temperature of 8.9 °C and an annual rainfall of 688.7 mm [18]. Lastly, it is mainly formed of dolomitic limestone and marbles [19].

3.2. Chemicals

General laboratory reagents were purchased from Sigma-Aldrich and Fisher Scientific. Deionized water was purified in loco with an Arium®purification system (Sartorius AG, Goettingen, Germany). Authentic standards of taxanes, namely 10-DAB, 10-DTAX, BAC, CEPH and PACL were purchased from TransMIT PlantMetaChem (Gießen, Germany). Stock solutions of taxanes were prepared by dissolving the standards in methanol (LC-MS grade).

3.3. Taxane Extraction

The extraction of taxanes was performed according to the method of [20] with modifications. Fresh needles collected from plants were freeze-dried (Freeze-dryer Alpha 1-2 LD plus, Christ, Germany; at −24 °C). The dried needles were milled into homogenous powders. Samples (0.5 g) were mixed with 4 mL of 60% acetone, stirred on an orbital shaker for 20 mins, and centrifuged (5 min; 1800× *g*; 4 °C). This procedure was repeated three times. The supernatants were pooled, and the acetone was evaporated under reduced pressure at T ≤ 38 °C. The remaining aqueous phase was mixed with 4 mL of dichloromethane. The mixture was vortexed for about 1 min and centrifuged (2 min; 900× *g*; 4 °C). This procedure was repeated twice. The organic layers were collected and dried under reduced pressure at T ≤ 38 °C. The dry residue was dissolved in 2 mL of LC-MS methanol, filtered through PTFE 0.22 µm membranes into glass vials, and stored at −80 °C until further analysis.

3.4. Ultra Performance Liquid Chromatography Mass Spectrometry (UPLC-MS/MS)

The UPLC-MS/MS analysis was adapted from [15] and performed on a UHPLC Dionex 3000 (Thermo Fisher Scientific Germany), equipped with a binary pump, an online vacuum degasser, and a column compartment. The separation of taxanes was carried out on a Kinetex C18 100Å column (2.1 mm × 100 mm, 2.6 µ), equipped with a column guard (Phenomenex, Torrance, CA, USA), kept at 35 °C. Samples were injected using an autosampler (Dionex Thermo Fisher Scientific Germany) set at 4 °C. The mobile phase was composed of water with 0.1% *v*/*v* formic acid (A) and acetonitrile with 0.1% *v*/*v* formic acid (B). Separation was carried out as follows: 80% A (0–0.5 min), 80–50% A (0.5–7 min), 50–28% A (7–10 min), 20–0% A (10–10.2 min), 0% A (10.2–12 min), 0–80%A (12.1–15 min). The flow rate was 0.3 mL min^{-1} and the injection volume was 5 µL.

The LC-MS/MS analysis was performed on an API 5500 triple-quadrupole mass spectrometer (Applied Biosystems/MDS Sciex, TOR, Canada). The instrument was operated using an electrospray source in positive ion mode. The electrospray ionization (ESI) parameters were as follows: the spray voltage was set at 5500 V for positive mode, the source temperature was set at 250 °C, the nebulizer gas (Gas 1) and heater gas (Gas 2) at 40 and 20 psi respectively. UHP nitrogen (99.999%) was used as both curtain and collision gas (CAD) at 20 and 9 psi respectively.) Analyst™ software version 1.6.1 (Applera Corporation, Norwalk, CT, USA) was used for instrument control and data acquisition. Compounds were identified by comparing the retention time and the spectral characteristic of the peaks with those of authentic standards. Multiple reactions monitoring (MRM) was used for quantification based on the peak area of the samples (Table 1).

The data of Table S1 were calculated based on the calibration curves presented in Figure 1. The Linear range for the taxanes studied (expressed in mg/L) was: 10-DAB, 0.03 to 57.8; BAC, 0.03 to 57.4; 10-DTAX, 0.03 to 7.23; PACL, 0.06 to 29.45; CEPH, 0.06 to 28.9. As internal standard, cinnamic acid was used and the recovery was 91.4%, with standard deviation (STDV) 2.50%. The reproducibility was estimated and expressed with a value of RSD = 1.72, as relative standard deviation. Samples were diluted ten times and analyzed for 10-DAB and 10-DTAX in order to retrieve intensities of absorbance within the linear range of the calibration curves. Concentrations are expressed as mg L-1.

Supplementary Materials: The following are available online at https://doi.org/10.5281/zenodo.3697868.

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References

1. Wheeler, N.C.; Jech, K.; Masters, S.; Brobst, S.W.; Alvarado, A.B.; Hoover, A.J.; Snader, K.M. Effects of genetic, epigenetic, and environmental factors on taxol content in *Taxus brevifolia* and related species. *J. Nat. Prod.* **1992**, *55*, 432–440. [CrossRef] [PubMed]
2. Benham, S.E.; Houston Durrant, T.; Caudullo, G.; de Rigo, D. *Taxus baccata* in Europe: Distribution, habitat, usage and threats. In *European Atlas of Forest Tree Species*; San-Miguel-Ayanz, J., de Rigo, D., Caudullo, G., Houston Durrant, T., Mauri, A., Eds.; Publications Office of the EU: Luxembourg, 2016; p. e015921+.
3. Wani, M.C.; Taylor, H.L.; Wall, M.E.; Coggon, P.; McPhail, A.T. Plant antitumor agents. VI. Isolation and structure of taxol, a novel antileukemic and antitumor agent from *Taxus brevifolia*. *J. Am. Chem. Soc.* **1971**, *93*, 2325–2327. [CrossRef] [PubMed]
4. Nadeem, M.; Rikhari, H.C.; Kumar, A.; Palni, L.M.S.; Nandi, S.K. Taxol content in the bark of Himalayan Yew in relation to tree age and sex. *Phytochemistry* **2002**, *60*, 627–631. [CrossRef]
5. Cope, E.A. Taxaceae: The genera and cultivated species. *Bot. Rev.* **1998**, *64*, 291–322. [CrossRef]
6. Ghassempour, A.; Rezadoost, H.; Ahmadi, M.; Aboul-Enein, H.Y. Seasons study of four important taxanes and purification of 10-deacetylbaccatin III from the needles of *Taxus baccata* L. by two-dimensional liquid chromatography. *J. Liq. Chromatogr. Relat. Technol.* **2009**, *32*, 1434–1447. [CrossRef]
7. van Rozendaal, E.L.M.; Lelyveld, G.P.; van Beek, T.A. Screening of the needles of different yew species and cultivars for paclitaxel and related taxoids. *Phytochemistry* **2000**, *53*, 383–389. [CrossRef]
8. Hao, D.C.; Xiao, P.G.; Ge, G.B.; Liu, M. Biological, chemical, and omics research of *Taxus* medicinal resources. *Drug Dev. Res.* **2012**, *73*, 477–486. [CrossRef]
9. Cameron, S.I.; Smith, R.E. Seasonal changes in the concentration of major taxanes in the biomass of wild Canada yew (*Taxus canadensis* Marsh.). *Pharm. Biol.* **2008**, *46*, 35–40. [CrossRef]
10. Hook, I.; Poupat, C.; Ahond, A.; Guénard, D.; Guéritte, F.; Adeline, M.-T.; Wang, X.-P.; Dempsey, D.; Breuillet, S.; Potier, P. Seasonal variation of neutral and basic taxoid contents in shoots of European Yew (*Taxus baccata*). *Phytochemistry* **1999**, *52*, 1041–1045. [CrossRef]
11. Glowniak, K.; Mroczek, T.; Zobel, A.M. Seasonal changes in the concentrations of four taxoids in *Taxus baccata* L. during the autumn-spring period. *Phytomedicine* **1999**, *6*, 135–140. [CrossRef]
12. Wang, X.; Huang, Y.; Mort Andrew, J.; Zeng, Y.; Tauer Charles, G.; Cochran Kenneth, D. Variation of taxane content in needles of *Taxus x media* cultivars with different growth characteristics. *Zeitschrift für Naturforschung C* **2006**, *61*, 619. [CrossRef] [PubMed]
13. Hampe, A.; Petit, R.J. Conserving biodiversity under climate change: The rear edge matters. *Ecol. Lett.* **2005**, *8*, 461–467. [CrossRef] [PubMed]
14. Fady, B.; Aravanopoulos, F.A.; Alizoti, P.; Mátyás, C.; von Wühlisch, G.; Westergren, M.; Belletti, P.; Cvjetkovic, B.; Ducci, F.; Huber, G.; et al. Evolution-based approach needed for the conservation and silviculture of peripheral forest tree populations. *For. Ecol. Manag.* **2016**, *375*, 66–75. [CrossRef]
15. Kobusiak-Prokopowicz, M.; Marciniak, A.; Ślusarczyk, S.; Ściborski, K.; Stachurska, A.; Mysiak, A.; Matkowski, A. A suicide attempt by intoxication with *Taxus baccata* leaves and ultra-fast liquid chromatography-electrospray ionization-tandem mass spectrometry, analysis of patient serum and different plant samples: Case report. *BMC Pharmacol. Toxicol.* **2016**, *17*, 41. [CrossRef] [PubMed]

16. Avramidou, E.; Abraham, E.; Mantzanas, K.; Sidiropoulou, A.; Chatzipaschali, A. Vegetation cover and species composition under different grazing intensity in mountainous grasslands of Northern Greece. In *Ecosystem Services and Socio-Economic Benefits of Mediterranean Grasslands*; Kyriazopoulos, A.P., López-Francos, A., Porqueddu, C., Sklavou, P., Eds.; CIHEAM: Zaragoza, Spain, 2016; Volume 114, pp. 95–98.
17. Ganatsios, H.P.; Tsioras, P.A.; Pavlidis, T. Water yield changes as a result of silvicultural treatments in an oak ecosystem. *For. Ecol. Manag.* **2010**, *260*, 1367–1374. [CrossRef]
18. Fyllas, N.M.; Troumbis, A.Y. Simulating vegetation shifts in north-eastern Mediterranean mountain forests under climatic change scenarios. *Glob. Ecol. Biogeogr.* **2009**, *18*, 64–77. [CrossRef]
19. Xystrakis, F. The Drought Tolerance Limit of European Beech (*Fagus sylvatica* L.) Stands on Mt. Olympus, NC Greece. Ph.D. Thesis, Faculty of Forest and Environmental Sciences, Albert-Ludwigs Universität, Freiburg im Breisgau, Germany, 2009.
20. Mubeen, S.; Li, Z.L.; Huang, Q.M.; He, C.T.; Yang, Z. Comparative transcriptome analysis revealed the tissue-specific accumulations of taxanes among three experimental lines of *Taxus yunnanensis*. *J. Agric. Food Chem.* **2018**, *66*, 10410–10420. [CrossRef] [PubMed]