Received: 5 April 2023 | Revised: 19 July 2023 | Accepted: 21 July 2023

DOI: 10.1002/pca.3273

RESEARCH ARTICLE

Phytochemical Analysis WILEY

TLC and HPTLC-APCI-MS for the rapid discrimination of plant resins frequently used for lacquers and varnishes by artists and conservators

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Funding information
ALAGO ART & STRINGS GmbH

Abstract

Introduction: Depending on their terpenoid and phenolic constituents plant resins can be classified as diterpenoid, triterpenoid or phenolic resins; thereby the profile of diterpenes and triterpenes is considered as genus- or even species-specific.

Objectives: We aimed to develop a simple, rapid, inexpensive, sensitive and specific method for the identification of resin-specific triterpenoid and phenolic compounds in plant resins using (HP)TLC [(high-performance) thin-layer chromatography] combined with APCI-MS (atmospheric pressure chemical ionisation mass spectrometry) and post-chromatographic detection reactions.

Methods: Twenty resin samples from different plant species were analysed. Different extraction procedures, post-chromatographic detection reagents as well as various sorbents and solvents for planar chromatography were tested. To evaluate the potential of the optimised (HP)TLC-APCI-MS methods, parameter such as limit of detection (LOD) was determined for selected marker compounds.

Results: Our protocol enabled qualitative analyses of chemotaxonomic molecular markers in natural resins such as dammar, mastic, olibanum and benzoin. For the first time, the application of thionyl chloride-stannic chloride reagent for a specific post-chromatographic detection of triterpenes is reported, sometimes even allowing discrimination between isomers based on their characteristic colour sequences. For triterpene acids, triterpene alcohols and phenolic compounds, detection limits of 2–20 ng/TLC zone and a system precision with a relative standard deviation (RSD) in the range of 3.9%–7.0% were achieved by (HP)TLC-APCI-MS. The applicability of the method for the analysis of resin-based varnishes was successfully tested on a mastic-based varnish. Thus, the method we propose is a helpful tool for the discrimination of resins and resin-based varnishes with respect to their botanical origin.

Marcel Schendzielorz and Theresa Schmidt contributed equally to this work.

KEYWORDS
benzoin balsam, HPTLC-MS, plant resin, triterpene, varnish

1 | INTRODUCTION

Plant resins are metabolic by-products of plant tissues that have been used for thousands of years for various applications, for example, as adhesives, hydro-repellents, coating and sealing agents or in the formulation of fragrances, flavours and pharmaceuticals.[1] They are very complex mixtures of different volatile as well as non-volatile compounds and can be classified as terpenoid or phenolic resins based on their constituents. Terpenoid resins make up the majority of these resins; they contain various monoterpenes, sesquiterpenes, diterpenes and triterpenes. Thereby, monoterpenes and sesquiterpenes are present in the volatile fraction of most resins, while diterpenes and triterpenes are non-volatile or very low volatile resin components. Since diterpenes and triterpenes are rarely found together in the same resin, a further subdivision into diterpene and triterpene resins is possible.[2,3]

Diterpenoid resins are produced by trees of the subfamily *Caesalpinioideae* or conifers such as pines of the genus *Pinus* (*Pinaceae* family). Sandarac, *Juniperus* and cypress resins, which are extracted from plants of the *Cupressaceae* family, also belong to the conifer resins and thus to the class of diterpenoid resins. These resins contain mainly bicyclic and tricyclic diterpenoids holding a labdane- or pimarane-type structure.[4,5] Resins of the *Pinaceae* family, such as pine resins, differ markedly in chemical composition from those of the *Cupressaceae* family. Pine resins, for instance, contain predominantly tricyclic diterpenoid acids with either abietane or pimarane skeletons.[5,6]

Triterpenoid resins are produced by various broad-leaved trees, for example, of the *Burseraceae*, *Dipterocarpaceae* or *Anacardiaceae* family. Resins from the *Burseraceae* family include olibanum or frankincense, obtained from several *Boswellia* species. *Boswellia* resins were already used by ancient cultures either in the preparation of perfumes, cosmetics and medicines or in fumigation during embalming ceremonies. In a few cases, the use of *Boswellia* resins as tempera paint binding media is also reported.[7] In the last decades, anti-inflammatory and antidepressant properties of triterpenoid boswellic acids and diterpenoid incensole derivates in *Boswellia* resins received large interest in the scientific world.[8] White dammar, however, is derived from various plants of the genera *Hopea* and *Shorea* (*Dipterocarpaceae* family) and mastic from trees of the genus *Pistacia* (*Anacardiaceae* family). Both, white dammar and mastic, are frequently used in the formulation of varnishes for easel paintings. In addition to their protective function, varnishes saturate the colours and give the painting a glossy appearance. Painting conservators in particular apply dammar and mastic on a large scale. Furthermore, mastic is utilised as incense or as an adhesive. Mastic holds many components in common with white dammar, but differs significantly in composition from *Boswellia* resins. In general, triterpenoid resins consist of pentacyclic and tetracyclic triterpenes belonging, for example, to oleanane, ursane, lupane, dammarane or euphane type molecules (Supporting Information Figure S1). Tetracyclic triterpenes based on the dammarane or euphane skeleton are characterised by the presence of a hydroxy or a keto group at position C-3. Furthermore, the lateral chain of tetracyclic triterpenic components often bears functional groups. A hydroxy or keto group at C-3 is also common for pentacyclic triterpenes based on oleanane, ursane or lupane type molecules. In addition, pentacyclic triterpenes are often oxidised at C-28 to alcohols, aldehydes or carboxylic acids.[5,6]

In addition to the terpenoid resins, there is a much smaller but also important group of phenolic resins. These resins contain no or hardly any terpenes, but esters of benzoic acid (**9**) and cinnamic acid (**10**) with benzenoid alcohols.[5,6] Benzoin as well as storax are well-known phenolic resins. The later results from *Liquidambar* spp. and *Altingia* spp. (*Altingiaceae* family).[6] Benzoin exudes from *Styrax* trees and shrubs (*Styracaceae* family). According to their origin, a distinction is made between Sumatra and Siam benzoin balsams. Siam benzoin balsam is mainly tapped in Laos and Thailand from *Styrax tonkinensis* (Pierre) Craib ex Hartwich. Sumatra benzoin balsam is produced by *Styrax benzoin* Dryand and *Styrax parallelloneurum* Perk, both native to tropical forests of North Sumatra. Analysis showed that Siam benzoin balsam is composed of **9** and esters of **9** and **10**. In contrast, Sumatra benzoin balsam contain **10** and its esters as well as a low content of **9** and respective esters.[9,10] Besides aromatic compounds, triterpenoid acids such as siaresinolic and sumaresinolic acid have also been identified in benzoin. A comparison of benzoin balsams shows that siaresinolic acid was found only in Siam benzoin balsam, while sumaresinolic acid was detected in both, Siam and Sumatra benzoin balsams.[10] Similar to benzoin balsams, storax resins obtained from *Liquidambar* species are also characterised by the presence of triterpenoid acids. For example, oleanonic acid and 3-epi-oleanolic acid have been identified in the resin of *Liquidambar orientalis* Mill.[11,12] Benzoin and storax are known for their pharmacological and odoriferous properties and used as incense in religious ceremonies, often in combination with *Boswellia* resins.[5,10] In addition, benzoin was used for the production of red lacquers. For instance, the red-coloured resin dragon's blood was often mixed with other red resins such as benzoin or shellac to intensify or modify the colour.[13]

The metabolic characterisation of plant resins has been the subject of several studies, and in particular the profile of diterpenes and triterpenes can be considered as genus-specific or sometimes even species-specific and allows distinction between different resin types.[5,6,14–16] Diterpenes and triterpenes as well as phenolic compounds in plant resins or varnishes can be analysed by numerous analytical methods. HPLC (high-performance liquid chromatography) in combination with MS (mass spectrometry) or UV (ultraviolet) detection as well as GC–MS (gas chromatography–mass spectrometry or THM-GC–MS (GC–MS with thermally-assisted hydrolysis and

methylation) are the most common techniques for the determination and quantification of diterpenoids/triterpenoids and phenolic compounds in various resins, with derivatisation of target structures required for GC applications.[4,10,12,17–23] The composition of varnishes on artworks as well as ageing products of plant resins has been studied, for instance, by MALDI-TOF-MS (matrix-assisted laser desorption/ionisation time-of-flight-mass spectrometry), GALDI-TOF-MS (graphite-assisted laser desorption/ionisation time-of-flight-mass spectrometry) or TOF-SIMS (time-of-flight secondary ion mass spectrometry).[24–28] These techniques allow the analysis of resins without any time-consuming sample preparation or derivatisation. However, the distinction between isomers is only possible to a limited extent, for example, by principal component analysis, which reveals the differences in the fragmentation pattern of the isomers.[28] Furthermore, planar chromatography is a powerful tool, especially for the screening of diterpenoids and triterpenoids in extracts of plant resins, thus enabling relatively simple, inexpensive and fast analyses without a time-consuming sample pretreatment.[29–31] However, diterpenoids and triterpenoids lack chromophores, so the sensitivity of UV detection is rather low. In addition, the characterisation of diterpenoid/triterpenoid compounds with similar structures and polarities remains a challenging task.

In this work, we report on the development of simple, rapid, sensitive and specific TLC (thin-layer chromatography) and (HP)TLC-APCI-MS [(high-performance) thin-layer chromatography-atmospheric pressure chemical ionisation-mass spectrometry) methods for the identification of triterpenoid and phenolic compounds in various plant resins to allow a discrimination of these resins with respect to their botanical origin. In this context, natural resins such as dammar, mastic, olibanum and benzoin, produced by different plant species, were analysed for resin-specific triterpenes and phenolic compounds. Furthermore, the potential of preliminary investigations using APCI-MS and specific post-chromatographic detection reactions was evaluated to provide first indications of resin composition. The applicability of the methods for the analysis of resin-based varnishes on wooden objects was tested on a wooden sample with a thin varnish film of mastic.

2 | MATERIAL AND METHODS

2.1 | Chemicals and materials

Acetone (HPLC grade), chloroform (HPLC grade), *n*-heptane (HPLC grade), *n*-hexane (HPLC grade), methanol (HPLC grade) and sulphuric acid (> 95%) were purchased from Fisher Scientific; acetonitrile (HPLC gradient grade) and formic acid (98%) were obtained from Riedel-de Haën; dichloromethane, ethanol and ethyl acetate from Carl Roth; acetic acid from J. T. Baker (99%); thionyl chloride (99.7%) from Acros Organics; **10** (97%), **9** (> 99.5%) and stannic chloride (99%) from Sigma-Aldrich; **1** from abcr GmbH; **2** (98.9%) from ChromaDex; **3** (99%) from PhytoLab GmbH & Co. KG Germany; **4** (≥ 97%) from Enzo Life Sciences Germany; **5** (98%) from Betulines Czech; **6** (> 96%) Tokyo Chemical Industry Co. Ltd; **7** (98.6%) from Carbone Scientific UK; **8** from ubichem UK; sumaresinolic acid (> 95%) from Biosynth Carbosynth Group; syringe filters [0.2 µm polytetrafluoroethylene (PTFE)], Normal-phase (NP)-TLC plates (silica gel 60, ALUGRAM Xtra SIL G/UV_{254}) as well as reversed-phase (RP)-HPTLC/TLC plates (partial octadecyl-modified silica, ALUGRAM RP-18 W/UV_{254}) from Macherey-Nagel; cerium(IV) sulphate (98%) was bought from Merck KGaA. Details of the resins used can be found in Supporting Information Table S1. Wooden panels (1 cm × 3.5 cm × 0.14 cm) with and without a mastic resin layer (see Figure S2) were prepared by Leonhard Rank (conservator, Cologne, Germany).

For natural ageing, dammar samples were kept in the dark in a closed box. Artificial ageing of resins was carried out under UV lamps (254 nm) for 163 h or in an oven for 21 h at 100°C. For ageing studies, powdered resins were stored on a glass surface.

2.2 | Preparation of stock solutions

Solutions (1 mg/mL) of triterpenic and phenolic standard compounds **1**–**10** were individually prepared in methanol, stored at −20°C and diluted with methanol to obtain working solutions down to a concentration of 1 µg/mL.

2.3 | Chromatography

Working solutions and extracts were applied on the (HP)TLC plates as 1 mm bands, in 2 aliquots using a Linomat 5 (CAMAG, Muttenz, Switzerland, track distance: 10 mm, distance from the lower edge: 15 mm, distance from left edge: 10 mm). Thereafter, each plate was developed in a pre-saturated ADC2 development chamber (CAMAG, Muttenz, Switzerland, migration distance: 85 mm) or a conventional TLC developing chamber using acetonitrile/water (95:5 v/v), acetonitrile/water (7:1 v/v) or 2% acetic acid in *n*-hexane/ethyl acetate (5:1 v/v) as developing solvent (see Section 3.2.3). For the optimisation of the chromatographic separation, different (HP)TLC plates as well as various developing solvents were tested, for example, various ratios of *n*-hexane/ethyl acetate, acetonitrile/water or methanol/water (with and without acetic acid addition).

(HP)TLC plates were inspected both under white light and with UV light at $\lambda = 254$ nm. Beside investigations by MS, different post-chromatographic detection reagents were also used for the identification of target structures (see Sections 2.4 and 2.5).

2.4 | Detection of triterpenes with thionyl chloride and stannic chloride (reagent A, modified according to Noller et al.[32])

The post-chromatographic detection reaction was performed with thionyl chloride and stannic chloride using working solutions of triterpenes and sample extracts applied on (HP)TLC plates. For the preparation of reagent A, anhydrous stannic chloride (0.2 mL) was dissolved

in thionyl chloride (20 mL). The reagent was prepared fresh daily. After development and drying, the (HP)TLC plates were immersed in the reagent for about 1 s and coloured bands became visible after some seconds or several minutes. For the optimisation of the detection reaction, pure thionyl chloride as well as various ratios of thionyl chloride and stannic chloride were tested (e.g., thionyl chloride/stannic chloride 1000:1, 100:1, 10:1 v/v).

2.5 | Detection of triterpenes with cerium-molybdenum reagent

Furthermore, post-chromatographic detection reactions were performed with cerium-molybdenum reagent (for the preparation of the cerium-molybdenum reagent see Schmidt et al.[33]). The reagent was stored in the fridge. After development and drying, the TLC plates were immersed in the reagent for about 1 s and blue bands became visible after exposure to heat.

2.6 | (HP)TLC-MS coupling

A TLC-MS interface (Plate Express from Advion combined with an isocratic pump) was utilised for the elution of compounds from the (HP)TLC plates into an expression^{L} CMS (compact mass spectrometer from Advion, Ithaca, NY, USA) system, equipped with an electrospray ionisation (ESI) ion source (negative and positive mode) or a APCI ion source (negative and positive mode, capillary temperature: 200/250°C, source voltage offset: 15/20 V, source voltage dynamic: 10/20 V, source gas temperature: 200/350°C, MS scan range *m/z* 100–600). Data acquisition and processing were performed with Mass Express and Data Express software (Advion). Prior to the measurements, substance-specific parameters were determined by direct inlet of respective working solutions. Methanol was used as eluent (flow rate 0.2 mL/min).

2.7 | Sample extraction and preparation (modified according to Mathe et al.[21] as well as Ganzera and Khan[18])

Resins (1 g) were powdered and an aliquot (250 mg) of each resin was extracted with methanol (3 mL) by sonicating the homogenised material for 10 min. After centrifugation (5 min, 22°C, 3000 rpm), the supernatant was filtered (0.2 µm, PTFE). This procedure was repeated three times. The supernatants were combined, and a defined volume (2 µL) of the sample extract was spotted onto a (HP)TLC plate. For the optimisation of the extraction procedure, further extracting agents were tested, for example, chloroform and *n*-hexane. For the calculation of extraction efficiency, the supernatant was concentrated to dryness and the residue was weighed (see Table S4). Furthermore, the insoluble residue was dried and weighed (see Table S5).

Thin wooden panels with and without a mastic-based varnish were also extracted three times with methanol (3 × 3 mL) by sonicating the homogenised material for 10 min. The supernatants were combined, and a defined volume (2 µL) of the sample extracts was spotted onto a (HP)TLC plate.

2.8 | Limit of detection

Limit of detection (LOD) was determined using the signal-to-noise ratio ($S/N \geq 3$). For calibration, stock solutions were diluted with methanol down to a concentration of 1 µg/mL. Calibration solutions were measured via direct injection (5 µL aliquots) by APCI-MS or applied on the (HP)TLC plates [NP- and RP-(HP)TLC plates; 2 µL aliquots]. (HP)TLC plates were developed using acetonitrile/water (95:5 v/v), acetonitrile/water (7:1 v/v) or 2% acetic acid in *n*-hexane/ethyl acetate (5:1 v/v) and investigated by (HP)TLC-APCI-MS. Experiments with NP-TLC plates were performed before and after development. Each calibration solution was measured three times, and analyses were executed with peak areas of characteristic mass peaks of the respective standard compound.

2.9 | System precision

For determining the system precision, methanolic solutions of **3**, **5**, **6**, **9** and **10** were used (1, 0.1 or 0.01 mg/mL). Each solution was measured six times by APCI-MS (direct injection) and (HP)TLC-APCI-MS. For (HP)TLC-APCI-MS experiments, solutions were spotted onto RP- or NP-(HP)TLC plates, (HP)TLC plates were developed using acetonitrile/water (95:5 v/v), acetonitrile/water (7:1 v/v) or 2% acetic acid in *n*-hexane/ethyl acetate (5:1 v/v) and investigated by (HP)TLC-APCI-MS. For the interpretation of the system precision, relative standard deviations (RSDs) of peak areas of characteristic mass peaks of **3**, **5**, **6**, **9** and **10** were used (positive mode; **3**: *m/z* 409, 453, 513; **5**: *m/z* 409, 423; **6**: *m/z* 409, 437, 455; negative mode; **9**: *m/z* 121; **10**: *m/z* 147). In addition, Dixon's Q test and Neumann trend test were applied for the identification of outliers or trends.

3 | RESULTS AND DISCUSSION

3.1 | Resin-specific triterpenoid and phenolic compounds

Discrimination between plant resins such as dammar, mastic, olibanum and benzoin is based on the selection of chemotaxonomic molecular markers, detectable in fresh/unaged and aged resins. The plant resins studied are commonly used triterpenoid or phenolic resins, whose molecular composition has already been investigated by several research groups. The photochemical and thermal ageing of triterpenoid resins have also been extensively studied. GALDI-MS studies by Dietemann et al. have shown, for example, that plant resins such as

dammar and mastic are oxidised quite quickly even when kept in darkness.[25] The commercially available resins are therefore usually in an advanced stage of oxidation and degradation.[26] Ageing of triterpenes results in a wide variety of products, formed by autoxidative chain reactions. Groups of signals spaced by 14 and 16 Da indicate the incorporation of different numbers of oxygen atoms as well as the simultaneous loss of hydrogen, for example, by allylic oxidation or oxidation from alcohols to acids.[25,26]

A selection of triterpenoid and phenolic compounds (for structural formula see Figure S3) that can be used as markers to distinguish plant resins is listed in Table 1.

3.2 | Development of a (HP)TLC-APCI-MS method for the identification of triterpenoid and phenolic marker compounds in plant resins

Identification of triterpenoid and phenolic marker compounds was performed by (HP)TLC-APCI-MS. For this purpose, plant resins were extracted; filtered extracts were applied on (HP)TLC plates, the plates were developed using a suitable developing solvent (see Section 3.2.3) and TLC zones were analysed by APCI-MS. In the course of method development, several parameters were optimised to allow detection of marker compounds in the presence of complex resin samples.

3.2.1 | Characterisation of triterpenoid and phenolic marker compounds by APCI-MS

Initially, the ability to ionise triterpenoid and phenolic marker compounds using ESI and APCI conditions [direct injection and ESI/APCI-(HP)TLC-MS experiments] was tested. Best results were obtained for the triterpenes by APCI in positive ionisation mode with a set of characteristic ions that provide information about the presence of functional groups ($[M + H]^+$, $[M + H - H_2O]^+$ and $[M + H - HCOOH]^+$; Table S2). In accordance with literature, dehydration of protonated triterpene alcohols as a dominating process in the APCI source resulting in $[M + H - H_2O]^+$ ions was observed.[35,38] Furthermore, fragment ions corresponding to $[M + H - HCOOH]^+$ are characteristic of triterpene acids with a carboxylic acid group in position 17 or 4 such as **2**, **6**, **7**, and **8**.[37–40] However, the carboxylic acid substituent present on **4** was not split off under APCI conditions. Instead, a fragment ion peak at *m/z* 441 was found for **4** in positive ionisation mode due to the presence of a hydroxy substituent and the resulting elimination of water ($[M + H - H_2O]^+$). In negative ionisation mode, triterpene acids were detected with acceptable peak intensities by APCI as deprotonated ions $[M - H]^-$. For the detection of the triterpene alcohols **1** and **5**, however, the negative ionisation mode is inappropriate. Phenolic compounds **9** and **10** were detected with satisfactory peak intensities by ESI and APCI as deprotonated ions $[M - H]^-$ in negative ionisation mode.

As a consequence, subsequent experiments were performed by [(HP)TLC]-APCI-MS in positive and negative ionisation mode.

3.2.2 | Characterisation of target structures by post-chromatographic detection reagents

Most triterpenoid marker substances cannot be detected by an inspection under UV light at $\lambda = 254$ nm or 366 nm. Therefore, different reagents were tested to confirm the presence of triterpenoid and phenolic marker compounds in extracts of plant resins. In addition to well-known reagents for the detection of terpenoids (e.g., anisaldehyde reagent[41,42] and vanillin–sulphuric acid reagent[43]), cerium-molybdenum reagent as well as the application of thionyl chloride and stannic chloride (reagent A) were tested. The cerium-molybdenum reagent is suitable for the detection of various oxidisable substances found in extracts of natural resins – this also includes triterpenoid and phenolic marker compounds. However, all stainable compounds showed a blue coloration. A more specific post-chromatographic detection reaction, suitable for the detection of triterpenes, can be performed with thionyl chloride and stannic chloride.

TABLE 1 Triterpenoid and phenolic marker compounds used to identify the plant resins studied.

Label	Name	Structure type	Biomarker for	Reference
1	α-Amyrin	Ursane	Various triterpenoid resins, e.g. elemi, white dammar, *Boswellia* resin, myrrh (unaged and aged)	6,15,24,34
2	β-Boswellic acid	Ursane	*Boswellia* resin (unaged and aged)	6,21
3	Acetyl-11-keto-β-boswellic acid	Ursane	*Boswellia* resin (unaged)	22
4	Dammarenolic acid	Dammarane	Dammar (white) (unaged and aged)	15,24,27,35
5	Lupeol	Lupane	Various triterpenoid resins, e.g. mastic, *Boswellia* resin (unaged and aged)	6,19,25,36
6	Moronic acid	Oleanane	Mastic (unaged and aged)	6,15,16,27,35
7	Oleanolic acid	Oleanane	Various triterpenoid resins, e.g. dammar, mastic (unaged)	25,28,37
8	Ursolic acid	Ursane	Dammar (unaged)	25,28,37
9	Benzoic acid	Phenolic compound	Benzoin (unaged)	6,10
10	Cinnamic acid	Phenolic compound	Benzoin (unaged)	6,10

Highly coloured solutions of triterpenes as well as characteristic sequences of colours, sometimes even allowing a distinction between isomeric compounds such as **1** and β-amyrin, using the thionyl chloride and stannic chloride reagent were first described by Noller et al.[32]

In this work, thionyl chloride and stannic chloride were used for the first time for post-chromatographic detection reactions of triterpenes. Initially, tests with different ratios of thionyl chloride and stannic chloride (1000:1, 100:1, 10:1 v/v) and thionyl chloride without addition of stannic chloride were carried out. A ratio of thionyl chloride/stannic chloride 100:1 v/v has proven to be suitable for the detection of triterpenoid marker compounds. Characteristic colour changes of seven triterpenes are listed in Table S3. As already described by Noller et al., the use of thionyl chloride in combination with stannic chloride allows a distinction between structural isomers.[32] For example, **2** and **8**, both triterpenes with the same molecular formula differing only in the positions of a methyl and a carboxylic acid group, show markedly different colour changes. Thus, for the characterisation of triterpenes, characteristic sequences of colours can be used in addition to retardation factor (R_F) values (see Table 2). However, phenolic marker compounds such as **9** and **10** as well as the triterpenoid compound **3** with an acetylated hydroxy group in position 3 cannot be stained with reagent A. A strong decrease in colour intensity was also found by Noller et al. for triterpenes holding esterified hydroxyl groups.[32] Studies on the stainability of acetylated triterpenes are the subject of additional investigations to be performed in future.

3.2.3 | Optimisation of mobile and stationary phase for planar chromatography

For the determination of triterpenoid and phenolic marker compounds in methanolic resin extracts, a variety of sorbents and solvents was tested. For this purpose, working solutions of standard compounds, resin extracts and extracts mixed with defined concentrations of standard compounds were applied on different (HP)TLC sorbents. After development and inspection under white light and with UV light at λ = 254 nm, (HP)TLC plates were investigated by (HP)TLC-APCI-MS or immersed in post-chromatographic detection reagents (see Section 3.2.2). (HP)TLC-APCI-MS studies showed that the use of RP-18 HPTLC plates results in a low background signal and an increase of peak areas for many triterpene acids – partly by a factor of 10–100 – as compared to TLC silica gel sorbent. Thus, octadecyl-modified silica HPTLC sorbent was preferred for HPTLC-APCI-MS experiments of triterpenoid resins.

For extracts of dammar and mastic, a satisfactory separation of triterpenoid marker and matrix compounds was found on partial octadecyl-modified silica HPTLC sorbent using acetonitrile/water (95:5 v/v) as developing solvent. Optimised TLC conditions for the analysis of **2** and **3** in the examined *Boswellia* resin extracts included the use of partial octadecyl-modified silica HPTLC plates and as a developing solvent methanol/water (7:1 v/v). For methanolic extracts of benzoin, no optimal separation of phenolic marker and matrix compounds was achieved with RP-18 (HP)TLC plates. Consequently, tests were performed with TLC silica gel sorbent. Here a satisfactory separation of marker and matrix compounds as well as suitable detection limits (see Section 3.2.5) for phenolic marker compounds **9** and **10** were observed with 2% acetic acid in *n*-hexane/ethyl acetate (5:1 v/v) as developing solvent. However, the R_F values for **9** (R_F = 0.32) and **10** (R_F = 0.30) are quite similar under these conditions. Thus, TLC-APCI-MS analysis is of great importance to distinguish between UV-active substances **9** and **10**, both of which cannot be stained with reagent A. The latter chromatographic system [TLC silica gel sorbent, 2% acetic acid in *n*-hexane/ethyl acetate (5:1 v/v)] also leads to an acceptable separation of extractable dammar, mastic and *Boswellia* resin components and can be used for preliminary investigations with regard to the composition of plant resins by a post-chromatographic

TABLE 2 Chromatographic data and retardation factor (R_F) values for triterpenoid and phenolic marker compounds. After dipping in cerium-molybdenum reagent all marker compounds show a blue coloration. For characteristic sequences of colours produced by dipping in reagent A see Supporting Information Table S3.

	Developing solvent and (high-performance) thin-layer chromatography [(HP)TLC] sorbent		
	Acetonitrile/water (95:5 v/v) RP-18 W	Methanol/water (7:1 v/v) RP-18 W	2% Acetic acid in *n*-hexane/ethyl acetate (5:1 v/v) Silica gel 60
Compound	R_F values		
1	0.31	–	0.38
2	0.55	0.41	0.28
3	0.67	0.60	0.25
4	0.67	–	0.22
5	0.37	–	0.37
6	0.66	–	0.20
7	0.57	–	0.28
8	0.54	–	0.27
9	–	–	0.32
10	–	–	0.30

detection reaction with reagent A. For the earlier reasons, RP-18 HPTLC plates were preferred for HPTLC-APCI-MS studies on dammar, mastic and *Boswellia* resins containing triterpene acids as marker compounds, whereas TLC silica gel plates were used for TLC-APCI-MS studies on benzoin balsam as well as for the preliminary study of the resin extracts.

The R_F values and coloration of marker compounds after derivatisation with reagent A or cerium-molybdenum reagent are presented in Tables 2 and S3.

3.2.4 | Optimisation of the extraction procedure

Extraction solvents of varying polarity were compared with respect to extraction efficiency, extract composition and the removal of polymeric fractions present in various resins such as dammar or mastic resins. For this purpose, powdered and homogenised plant resins (250 mg) were extracted three times by sonicating using, for instance, methanol, *n*-hexane or chloroform as extraction solvent. Afterwards, filtered supernatants were applied on the (HP)TLC plates; the (HP)TLC plates were developed and investigated by (HP)TLC-APCI-MS. For comparison, average peak areas of characteristic mass peaks of triterpenoid and phenolic marker compounds were determined. Furthermore, the extraction efficiency in terms of weight (Tables S4 and S5) as well as the chromatographic separation of marker and matrix compounds were considered. In accordance with the literature, methanol has proven to be a suitable solvent for the extraction of triterpenoid and phenolic marker compounds from the examined resins.[17,18,21,29,37] In addition, experiments were carried out with significantly lower sample amounts (8–9 mg resin). For this purpose, powdered resins were extracted with methanol. After centrifugation and filtration, the supernatants were concentrated to dryness. Subsequently, the residues were dissolved in methanol (300–500 µL) and used for analyses. Detection of marker compounds was possible even when using the lower sample amounts.

3.2.5 | Evaluation of the limit of detection and the system precision

LOD and system precisions were evaluated for selected triterpenoid and phenolic marker compounds by APCI-MS (direct injection) and (HP)TLC-APCI-MS (see Table 3). In this context, the influence of the chromatographic system on detection limits was investigated. For **3**, **5** and **6**, detection limits of 5–50 ng/injection and 2–20 ng/TLC zone were determined by APCI-MS (direct injection) and RP-HPTLC-APCI-MS using acetonitrile/water (95:5 v/v) or methanol/water (7:1 v/v) as developing solvent. For **5**, a detection limit of 20 ng **5**/TLC zone could also be determined with NP (in this case it means silica gel sorbent) plates. The use of NP-TLC plates, however, increases the detection limits for triterpene acids such as **3** and **6** to 2000 ng **3**/TLC zone [developing solvent: 2% acetic acid in *n*-hexane/ethyl acetate (5:1 v/v)], 200 ng **3**/TLC zone (without development of the TLC plates) and 200 ng **6**/TLC zone (with and without development of the TLC plates). As mentioned earlier, octadecyl-modified silica HPTLC sorbent was preferred for HPTLC-APCI-MS experiments on triterpenoid compounds (see Section 3.2.3). For phenolic compounds **9** and **10**, satisfactory detection limits of 5 ng/injection and 2 ng/TLC zone were determined by APCI-MS (direct injection) and NP-TLC-APCI-MS using 2% acetic acid in *n*-hexane/ethyl acetate (5:1 v/v) as developing solvent. System precisions were determined for **3**, **5**, **6**, **9** and **10** with an RSD in the range of 0.8%–10.4%. Thus, the data were considered adequate for the purposes of the present study.

3.3 | Preliminary investigations by APCI-MS (direct injection)

For preliminary investigations with regard to the composition of plant resins by APCI-MS, an aliquot of the respective methanolic resin extract was directly injected into the mass spectrometer and analysed without prior chromatographic separation. Obtained mass spectra

TABLE 3 Limit of detection (LOD) for selected marker compounds and relative standard deviations (RSDs) of peak areas of characteristic mass peaks of **3**, **5**, **6**, **9** and **10,** determined by atmospheric pressure chemical ionisation mass spectrometry (APCI-MS) (direct injection), normal-phase thin-layer chromatography (NP-TLC)-APCI-MS and reversed-phase high-performance thin-layer chromatography (RP-HPTLC)-APCI-MS.

	LOD (ng/injection or ng/TLC zone)				RSD (%)	
Compound	APCI-MS (direct injection)	NP-TLC-APCI-MS	NP-TLC-APCI-MS (without development of the TLC plates)	RP-HPTLC-APCI-MS	APCI-MS (direct injection)	(HP)TLC-APCI-MS
3	5	2000	200	2	10.4	3.9 (RP-HPTLC)
5	5	20	20	20	7.6	12.3
6	50	200	200	20	4.7	5.5 (RP-HPTLC)
9	5	2	–	–	4.3	6.0 (NP-TLC)
10	5	2	–	–	0.8	7.0 (NP-TLC)

were analysed with respect to the presence (or absence) of characteristic peaks for triterpenoid and phenolic marker compounds. Further information on the resins studied is given in Table S1.

In APCI mass spectra of dammar samples **D1–D4** (plant family *Dipterocarpaceae*), recorded in positive and negative ionisation mode, characteristic peaks of the triterpenoid marker compound **4** at *m/z* 441 ($[M + H - H_2O]^+$) and 457 ($[M\text{-}H]^-$) can be seen as base peaks or intense signals (Figure 1). Furthermore, intense signals at *m/z* 425 and 409 indicate the presence of white dammar ingredients such as dammaradienone (*m/z* 425 $[M + H]^+$), dammaradienol (*m/z* 409 $[M + H - H_2O]^+$), **1** (*m/z* 409 $[M + H - H_2O]^+$) or hydroxydammarenone (*m/z* 425, $[M + H - H_2O]^+$).[25,35,37,44] The latter is a major component of both, dammar and mastic. Signals with mass differences of 16 Da indicate the presence of corresponding oxidation products (see Section 3.1). A similar oxidation pattern resulting from autoxidation of hydroxydammarenone during storage was observed, for example, by Dietemann et al.[25] However, in naturally and thermally aged as well as photoaged dammar samples, the relative intensity of the signals, for example, at *m/z* 425, 441, 457 and 473, does not increase significantly (positive mode, Figure S4). In addition, signals at *m/z* 457 and 473 can also be detected in the extracts of other resin samples and are consequently not characteristic for aged dammer or mastic samples. Further investigation on oxidation products should be the subject of future studies. Mass spectra of sample **D5** differ significantly from spectra of **D1–D4**. Characteristic signals of **4** are absent or show a much lower intensity in mass spectra of **D5** (Figure 1). However, an intensive signal at *m/z* 409 can also be observed for **D5** in positive ionisation mode (Figure 1). **D5** is traded under the name "dammar dark" and probably originates from a *Canarium* species (plant family *Burseraceae*). Several resins of the genus *Canarium* have been marketed under the name elemi, with Manila elemi from *Canarium luzonicum* (Blume) A. Gray or *Canarium commune* L. in particular being used in Fine Arts.[34] Furthermore, a brownish-black resin with the commercial name black dammar can be obtained from trees of the species *Canarium strictum* Roxb.[37,45] Both resins, Manila elemi and black dammar, contain various triterpenes such as **1** or β-amyrin, to which an intensive signal at *m/z* 409 as well as less intense signals at *m/z* 425 and 423 can be attributed (see Table S2).[5,34,46] Moreover, **4** does not represent a triterpenoid marker compound for these *Canarium* resins. Thus, **D5** is correctly identified as no (white) dammar resin sample in the preliminary investigations by APCI-MS.

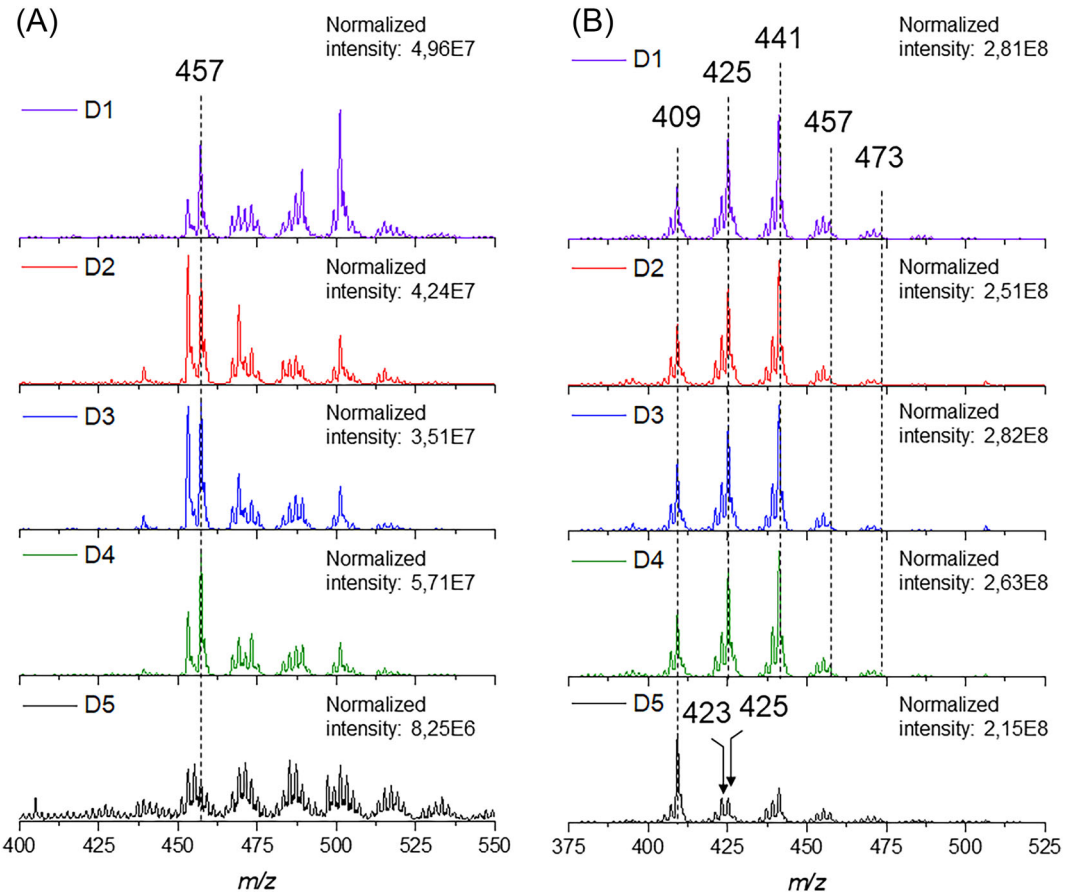

FIGURE 1 APCI mass spectra (direct injection) of methanolic extracts of dammar samples **D1–D5**. MS detection was performed in (A) negative mode (molecular ion of **4**: *m/z* 457) and (B) positive mode (fragment ions of **4**: *m/z* 441; **1** and β-amyrin: *m/z* 425, 423, 409; triterpenes contained in dammar with a dammarane skeleton such as hydroxydammarenone or dammaradienol: *m/z* 425, 409; putative products of autoxidative chain reactions: *m/z* 457, 473).

Intense signals at *m/z* 425 and 409 can also be detected in positive ionisation mode under APCI conditions in extracts of mastic samples **M1–M4** (Figure 2). As mentioned earlier, components such as hydroxydammarenone occur both in dammar and mastic. However, these signals can also be assigned to other triterpenoid components of mastic such as **5** (fragment ions of **5**: *m/z* 425, 409) or β-amyrin (expected fragment ions of β-amyrin: *m/z* 425, 423, 409[42,47]).[25] Furthermore, characteristic signals of the protonated and deprotonated marker compound **6** at *m/z* 455 ($[M + H]^+$) and 453 ($[M - H]^-$) are found as intense peaks in APCI mass spectra of **M1–M4** (Figure 2). For isomeric triterpene acids contained as minor components in mastic, for example, masticadienonic acid, isomasticadienonic acid or oleanonic acid, however, signals at *m/z* 455 (positive mode) or 453 (negative mode) would also be expected.[37]

Mass spectra of *Boswellia* resin extracts are more heterogeneous. However, in APCI mass spectra of samples *Boswellia* **1–5** characteristic peaks at *m/z* 513 ($[M + H]^+$) and 511 ($[M - H]^-$) assigned to protonated and deprotonated **3** are found as base or intense peaks (Figures S5A–D, S5A and S6A). In addition, signals at *m/z* 455 ($[M - H]^-$) and 497 ($[M - H]^-$) corresponding, for instance, to deprotonated boswellic acid (α-BA and **2**) or acetylated boswellic acid (α-ABA and β-ABA), are detected.[14,21] A characteristic signal of deprotonated boswellic acid at *m/z* 455 ($[M - H]^-$) is also prominent in mass spectra of sample *Boswellia* **6**, which is traded as "Weihrauch Borena (*Boswellia neglecta*)" (Figure S5E). As expected for a *Boswellia neglecta* resin, characteristic signals of **3** (negative and positive mode, Figures S6B and S7B) as well as the signal at *m/z* 497 (negative mode) were missing in mass spectra of this sample. Resins of *Boswellia neglecta* are characterised by very low concentrations of **3** and ABA and a medium concentration of boswellic acid compared to other *Boswellia* species.[22,48] Thus, first indications of the presence or absence of boswellic acids can be obtained by APCI-MS. Furthermore, characteristic signals at *m/z* 425, 423 and 409 of triterpenes such as **1** and **5**, also contained in *Boswellia* resins (see Table 1), were detected by APCI-MS in positive mode (Figure S7).

Characteristic peaks at *m/z* 121 ($[M - H]^-$) and 147 ($[M - H]^-$), indicating the presence of **9** and **10**, are found in APCI mass spectra of the benzoin extracts of **B1–B3** (**B1**: designated as Gummi Benzoe, without further details; **B2** and **B3**: traded as a Sumatra benzoin balsam, Figure S8). As expected, a characteristic peak of **9** at *m/z* 121 and no evidence for the presence of free **10** was found in sample **B4**, traded as a Siam benzoin balsam (Figure S8). Previous studies have shown that **9** was detected in both, Sumatra and Siam benzoin balsam, while quite important proportions of **10** were detected rather

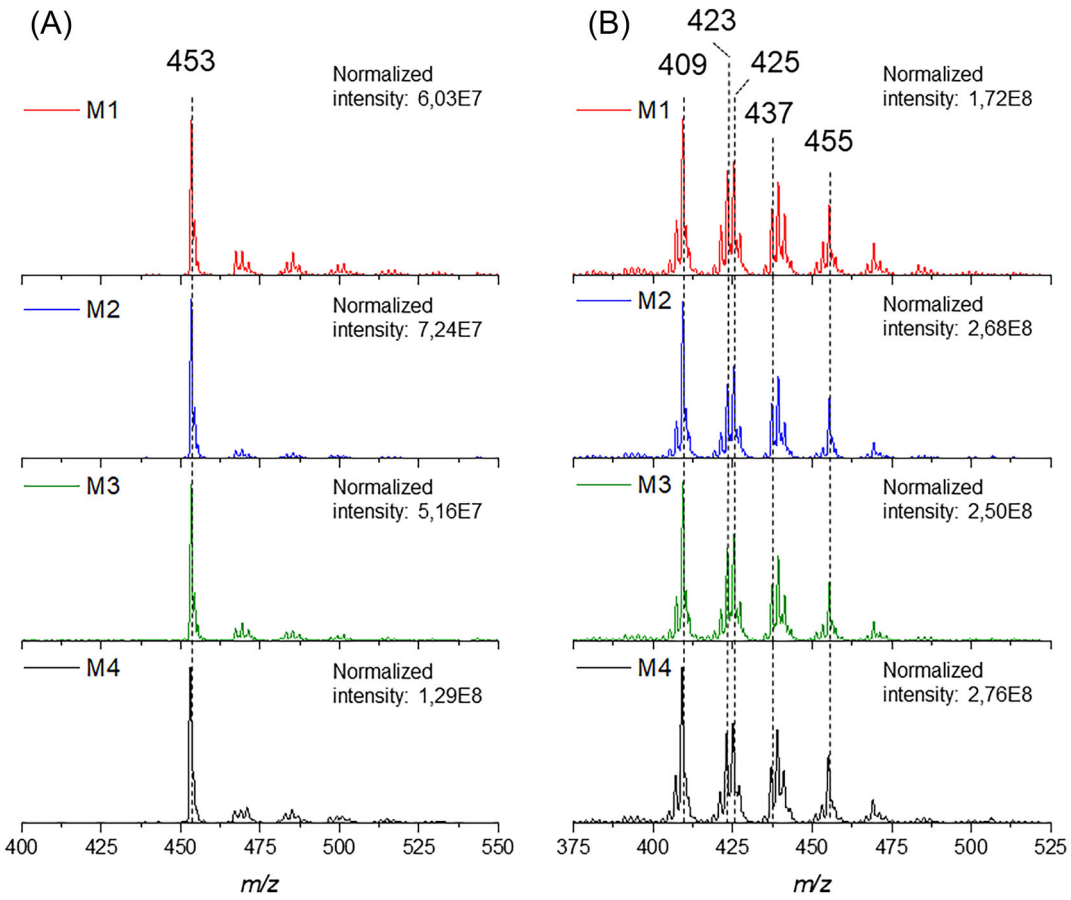

FIGURE 2 APCI mass spectra (direct injection) of methanolic extracts of mastic samples **M1–M4**. MS detection was performed in (A) negative mode (molecular ion of **6**: *m/z* 453) and (B) positive mode [(fragment) ions of **6**: *m/z* 455, 437, 409; **5**: *m/z* 425, 409; β-amyrin: *m/z* 425, 423, 409; hydroxydammarenone as a major component of mastic: *m/z* 425].

in Sumatra benzoin balsams.[10] However, in addition to a signal for **9** at *m/z* 121, an intensive characteristic signal of **10** at *m/z* 147 was also observed for sample **B5**, designated as Siam benzoin balsam. This finding is surprising, as the intensive characteristic signal of **10** is atypical for a Siam benzoin balsam and suggest a Sumatra benzoin balsam or an adulterated Siam benzoin balsam. However, further investigations, for example, by GC–MS or HPLC-ELDS (evaporative light scattering detector; see Burger et al.[10]), are required to verify the results. These investigations will be the subject of future studies.

Furthermore, APCI mass spectra of **B1–B5**, recorded in negative ionisation mode, show intense signals at *m/z* 469 and 471. These signals can probably be assigned to deprotonated siaresinolic or sumaresinolic acids (isomeric structures, both 472 Da) and deprotonated oxidised derivatives with a carbonyl group instead of a hydroxy group (470 Da). Sumaresinolic acid is well-known as a minor component in Sumatra and Siam benzoin balsams. Siaresinolic acid, however, is found only in Siam benzoin.[10,49] For a more detailed characterisation of **B1–B5**, further investigations by APCI-TLC-MS are required. It should be noted that **9**, **10** and respective ester derivatives are not reliable markers for a discrimination between ancient phenolic resins due to their high volatility and water solubility as well as cross-reactions (transesterification) with other constituents of the sample during ageing processes. Furthermore, the double bond of cinnamates might be cleaved by oxidative processes.[12] In contrast to **9** and **10** as well as respective ester derivatives, triterpenes such as sumaresinolic and siaresinolic acid are promising marker compounds for strongly aged phenolic resins, for example, from archaeological sites (see Section 1 and Courel et al.[12]).

For all extracts studied, characteristic signals of triterpenoid and phenolic marker compounds can be detected by APCI-MS. However, the distinction between isomeric structures in these complex multi-component mixtures is possible only to a limited extent without a chromatographic separation of these components. Consequently, (HP) TLC-APCI-MS studies are indispensable for further characterisation of natural resins (see Section 3.5).

3.4 | Preliminary investigations by planar chromatography and post-chromatographic detection reactions

For preliminary investigations with regard to the composition of the plant resins by TLC and post-chromatographic detection reactions, methanolic resin extracts were applied on TLC silica gel 60 sorbent and TLC plates were developed using 2% acetic acid in *n*-hexane/ethyl acetate (5:1 v/v). After an inspection under white light and with UV light at $\lambda = 254$ nm, TLC plates were immersed in reagent A.

Samples **B1–B5** and *Boswellia* **1–6** show characteristic bands and colour changes allowing a distinction between these resins. The obtained chromatograms of **B1–B3** (**B1**: designated as Gummi Benzoe, without further details; **B2** and **B3**: Sumatra benzoin balsams) show a uniform spot pattern with characteristic UV-active bands for **9** and/or **10**, however, with similar R_F values as already noted earlier (see Section 3.2.3, Figure S9). A characteristic UV-active band for **9** is also present in track 6 of sample **B4** (Siam benzoin balsam). Furthermore, a band stainable with reagent A (sequence of colours: red-brown → purple → purple-grey → grey → grey-green) and a R_F value of 0.15 indicates the presence of sumaresinolic acid in **B1–B4** (Figure S10). As already mentioned, sumaresinolic acid is contained in benzoin balsam from Sumatra and Siam. It should be noted that sample **B5** (designated as Siam benzoin balsam) shows a similar pattern to samples **B1–B3**, which is distinctly different from sample **B4** (Figures S9 and S10). This finding supports the assumption that sample **B5** is a Sumatra benzoin balsam or an adulterated Siam benzoin balsam (see Section 3.3).

Characteristic bands at R_F 0.28 with colour changes typical for **2** can be observed for *Boswellia* resins *Boswellia* **1–5** (Figure S11). In addition, the chromatograms of *Boswellia* **1–5** show at a R_F value of 0.25 a characteristic UV-active band for **3** that is not stainable with reagent A (Figure S12). An exception are extracts of the resin sample *Boswellia* **6**. These sample extracts show a spot pattern different from other *Boswellia* resins; for example, no indication of the presence of **3** and only a small amount of **2** was found (Figures S13 and S14). As mentioned earlier, *Boswellia* species such as *Boswellia neglecta* are characterised by a rather low concentration of boswellic acids (see Section 3.3).

Chromatograms of mastic samples **M1–M4** and dammar samples **D1–D4** are very similar (Figures 3 and S15–S17). However, different colour sequences can be observed for some stainable bands. Furthermore, marker compound **6** is not sufficiently separated from matrix components of samples **M1–M4** under these conditions (Figure S15), whereas marker compound **4** is clearly detectable in extracts of **D1–D4** (Figure 3). Artificially and naturally aged dammar samples also show characteristic bands with colour changes typical for **4** (see Section 2.1). Compared to **D1–D4**, the chromatogram of sample **D5** (*Canarium* resin) shows only one intense stainable band with a R_F value of 0.44 (sequence of colours: orange → red-brown → brown → grey-brown → grey-purple, Figure 3) and, as expected, differs significantly from chromatograms of **D1–D4** (white dammar).

Thus, characteristic spot patterns obtained after a planar chromatographic separation of extract ingredients, in combination with the sequence of colours observable after derivatisation with reagent A, are helpful to obtain the first hints about the resin composition.

3.5 | Characterisation of triterpenoid and phenolic resins by (HP)TLC-APCI-MS and post-chromatographic detection reactions

The characterisation of plant resins was performed by (HP)TLC-APCI-MS. Results of preliminary investigations using APCI-MS (direct injection) and post-chromatographic detection reactions provide initial information on the composition of the resins studied and help to choose suitable chromatographic conditions enabling a separation of

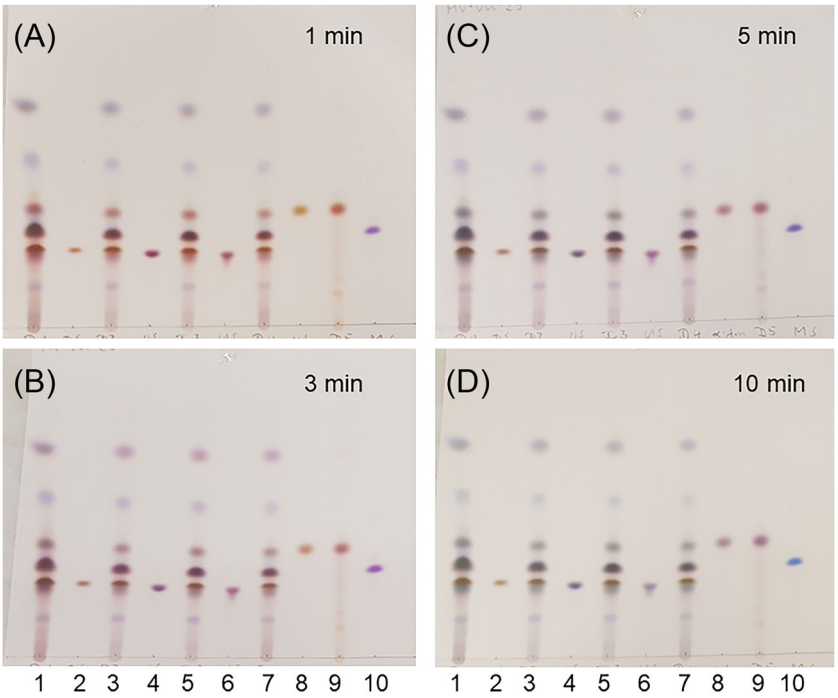

FIGURE 3 TLC chromatogram of methanolic dammar extracts and respected marker compounds developed on silica gel plates using 2% acetic acid in *n*-hexane/ethyl acetate (5:1 v/v) as mobile phase. TLC plates were viewed under white light (A) 1 min, (B) 3 min, (C) 5 min and (D) 10 min after derivatisation with reagent A. Tracks 1, 3, 5, 7, 9 = methanolic resin extracts (1: **D1**; 3: **D2**; 5: **D3**; 7: **D4**; 9: **D5**). Tracks 2, 4, 6, 8, 10 = methanolic solutions of marker compounds [2: **4** (dammarenolic acid); 4: **7** (oleanolic acid); 6: **8** (ursolic acid); 8: **1** (α-amyrin), 10: **6** (moronic acid); 2 μg/TLC zone each].

resin components. Identification of selected triterpenoid and phenolic compounds was achieved by APCI-MS spectra, R_F values and the presence or absence of characteristic colour changes after derivatisation with reagent A.

For dammar samples **D1–D4**, APCI mass spectra of the stainable band (typical colour sequence for **4**, see Table S3) at R_F 0.67 show intense peaks at *m/z* 441 (positive mode) and 457 (negative mode) correspond to the triterpenoid marker compound **4**. In contrast, **4** cannot be detected in the extract of sample **D5**. As already discussed, the composition and thus MS spectra and (HP)TLC chromatograms of white dammar (**D1–D4**) and the *Canarium* resin **D5** differ significantly (see Sections 3.3 and 3.4).

Compound **6** with a R_F value of 0.66 (colour sequence see Table S3) and characteristic signals at *m/z* 455, 437, 409 (positive mode) and 453 (negative mode) was identified in mastic samples **M1–M4**.

Characteristic signals of the triterpenoid marker compound **3** at *m/z* 513 (positive mode) and 511 (negative mode) are found at R_F 0.60 (non-stainable with reagent A) as intense peaks in APCI mass spectra of *Boswellia* **1–5**. The simultaneous presence of smaller peaks at *m/z* 453 and 409 in positive mode corresponding to fragment ions of **3** confirms the detection of **3** (see Table S2). Furthermore, bands with R_F values of 0.41 (typical colour sequence for **2**, see Table S3) and a characteristic signal of **2** at *m/z* 455 (negative mode) are found for *Boswellia* **1–5**. In contrast, no evidence of the presence of **3** was found in extracts of *Boswellia* **6**. Thus, **2** and **3** are suitable marker compounds for *Boswellia* resins, with the exception of sample *Boswellia* **6**. *Boswellia* **6** is traded as a *Boswellia neglecta* resin, which has a very low concentration of **3** compared to other *Boswellia* species (see Section 3.3). However, the composition of *Boswellia* **6** extracts should be verified by another method, for example, GC–MS, in future studies.

Phenolic marker compounds **9** and/or **10** with characteristic peaks at *m/z* 121 and 147 (negative mode) were detected in benzoin extracts **B1–B5** at R_F values of 0.32 and 0.30, respectively (NP-TLC-MS experiments). As noted earlier, bands of **9** and **10** can be detected under UV light at 254 nm, however, cannot be stained with reagent A (see Section 3.2.2 and Figures S9 and S10). The presence of sumaresinolic acid in benzoin extracts **B1–B5** can be confirmed by RP-HPTLC-APCI-MS. A characteristic signal of sumaresinolic acid at *m/z* 471 is found at R_F 0.70 as an intense peak in APCI mass spectra of **B1–B5** (Figure S18). As expected, the use of silica gel plates is unsuitable for the detection of the triterpene acid sumaresinolic acid by TLC-MS and results in a low intensity signal at *m/z* 471. Unfortunately, no optimal separation of **9**, **10** and matrix compounds was achieved with octadecyl-modified silica HPTLC sorbent. Therefore, two independent experiments using NP- and RP-(HP)TLC plates are required for the detection of **9**, **10** and sumaresinolic acid in benzoin extracts by TLC-MS.

Thus, the identification of triterpenoid and phenolic marker compounds, which allow discrimination of plant resins, by (HP)TLC-APCI-MS and specific detection reactions with reagent A was successful for all natural resins tested. In summary, (HP)TLC-APCI-MS in combination with specific post-chromatographic detection reactions is a simple, fast, inexpensive, sensitive and specific method for screening resins and resin-based varnishes for botanical origin that can analyse a large number of samples simultaneously (for a comparison with other techniques see Table S6).

3.6 | Case study: Analysis on a mastic varnish

Extracts of a 1-year-old mastic varnish – naturally aged as a thin film on a wooden panel – and an unvarnished wooden panel were

analysed by HPTLC-APCI-MS. Initially, the preliminary investigations described earlier were carried out. APCI mass spectra and (HP)TLC chromatograms of the mastic-based varnish look very similar to the studied mastic samples **M1–M4** (Figures S15 and S16). Thus, for the subsequent studies by HPTLC-APCI-MS, RP-18 HPTLC plates and acetonitrile/water (95:5 v/v) as developing solvent were used for the chromatographic separation of the resin components. Corresponding analyses confirm the presence of the triterpenoid marker compound **6** for the varnished sample and thus the presence of mastic, a natural triterpenoid resin often used by conservators. In comparison, no marker compound was detected in the extract of the unvarnished wooden panel.

ACKNOWLEDGEMENTS

This work was supported by ALAGO ART & STRINGS GmbH. The authors would like to thank Leonhard Rank (Cologne, Germany) for providing natural resins and for preparing a varnished wooden sample. Open Access funding enabled and organized by Projekt DEAL.

DATA AVAILABILITY STATEMENT

All data are included in the Supporting Information section.

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How to cite this article: Schendzielorz M, Schmidt T, Puchalla N, Csuk R, Kramell AE. TLC and HPTLC-APCI-MS for the rapid discrimination of plant resins frequently used for lacquers and varnishes by artists and conservators. *Phytochemical Analysis*. 2024;35(1):64-76. doi:10.1002/pca.3273