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RESEARCH ARTICLE

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Exploring the polysaccharide composition of plant cell walls in succulent aloes

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Societal Impact Statement

Aloes are iconic succulent plants native to Africa, Madagascar and the Arabian Peninsula. The succulent leaf mesophyll of aloes has been used extensively as a herbal product for centuries, contributing to their overexploitation. Health benefits are attributed to their polysaccharide content. We present a comprehensive comparison of the polysaccharide composition of succulent tissues from 93 *Aloe* species. We found polysaccharide composition primarily related to leaf morphology in alignment with the broad range of *Aloe* species used medicinally. All aloes except *Aloe ferox* and *Aloe vera* are endangered raising concern about over-harvesting of wild species.

Summary

- Aloes are iconic succulent plants native to Africa, Madagascar and the Arabian Peninsula. All aloes except the commercially grown *Aloe ferox* and *Aloe vera* are protected according to the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). Major factors contributing to their overexploitation are their ornamental value and medicinal use with more than 25% of *Aloe* species being utilised. The succulent inner leaf mesophyll of aloes is used in traditional medicine, with the healing effect ascribed to the properties of their structural polysaccharides.
- To explore the correlation between *Aloe* polysaccharide profiles and other biologically relevant traits across the genus, we (1) extracted polysaccharides and created profiles for nearly 100 representative species using carbohydrate microarrays and molecular probes. We targeted six major plant cell wall polysaccharide groups using 27 different molecular probes. We (2) tested for phylogenetic signal in the polysaccharide profiles and (3) assembled an exhaustive database from literature on the geographic region, level of endemism, altitude, habitat, habit, medicinal use and leaf morphology of the individual species of *Aloe*.
- In the absence of phylogenetic signal of polysaccharide profiles, multivariate linear modelling without phylogenetic correction was used and showed that

polysaccharide composition primarily correlated with leaf morphology, highlighting the fundamental role of polysaccharides as the building blocks of plants. No correlations between polysaccharide composition of commercial and non-commercial species were found.
- We found polysaccharide composition to primarily relate to leaf morphology emphasising the fundamental and structural role of polysaccharides.

KEYWORDS
aloe, *Aloe vera*, inner leaf mesophyll, medicinal plants, MicroArray Polymer Profiling (MAPP), plant cell walls, polysaccharides, succulent

1 | INTRODUCTION

Aloes are iconic succulent plants native to the South and Eastern parts of the African continent, Madagascar and the Arabian Peninsula (Newton, 2004). The family Asphodelaceae (APG IV, 2016) comprises over 600 species in the genus *Aloe* together with the smaller genera *Aloiampelos*, *Aloidendron*, *Aristaloe*, *Gonialoe* and *Kumara* (Newton, 2020). Aloes evolved in southern Africa, and their diversity is concentrated within four major regional centres in southern Africa, Madagascar, East Africa/Zambezia and the Horn of Africa/Ethiopia-Somalia (Carter et al., 2011; Grace et al., 2015). These areas span seven geographical zones (Brummitt, 2001) across Africa and the Arabian Peninsula, and the plants are found growing from sea level to 2800 m in altitude (Carter et al., 2011). Within these areas, the natural habitats of aloes range from open savannas and grasslands to forests, exposed rock surfaces and hill sides. The genus is tolerant to a range of different soil types, but most species have a preference to one or a few ground compositions (Carter et al., 2011). Aloes are slow growing narrow endemics and are therefore very vulnerable to overexploitation and habitat destruction (Grace, Klopper, et al., 2013), with all species except for the commercially grown *Aloe ferox* and *Aloe vera* being protected according to the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) (2021). Habitat destruction is one aspect, but other factors contributing to their overexploitation is their ornamental value and medicinal use. Aloes are often subject to illegal harvesting for succulent collectors (Grace, 2011), and more than 20% of *Aloe* species are utilised for their medicinal properties. The first European description of an *Aloe* as a medicinal plant was recorded by Dioscorides in 41–69 AD in the herbal *De materia medica* (Upton et al., 2012). In his and later works, two medicinal components from aloes have been defined—the yellow exudate excreted from aloitic cells and the clear inner leaf mesophyll. The yellow exudate is used as a laxative, and the inner leaf mesophyll is used as a herbal remedy to treat skin conditions like radiation and sun burns, as well as minor cuts and scrapes (Grindlay & Reynolds, 1986; Hodge, 1953; Reynolds & Dweck, 1999; Tyler et al., 1981). *A. vera* is a considerable global commodity, and in 2016, the annual revenue of *A. vera* extracts worldwide hit 26 billion $US (Future Market Insights, 2016). Unfortunately, *A. vera* has likely gone extinct in the wild, probably due to overexploitation (Grace et al., 2015).

Morphologically, aloes form nine distinct habit types based on leaf arrangement, plant size and inflorescence (Carter et al., 2011). Five of the nine morphological habit types are depicted in Figure 1 to illustrate their diversity. Generally, aloes are either caulescent (visible stem) or acaulescent (without a visible stem), growing in small or large clumps, and having few- or multi-branched inflorescences. An additional three habit types are not completely aligned with the above criteria, with the grass aloes (*Aloe* section *Leptaloe*) having very thin and narrow leaves, the scrambling aloes (*Aloiampelos*) growing in a more irregular pattern and tree aloes (*Aloidendron* and *Kumara*) having a distinct trunk of up to several metres (Carter et al., 2011).

Succulent plants are commonly described as species with the ability to store larger amounts of water in leaves, stems, roots or pseudobulbs (Raven et al., 2005). Succulence has developed independently in several plant lineages as an adaptation to environmental conditions where water can be periodically limited (Grace, 2019; Males, 2017). All aloes are leaf succulent perennials with the structural and physiological adaptations needed to survive in arid regions (Newton, 2004). As a morphological or physiological trait, succulence can be in the form of either all-cell-succulence or storage-succulence, with aloes falling into the latter category (von Willert et al., 1990; Eggli & Nyffeler, 2009; Grace, 2019). While all aloes are leaf storage-succulents, the amount of hydrenchyma tissue varies significantly between the more pronounced succulent species like *A. ferox* with a thick hydrenchyma and the lesser succulent species like *Aloe chortolirioides* with a thin hydrenchyma (Carter et al., 2011; Cutler, 2004). The overall *Aloe* leaf anatomy consists of epidermis, chlorenchyma and a parenchymatous hydrenchyma in the centre of the leaf, which is also referred to here as the inner leaf mesophyll (Figure 1). One of the ways aloes have adapted to dry conditions is by developing a flexible hydrenchyma tissue capable of storing large amounts of water (Ahl, Mravec, et al., 2019). Photosynthesis is conducted in the chlorenchyma below the epidermis, and the water storing abilities of aloes allow for photosynthesis to continue even during extended periods of drought (Kluge et al., 1979).

Structural polysaccharides play a primary role in cell wall composition and are classically divided into three broad groups—cellulose, hemicelluloses and pectic polysaccharides (Albersheim et al., 2010). Cellulose is a principal load-bearing component of the plant cell wall, and the strands are cross-linked together by hemicelluloses to form a robust mesh. The cellulose-hemicellulose scaffold is then embedded

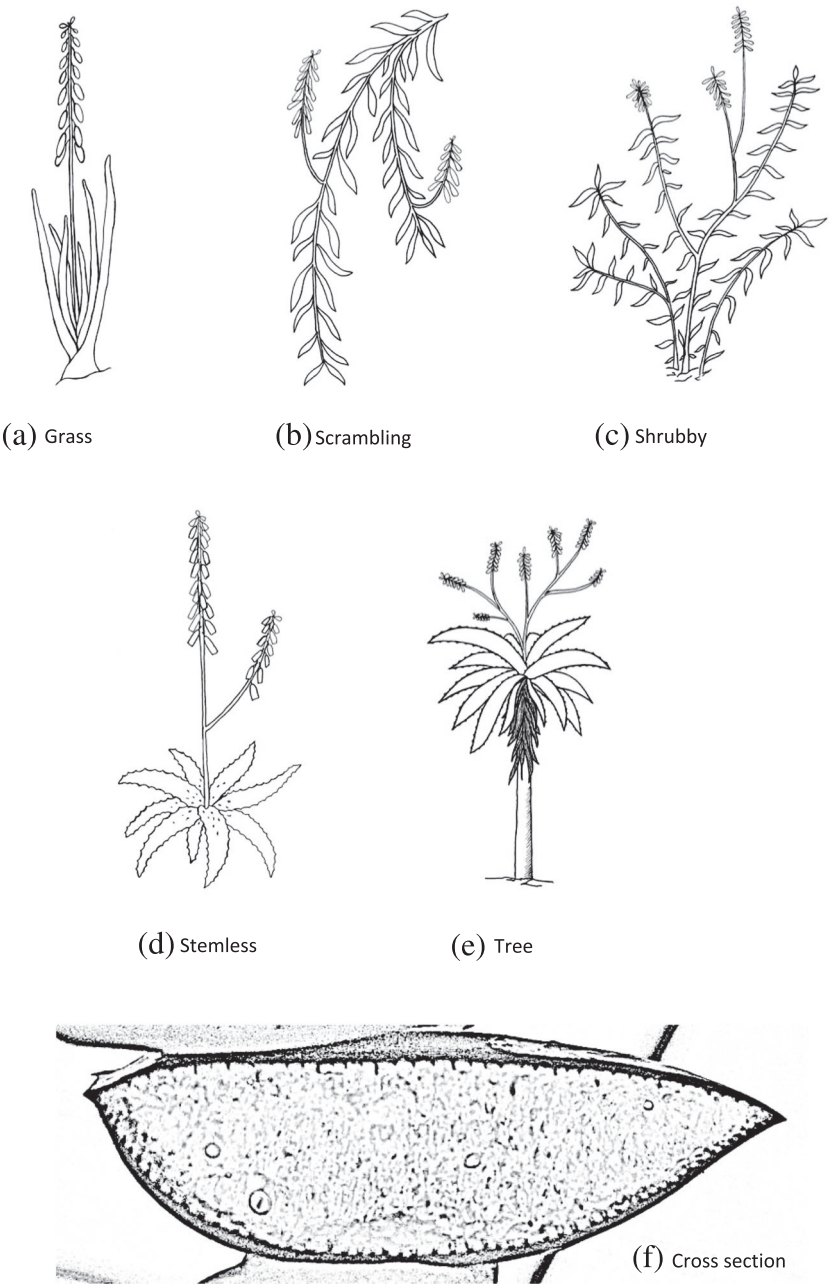

FIGURE 1 Simplified representation of five of the nine morphological habit types (a–e) and a cross section of a well hydrated *Aloe vera* leaf (f). Each habit type, except for grass (a), can be found in forms with either few (as shown here) or multi-branched inflorescences. Drawings by Louise Isager Ahl, inspired by Carter et al. (2011). Photo (f) by Louise Isager Ahl converted into a sketch using: https://pencilsketch.imageonline.co/index.php.

in a matrix of pectic polysaccharides. Cellulose is constructed of long linear polymers made of β-(1-4)-linked D-glucose residues collected into microfibrils of approximately 30–50 strands that are tied together via hydrogen bonds (Cosgrove, 2005). Hemicelluloses are more varied in their appearance, but they all contain a β-linked backbone (Popper & Fry, 2003). In aloes, the dominating hemicelluloses derived from the inner leaf mesophyll are mannans and xyloglucans (Reynolds & Dweck, 1999; Talmadge et al., 2004). Pectic polysaccharides embedding the cellulose-hemicellulose scaffold are the most complex polymers of the cell wall, both in terms of structure and function (Willats et al., 2006). The backbone of pectic polymers is dominated by D-galacturonic acid, accounting for more than 65% of the total number of residues in the polymer (Caffall & Mohnen, 2009). Pectic polysaccharides are cross-linking to each other via calcium-bridges and hydrogen bonds thus making the embedding matrix a flexible construction (Caffall & Mohnen, 2009; Cosgrove, 2005). Calcium bridges and hydrogen bonds are easily remodelled or broken down allowing changes in the cell wall conformation in response to external or internal stimuli (Albersheim et al., 2010). This ability to make conformational alterations is often referred to as plasticity, and it is an essential ability enabling plants to survive in changing environments. It has been shown that the inner leaf mesophyll of aloes can undergo a controlled folding in response to drought (Haberlandt, 1914; Pfitzer, 1877), with changes in the cell wall likely facilitated by changes in the polysaccharide composition in response to drought conditions (Ahl, Mravec, et al., 2019). The composition and structure of the cell wall varies between species and between cell types and developmental stages (Gigli-Bisceglia et al., 2020).

The inner leaf mesophyll has been widely studied in *A. vera* (Reynolds & Dweck, 1999) due to its extensive use in traditional and herbal medicine, and it has been proposed that an acetylated form of mannan or glucomannan (hemicelluloses) is the main bioactive component in aloes (Femenia et al., 1999; Minjares-Fuentes et al., 2018). Mannans are commonly known as storage polysaccharides in roots and tubers, where they are involved in the plants ability to preserve water during drought (Handford et al., 2003). Due to their structural complexity, comparative studies of plant cell wall polysaccharide compositions are limited. This is also the case for comparative polysaccharide studies of aloes and of the reasons why their role in maintaining succulence is poorly understood (Ahl, Mravec, et al., 2019; Grace, Dzajic, et al., 2013). Earlier studies have used composite monosaccharide analyses of the inner leaf mesophyll as a proxy for the more complex polysaccharides, but this approach does not adequately represent the tertiary structure of the functional compounds (Grace, Dzajic, et al., 2013). A newer approach to target tertiary structures of polymers is MicroArray Polymer Profiling (MAPP) (Fangel et al., 2021) formerly known as Comprehensive Microarray Polymer Profiling (CoMPP) (Moller et al., 2007). This method uses a combination of carbohydrate microarrays and molecular probes to directly target the presence of polysaccharides where several monomers assembled in a specific and composition can be identified. With this approach, distinctive differences in the polysaccharide composition have been found between different *Aloe* species (Ahl, Al-Husseini, et al., 2019).

In this study, we are exploring if the polysaccharide composition of aloes varies with a range of traits from geographic distribution to medicinal use, leaf morphology and ecological and evolutionary life history (Table 1). To address this question, we (1) produced polysaccharide profiles for 93 *Aloe* species using the MAPP method, (2) tested for phylogenetic signal in the polysaccharide profiles and (3) tested for correlation of the polysaccharide profiles against—geographic region, level of endemism, altitude, habitat, habit, medicinal use and leaf morphology (degree of succulence) of the individual species of *Aloe*, based on an exhaustive trait database assembled from literature (Table 2 and Table S1).

2 | MATERIALS AND METHODS

2.1 | Plant material

A comprehensive trait database of aloes and closely related species was assembled containing over 200 species. From this database, 93 species of aloes were selected for this study to represent both medicinal (40%) and non-medicinal species (60%), as well as a diversity of biogeography, level of endemism, habit and considering availability of material (Figure 2, Tables 1 and 2 and S1). Plant material was sampled from the living collections in the Botanical Garden of the Natural History Museum of Denmark, University of Copenhagen and Royal Botanical Gardens, Kew, United Kingdom. Vouchers were deposited in herbaria C and K, respectively (Table S1). All samples were collected in triplicates from mature plants (biological replicates). For most species in this study, three separate leaves from the same accession (a single plant) were collected, but whenever possible, three leaves were collected from three different plants. All tissue samples were collected over a 2-month period to limit the noise of seasonal variation. Inner leaf mesophyll was collected from all species for polysaccharide analyses using the approach described in the MAPP methodology tailored for *Aloe* species (Ahl et al., 2018).

2.2 | Polysaccharide analysis

Sampling and polysaccharide data were obtained following the MAPP protocol by Ahl et al. (2018) modified from Moller et al. (2007). The

TABLE 1 Trait types and their description included in the statistical analyses. The trait types were selected to enable a comprehensive analysis of how the composition of polysaccharides obtained from *Aloe* inner leaf mesophyll relates to other biological factors that may or may not influence polysaccharide composition.

Trait type	Trait description
Geographic region	This includes 6 major geographical regions mainly in Africa, as well as one Arabian, and if the species is a native to more than 1 region, it is registered as 'cosmopolitan'. See Figure 2.
Altitude	Trait information included as a range from the lowest to the highest altitudes the specific species is present, and afterwards, the different ranges were combined and entered as 'low' (0–1000 m), 'mid' (1000–2000 m), 'high' (over 2000 m), 'ubiquitous' (when the range is over 1000 m) or NA (when data are not available).
Level of endemism	The distribution of the species ranging from endemic and common locally to widespread across geographic regions.
Habitat	A more extensive description of the habitat in which the plant is most commonly found. This category is further divided into 7 sub-categories with the most common ones being bushland, coastal, forests, grassland, rocky, sandy and shrublands. The category also includes soil type.
Habit	This refers to the growth form of the plants.
Leaf morphology	Length and width of leaves in cm. Data here were found in the literature for all species, and for a subset, they were also measured on living specimens together with the thickness of the leaf referred to as the degree of succulence (thickness: width ratio).
Medicinal use	A yes or no category that reflects whether the species is used medicinally for either its yellow exudate or its succulent tissue.

TABLE 2 Metadata including all species tested in this study along with trait information on their geographic region, level of endemism, medicinal use, altitude at which they occur in the wild, habitat and habit types. Trait descriptions can be found in Table 1. Altitudinal ranges were not included in descriptions for some of the species and have been marked with an 'NA' meaning 'not available'.

Species name	Geographic region	Level of endemism	Medicinal	Altitude	Habitat	Habit type
Aloe aageodonta	Northeast tropical Africa	Type locality	Yes	Mid	Slopes	Shrubby
Aloe acutissima	Western Indian Ocean	Island	Yes	Low	Rocks	Shrubby
Aloe adigratana	Northeast tropical Africa	Type locality	No	High	Hills	Shrubby
Aloe ahmarensis	Arabian	Widespread	No	Low	Rocks	Stemless
Aloe albiflora	Western Indian Ocean	Type locality	No	NA	Grassland	Stemless
Aloe andringitrensis	Western Indian Ocean	Island	No	High	Slopes	Stemless
Aloe antandroi	Western Indian Ocean	Island	No	Low	Rocks	Shrubby
Aloe arborescens	Cosmopolitan	Widespread	Yes	Ubiquitous	Slopes	Tree
Aloe babatiensis	East tropical Africa	Limited	No	High	Outcrops	Shrubby
Aloe ballyi	Cosmopolitan	Limited	Yes	Mid	Thickets	Tree
Aloe bellatula	Western Indian Ocean	Island	No	High	Outcrops	Stemless
Aloe brevifolia	Southern Africa	Limited	Yes	NA	Rocks	Stemless
Aloe buettneri	Cosmopolitan	Widespread	No	Ubiquitous	Grassland	Grass
Aloe burgersfortensis	Southern Africa	Common locally	Yes	Mid	Valley	Stemless
Aloe cameronii	Cosmopolitan	Widespread	Yes	Ubiquitous	Rocks	Shrubby
Aloe chabaudii	Cosmopolitan	Widespread	Yes	NA	Slopes	Stemless
Aloe cheranganiensis	Cosmopolitan	Unspecified	Yes	High	Open	Shrubby
Aloe chortolirioides	Southern Africa	Widespread	No	High	Grassland	Grass
Aloe comosa	Southern Africa	Widespread	No	Low	Valley	Tree
Aloe comptonii	Southern Africa	Widespread	Yes	NA	Gorges	Shrubby
Aloe confusa	Cosmopolitan	Limited	Yes	Mid	Slopes	Scrambling
Aloe cryptopoda	Cosmopolitan	Widespread	No	Ubiquitous	Slopes	Stemless
Aloe davyana	Southern Africa	Widespread	Yes	Ubiquitous	Outcrops	Stemless
Aloe dawei	Cosmopolitan	Widespread	Yes	Mid	Grassland	Shrubby
Aloe decaryi	Western Indian Ocean	Island	No	Low	Coastal	Scrambling
Aloe desertii	Cosmopolitan	Widespread	Yes	Mid	Thickets	Shrubby
Aloe dhufarensis	Arabian	Widespread	Yes	Low	Mountain	Stemless
Aloe dorotheae	East tropical Africa	Type locality	Yes	Mid	Slabs	Stemless
Aloe dyeri	Southern Africa	Limited	Yes	Mid	Valley	Stemless
Aloe elegans	Northeast tropical Africa	Widespread	Yes	Mid	Slopes	Stemless
Aloe ellenbeckii	Northeast tropical Africa	Limited	Yes	Low	Bushland	Stemless
Aloe ferox	Southern Africa	Widespread	Yes	Ubiquitous	Hills	Tree
Aloe fleurentinorum	Arabian	Limited	Yes	High	Slopes	Stemless
Aloe forbesii	Arabian	Limited	No	Low	Embarkments	Stemless
Aloe fosteri	Southern Africa	Limited	Yes	Low	Woodland	Stemless
Aloe globuligemma	Cosmopolitan	Widespread	Yes	NA	Bushveld	Stemless
Aloe greatheadii	Cosmopolitan	Widespread	Yes	Mid	Grassland	Stemless
Aloe hardyi	Southern Africa	Limited	No	NA	Cliff	Scrambling
Aloe haworthioides	Western Indian Ocean	Island	No	Mid	Mountain	Stemless
Aloe hildebrandtii	Northeast tropical Africa	Limited	No	Mid	Mountain	Scrambling
Aloe humilis	Southern Africa	Widespread	Yes	NA	Bush	Stemless
Aloe imalotensis	Western Indian Ocean	Limited	No	Low	Rocks	Stemless
Aloe inermis	Arabian	Widespread	Yes	Low	Slopes	Shrubby
Aloe jucunda	Northeast tropical Africa	Limited	No	Mid	Slopes	Stemless
Aloe juvenna	Northeast tropical Africa	Limited	Yes	High	Ridges	Shrubby
Aloe kedongensis	Northeast tropical Africa	Unspecified	Yes	High	Bush	Shrubby

(Continues)

TABLE 2 (Continued)

Species name	Geographic region	Level of endemism	Medicinal	Altitude	Habitat	Habit type
Aloe leachii	East tropical Africa	Limited	No	Low	Grassland	Stemless
Aloe macrocarpa	Cosmopolitan	Widespread	Yes	Mid	Grassland	Stemless
Aloe maculata	Southern Africa	Widespread	Yes	Ubiquitous	Grassland	Stemless
Aloe massawana	East tropical Africa	Limited	Yes	NA	Seashore	Stemless
Aloe melanacantha	Southern Africa	Limited	No	Low	Hills	Stemless
Aloe microstigma	Southern Africa	Widespread	Yes	Ubiquitous	Slopes	Shrubby
Aloe mitriformis	Southern Africa	Limited	No	NA	Mountain	Shrubby
Aloe morijensis	Cosmopolitan	Limited	Yes	High	Slopes	Shrubby
Aloe mubendiensis	West-central tropical Africa	Limited	No	Mid	Outcrops	Stemless
Aloe mutabilis	Southern Africa	Unspecified	Yes	Mid	Cliff	Scrambling
Aloe mzimbana	Cosmopolitan	Widespread	No	High	Outcrops	Stemless
Aloe niebuhriana	Arabian	Limited	Yes	Low	Slopes	Stemless
Aloe officinalis	Arabian	Widespread	Yes	Low	Coastal	Stemless
Aloe peckii	Northeast tropical Africa	Limited	Yes	High	Slopes	Stemless
Aloe pendens	Arabian	Limited	Yes	High	Cliff	Scrambling
Aloe penduliflora	Northeast tropical Africa	Island	No	Low	Slopes	Scrambling
Aloe polyphylla	Southern Africa	Limited	No	High	Slopes	Stemless
Aloe porphyrostachys	Arabian	Limited	No	High	Mountain	Stemless
Aloe reitzii	Southern Africa	Limited	No	Mid	Slopes	Stemless
Aloe reynoldsii	Southern Africa	Limited	No	Low	Slopes	Stemless
Aloe sabaea	Arabian	Widespread	Yes	Ubiquitous	Slopes	Tree
Aloe secundiflora	Northeast tropical Africa	Widespread	Yes	Mid	Grassland	Stemless
Aloe sinkatana	Northeast tropical Africa	Common locally	Yes	Mid	Riverbeds	Stemless
Aloe somaliensis	Northeast tropical Africa	Widespread	No	High	Mountain	Stemless
Aloe squarrosa	Western Indian Ocean	Island	No	Low	Cliff	Scrambling
Aloe striata	Southern Africa	Widespread	Yes	Ubiquitous	Slopes	Stemless
Aloe suffulta	Cosmopolitan	Widespread	No	Low	Flat country	Scrambling
Aloe suprafoliata	Southern Africa	Limited	Yes	Mid	Slopes	Stemless
Aloe swynnertonii	Southern Africa	Limited	Yes	Mid	Mountain	Stemless
Aloe thorncroftii	Southern Africa	Limited	No	Mid	Slopes	Stemless
Aloe tororoana	West-central tropical Africa	Type locality	No	Mid	Cliff	Scrambling
Aloe trichosantha	Northeast tropical Africa	Limited	Yes	Ubiquitous	Slopes	Stemless
Aloe tweediae	West-central tropical Africa	Widespread	Yes	High	Bushland	Stemless
Aloe vanbalenii	Southern Africa	Limited	No	Low	Outcrops	Stemless
Aloe vaombe	Western Indian Ocean	Widespread	Yes	Ubiquitous	Thorn-bush	Tree
Aloe vaotsanda	Western Indian Ocean	Island	No	Low	Outcrops	Tree
Aloe vera	Cosmopolitan	Naturalised	Yes	NA	Cultivation	Stemless
Aloe volkensii	Cosmopolitan	Unspecified	Yes	Ubiquitous	Slopes	Tree
Aloe wrefordii	West-central tropical Africa	Unspecified	No	Mid	Slopes	Stemless
Aloe zanzibarica	Western Indian Ocean	Island	No	Low	Cliff	Scrambling
Aloe zebrina	Cosmopolitan	Widespread	Yes	Low	Grassland	Stemless
Aloiampelos ciliaris	Southern Africa	Widespread	Yes	NA	Bushland	Shrubby
Aloiampelos commixta	Southern Africa	Limited	Yes	Low	Lowland	Shrubby
Aloidendron dichotomum	Southern Africa	Unspecified	Yes	Mid	Slopes	Tree
Aloidendron ramosissimum	Southern Africa	Limited	Yes	NA	Mountain	Tree
Aristaloe aristata	Southern Africa	Widespread	Yes	Mid	Slopes	Stemless
Kumara plicatilis	Southern Africa	Limited	No	Mid	Slopes	Tree

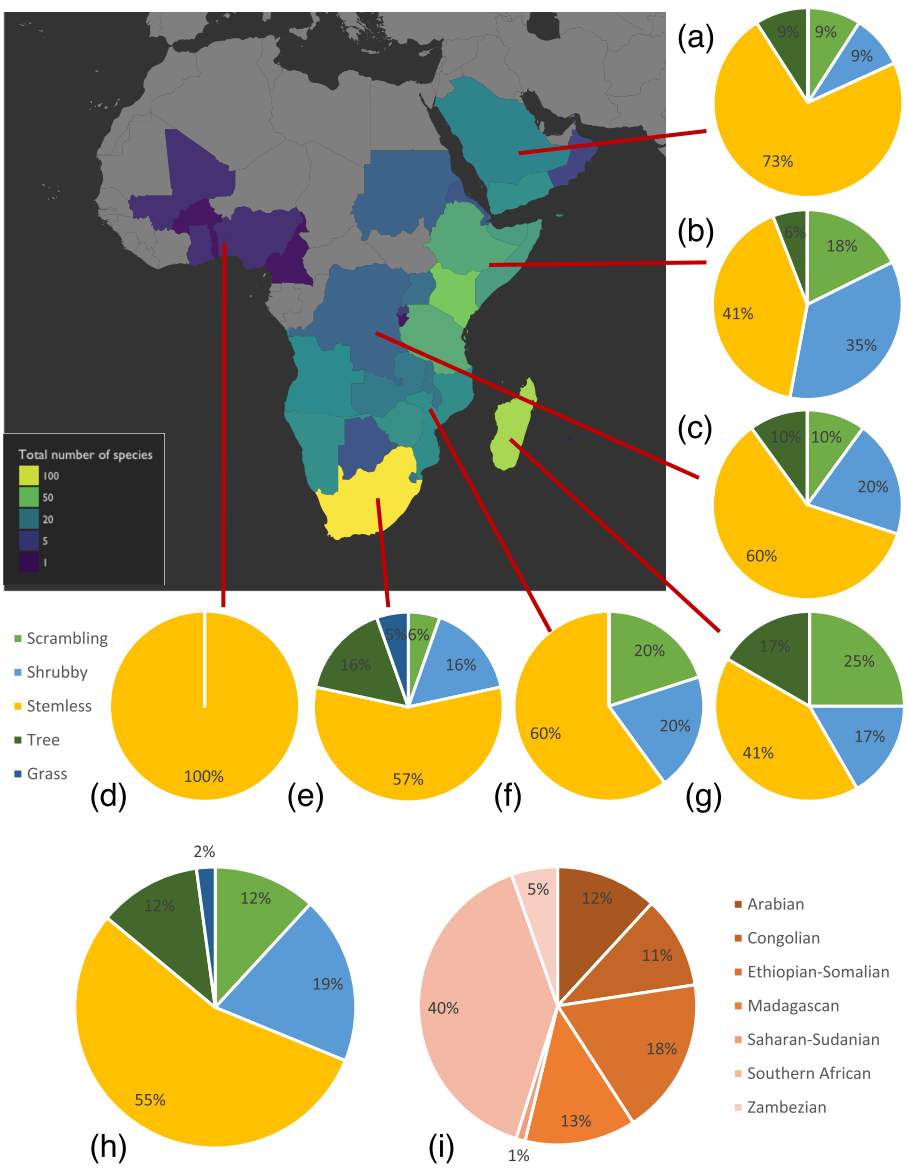

FIGURE 2 Aloe distribution map with pie charts of the sample distribution based on habit from each of the 7 geographic regions (Hollis et al. 2001): Arabian (a), Ethiopian-Somalian (b), Congolian (c), Saharan-Sudanian (d), southern African (e), Zambezian (f) and Madagascan (g). The total distribution between habit types in the samples set (h) and the distribution of species originating from the different geographic regions (i). Species overlapping in geographical distributions have only been included once.

inner leaf mesophyll was collected in three technical replicates (i.e., three technical replicates for each of the three biological replicates) and kept separate throughout the experiment. The succulent tissue was carefully excised from mature leaves and immediately snap-frozen in liquid nitrogen in labelled Falcon tubes (Corning, New York, USA). Samples were kept at −20°C for a minimum of 24 h before they were freeze dried, milled and weighed prior to extractions. Samples were homogenised using a Tissuelyser II (Gentec Biosciences, Envigado, Colombia), and approximately 5 mg of each sample was weighed to 1 decimal accuracy from each technical replicate and placed in Corning 8-strip cluster tubes (Merck Life Science, Darmstadt, Germany). A glass bead was then added to each tube prior to extractions to ensure proper mixing during the extraction process. Extractions were carried out in three-step sequential series on three separate days. Material from each test species was divided into three test tubes referred to as MAPP samples or samples in the following paragraphs. Each MAPP sample was extracted three times with three different solvents, and the extractant volume was adjusted to accommodate the exact weight of each sample reaching a ratio of 10-mg sample to 300-µL extraction solvent. The extraction series was based on the protocol by Moller et al. (2007) and Fry (1988) and adjusted according to Ahl et al. (2018) to accommodate for the high gelling abilities of the samples. Extractions were performed using three different solvents: (1) distilled H_2O—targeting soluble un- or loosely bound polysaccharides in the cytosol and embedded in the primary cell wall; (2) 50-mM CDTA (trans-1,2-diaminocyclohexane-*N*,*N*,*N*′,*N*′-tetraacetic acid monohydrate, pH 7.5, Merck Life Science, Darmstadt, Germany)—targeting pectins and some hemicelluloses contained in the primary cell wall; (3) 4-mM NaOH—primarily targeting hemicelluloses in the primary cell wall. Samples were constantly shaken during the extraction steps in a Tissuelyser II (Gentec Biosciences, Envigado, Colombia) first at a speed of 27 s for 2 min and then the speed was reduced to 6 s for 2 h. All extractions were carried out at room temperature. After the extractions, samples were centrifuged at 4000

RPM (Thermo-Fisher Scientific, Waltham, MA USA) for 10 min before the supernatant was carefully removed and transferred to a labelled 0.5-mL Eppendorf tube (Eppendorf, Hamburg, Germany). Subsequent extractions were carried out on the pellet left after the supernatant was removed. Supernatants were kept at 4°C during the subsequent extractions to minimise degradation.

Four-fold dilution series were made for each technical replicate in 384-well microtiter plates (Merck Life Science, Darmstadt, Germany), and a total of three 384-well microtiter plates were used each day to accommodate for all the MAPP samples. Dilutions in the plates were made using Arrayjet buffer (55.2% glycerol, 44% water, 0.8% Triton X-100), and the plates with the diluted extracts were centrifuged at 3000 RPM (Thermo-Fisher Scientific, Waltham, MA, USA) for 10 min before they were printed on a 0.45-μm nitrocellulose membrane (Whatman, Maidstone, UK) using an Arrayjet Marathon (Arrayjet, Edinburgh, UK) piezoelectric robotic printer. For each sample, the dilution series was printed in two technical replicates on each microarray. The three technical replicates were extracted and printed within a period of 2 weeks using the approach described above. Additionally, samples were kept at 4°C until the plating, which was done during the subsequent extraction step. This was done to allow printing to happen as fast as possible after extraction to minimise potential natural degradation of the extracts.

Twenty-seven primary monoclonal antibodies were selected to investigate the presence of as many types of pectic and hemicellulotic epitopes as possible (Figure 3; Table S2). The primary antibodies were paired with either alkaline phosphatase conjugated anti-rat or anti-mouse as secondary antibody (Merck Life Science, Darmstadt, Germany) corresponding to the origin of the primary antibody. The printed arrays from all three identical extraction rounds were developed, quantified and analysed simultaneously following the procedures described by Ahl et al. (2018). Quantification was carried out in accordance with Moller et al. (2007) using the program ArchSoft PhotoStudio at a resolution of 2400 dpi. The program measures the colour intensity of each spot before the background noise is subtracted (Moller et al., 2007). In total, 47 arrays were developed for this study: one for each antibody and extraction round, plus two for negative controls of the secondary antibodies. Normalised data from all antibodies and extractions can be found in Tables S3–S5.

2.3 | Statistical analysis of polysaccharide arrays

All statistical analyses were performed within the *R* statistical computing environment (version 2.14.9), with visualisations performed using the ggplot2 package within it (Wickham, 2016), with the exception of heatmaps that were constructed using Microsoft Excel for Mac, version 16.16.4 (181110), 2018.

For downstream analyses of the carbohydrate microarray, data averages were calculated using both the dilution series for each sample and the array triplicates (total of 48 data points per technical replicate) following the protocol of Ahl et al. (2018). The full polysaccharide dataset was visualised in a heatmap format with all antibodies and their binding shown in Tables S3–S5. The highest mean value of the dataset (for each extraction solvent separately) was assigned the value of 100%, and the remainder of the data were adjusted accordingly and normalised, with a 5% cutoff assigned to reduce noise in the datasets (Figure 3, Tables S3–S5). All replicates from all plants from the three different extraction solvents were visualised together using non-metric multidimensional scaling (nMDS) using the Vegan package (Oksanen et al., 2019), which demonstrated clear clustering by extraction solvent, and highly significant effects found using multivariate response linear models (MLM [Df = 2, Dev = 8990, $P = .002$]) using the MVABUND package. Data from the three extraction solvents were therefore partitioned and re-normalised (based on peak values for each individual dataset), and noise was reduced (using the 5% cutoff). Furthermore, there was a clear and large species effect when visualising technical replicates (this was highly significant in all three datasets extracted with the different solvents). Therefore, the mean values for each polysaccharide for each species were calculated and data renormalised once again (100% of maxima and denoising cutoff). Single mean averages (across technical and biological replicates) for each polysaccharide were then used in downstream ecological and evolutionary analyses (Tables 3, 4 and S2).

2.4 | Trait variables

A trait database was assembled to explore the potential correlation of polysaccharide composition with geographical, environmental and plant morphological trait variables. The included traits as they are used in this study are defined in Table 1.

Information on the traits listed above was obtained from the literature (Bjorå et al., 2015; Carter et al., 2011; Cock, 2015; Grace et al., 2009, 2015; Loewenthal, 1949; Nasar & Narasegowda, 2016; Oda & Erena, 2017; Osman & Asgedom, 2016; de Rodriguez et al., 2006) (overview of selected traits in Table 2 and detailed in Table S1). Geographical regions were defined by the *World Geographical Scheme for Recording Plant Distributions* (Brummitt, 2001), and habitat and habit were modified according to Carter et al. (2011), for subsequent analyses. As an example of how information has been recorded, this is the description of *Aloe dorothea* Berger: This species is stemless and occurs around Kideliko Rock, Tanzania. It has therefore been coded as 'stemless' and belonging to the geographic region of 'East tropical Africa'. *A. dorothea* occurs only at the type locality growing on 'slabs' at 'mid altitude'. Here, the level of endemism refers to how widely a specific species can be found and ranges from species only occurring at their 'type locality' to the more 'widespread' species that can be found in many areas and sometimes across different 'geographic regions'. Altitudinal ranges have been combined for simplicity and entered as 'low' (0–1000 m), 'mid' (1000–2000 m), 'high' (over 2000 m), 'ubiquitous' (when the range is over 1000 m) or NA (when data are "Not Available"). Several species cover more than one range and will be placed in the altitude group in which they occur most. An example could be a species found from 850 to 1900 m. This species

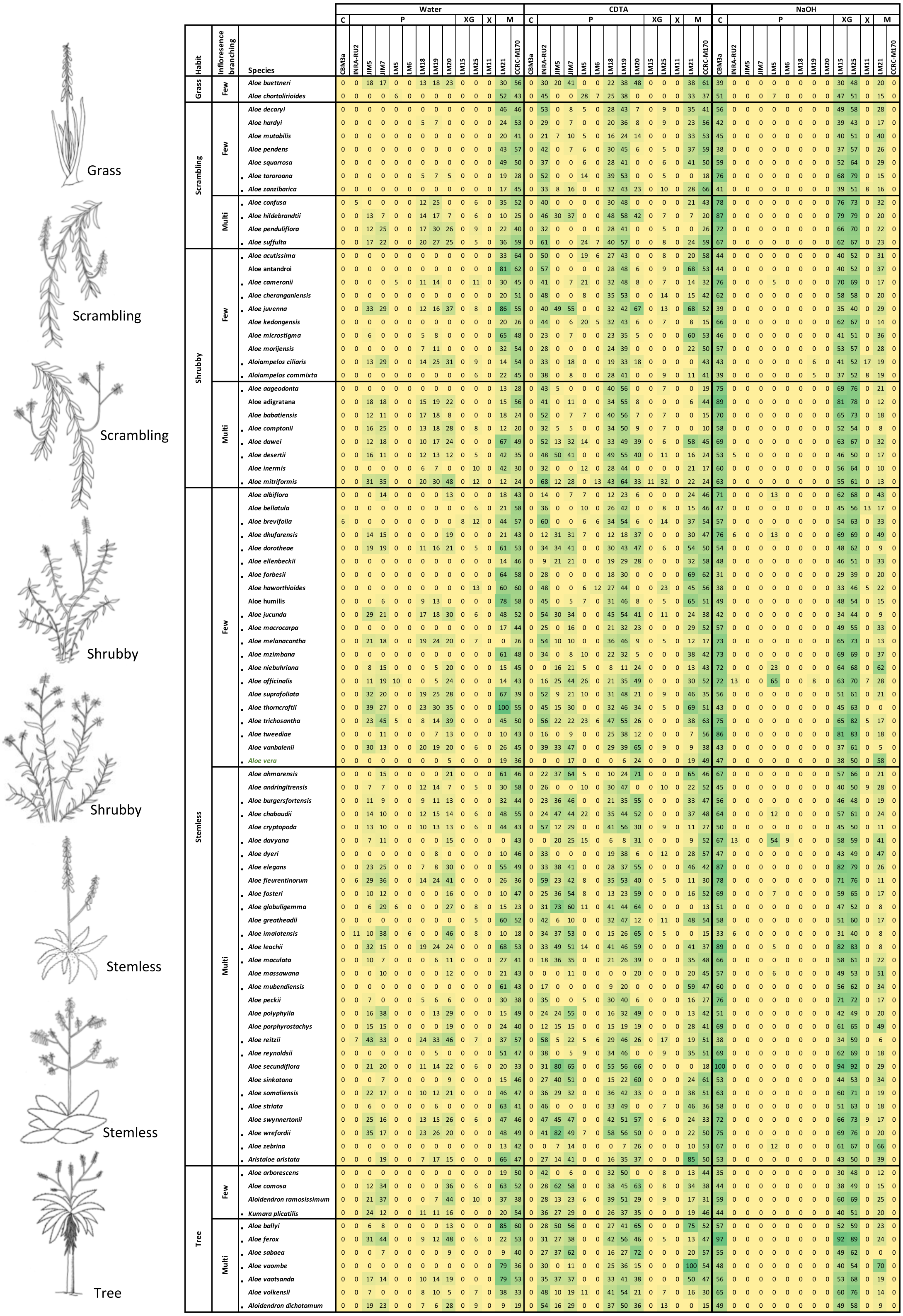

FIGURE 3 Heatmap of polysaccharide composition in *Aloe* species arranged by growth habit. Antibodies are arranged according to the polysaccharide group they target: cellulose (C), pectin (P), xyloglucan (XG), xylan (X) and mannan (M) for each extraction solvent—water, cyclo-hexane-diamine-tetraacetic acid (CDTA) and NaOH. Medicinal aloes are marked with ●, and *Aloe vera* is marked with green. Heatmap colouring goes from yellow (low relative abundance) to dark green (high relative abundance). Drawings by Louise Isager Ahl, inspired by Carter et al. (2011).

TABLE 3 Individually analysed variables including degrees of freedom (DF) and deviation and *P* values for each of the three extraction solvents—water, cyclo-hexane-diamine-tetraacetic acid (CDTA) and NaOH. Variables have been analysed individually rather than as combined, and all numbers highlighted in bold are significant.

		Water		CDTA		NaOH	
Variable	**DF**	**Deviation**	***P* value**	**Deviation**	***P* value**	**Deviation**	***P* value**
Geographic region	86	125.2	.086	129.2	.124	**199.3**	**.002**
Level of endemism	86	117	.084	**182.2**	**.006**	137.1	.114
Altitude	77	45.21	.505	53.33	.383	77.48	.226
Habitat	86	104.9	.198	102.4	.495	131.3	.218
Medicinal use	91	12.87	.603	16.67	.543	28.09	.238
Habit	88	69.97	.246	**137.1**	**.008**	90.78	.281
Leaf thickness	31	**41.98**	**.034**	**41.33**	**.024**	**64.4**	**.002**
Leaf width	31	28.71	.058	**43.38**	**.012**	**48.23**	**.008**
Leaf thickness:width	31	10.77	.591	8.901	.814	**21.17**	**.206**

will then be placed in the 'mid' group as the bigger part of its range is between 1000 and 2000 m. The words used to describe similar habitats and ecological adaptations (represented in the database under the habitat category) were inconsistent in the plant descriptions by Carter et al. (2011). These were therefore re-categorised under one term to aggregate similar habitats, for example, 'rocks' covers descriptions like 'growing on thin soil on rocks', 'growing among lava rocks', 'dry limestone rocks or rubble', 'shallow soil pockets on granite rocks' and so forth. 'Habit' in this dataset is strictly a description of the growth form, meaning the 'maculate' aloes that Carter et al. (2011) have included as a category of its own have been terminated, and the aloes have been sorted after how they grow. An example being *Aloe davyana* Schönland is entered as a 'stemless' *Aloe* rather than a 'maculate' *Aloe*. Succulence as a trait is defined, but no standard measure or method currently exists for comparisons of how succulent a species is (Eggli & Nyffeler, 2009; Grace, 2019; Reynolds & Dweck, 1999). To accommodate for the lack of a measure of succulence, we tested if leaf length and width could be of use. It was possible to obtain the measures from the literature, and these were used to determine an estimated 'degree of succulence'. For the literature reference material, we used the midpoint between the minimum and maximum leaf lengths and widths when ranges were available. Additionally, we measured leaf thickness and width for a subset to test if this could be used as a proxy for leaf succulence. Finally, medicinal use has previously been found to be correlated with the presence of succulent leaves using a phylogenetic approach (Grace, 2011. Grace et al., 2015) and was therefore also included here as a trait in the analysis (see Table 1).

2.5 | Correlation of traits and phylogeny with polysaccharide composition

Initially, differences in the overall polysaccharide composition of aloes (datasets separated by extraction method and using the mean average of technical and biological replicates) were investigated by performing nMDS and visual inspection. Significant variation associated with geographic region, altitude, level of endemism, medicinal use, habitat, habit and leaf morphology (both a measured 'degree of succulence' using leaf width and thickness and a referenced one with data on leaf length and width from the literature) (trait descriptions in Table 1). Both measures were correlated against the polysaccharide composition using Multivariate Generalised Linear Modelling (MGLM; 500 permutations; using the mvabund package).

Additionally, the evolutionary history of the polysaccharides was explored using the most complete *Aloe* phylogeny to date produced by Grace et al. (2015), which included a total of 65 species represented in both phylogenetic and MAPP analyses. The polysaccharide datasets were simplified prior to the phylogenetic analyses using principal component analysis (PCA), with principal components (PCs) explaining a cumulative total of over 50% variation within each within the first five PCs (Table S6). Subsequently, 999 Bayesian phylogenetic trees from the Grace et al. (2015) study were randomly subsampled from the 95% highest posterior density region and pruned to include only the 65 species shared within this study. Significant phylogenetic effects were subsequently assessed by correlating PC1-5 individually against each of the 999 trees using the phylosignal function within the Picante package (Kempel et al., 2010), which performed 999 randomisations per tree, with significance determined using the 50%-Majority Rule. This process was repeated for the data produced via all three extraction solvents. Blomberg's *K*, a standard index developed to correlate continuous traits against phylogenies, was applied, where *K* values close to 0 ($K \approx 0$) suggest random dispersal of the trait across the tree and therefore no correspondence with phylogeny, whereas values of *K* between 0 and 1 suggest a correlation between the phylogeny and the evolution of the trait under Brownian motion, and values of *K* larger than 1 ($K > 1$) indicates that close relatives are more similar than expected under Brownian motion (Blomberg et al., 2003; Molina-Venegas & Rodríguez, 2017). The full value of Bloomberg's *K* values and *P* values for every PC for each extraction method has been included in Table S7.

TABLE 4 Data analyses for traits and antibodies used to detect various polysaccharides present in *Aloe* inner leaf mesophyll. All data highlighted in bold are significant. Three different solvents were used to extract polysaccharides from plant tissue—water, cyclo-hexane-diamine-tetraacetic acid (CDTA) and NaOH. For a description of the specificity for each of the antibodies, see Table S2.

	CBM3a		JIM5		JIM7		LM5		LM15		LM18	
Water	**Test stat**	***P* value**	**Test stat**	***P* value**	**Test stat**	***P* value**	**Test stat**	***P* value**	**Test stat**	***P* value**	**Test stat**	***P* value**
Geographical region			**3.065**	**.009**	1.817	.105					**2.366**	**.037**
Level of endemism			1.879	.094	1.181	.324					1.446	.207
Altitude			1.317	.275	0.149	.930					1.943	.130
Habitat			1.278	.276	0.949	.464					1.867	.096
Medicinal use			−1.409	.163	−1.042	.300					−0.891	.376
Habit			1.280	.284	1.447	.225					0.379	.823
Length (cm; literature)			0.977	.331	1.005	.318					0.263	.793
Width (cm; literature)			1.784	.078	**2.048**	**.043**					0.546	.587
CDTA												
Geographical region			0.715	.639	0.465	.832	0.578	.747			2.192	.051
Level of endemism			1.002	.430	1.356	.242	1.038	.407			2.102	.061
Altitude			0.895	.448	0.766	.516	1.141	.338			1.897	.137
Habitat			0.509	.800	0.557	.763	0.627	.708			1.167	.331
Medicinal use			−1.685	.096	−1.375	.173	−0.184	.854			−0.766	.446
Habit			**3.038**	**.021**	**4.394**	**.003**	1.921	.114			1.839	.129
Length (cm; literature)			**2.856**	**.005**	**3.063**	**.003**	0.036	.972			0.148	.883
Width (cm; literature)			**3.711**	**.000**	**3.650**	**.000**	0.250	.803			1.434	.155
NaOH												
Geographical region	**4.255**	**.001**							**4.205**	**.001**		
Level of endemism	1.030	.412							1.088	.376		
Altitude	0.876	.458							0.789	.504		
Habitat	0.956	.460							0.739	.620		
Medicinal use	−0.926	.357							−0.965	.338		
Habit	0.753	.559							0.726	.577		
Length (cm; literature)	**2.573**	**.012**							**2.322**	**.022**		
Width (cm; literature)	**3.329**	**.001**							**3.100**	**.003**		

TABLE 4 (Continued)

	LM19		LM20		LM21		LM25		INRA-RU2		CCRC-M170	
Water	**Test stat**	***P* value**	**Test stat**	***P* value**	**Test stat**	***P* value**	**Test stat**	***P* value**	**Test stat**	***P* value**	**Test stat**	***P* value**
Geographical region	2.121	.059	1.871	.095	0.862	.526					0.822	.556
Level of endemism	1.136	.349	1.310	.262	0.884	.510					1.659	.141
Altitude	1.506	.220	0.062	.980	1.186	.321					0.117	.950
Habitat	1.925	.086	0.716	.638	0.709	.643					0.686	.661
Medicinal use	−0.920	.360	−1.219	.226	0.445	.657					0.342	.734
Habit	0.627	.644	1.570	.189	0.467	.760					0.324	.861
Length (cm; literature)	−0.129	.897	1.141	.257	−0.826	.411					−1.145	.255
Width (cm; literature)	0.118	.907	1.940	.055	−0.508	.613					−1.742	.085
CDTA												
Geographical region	1.466	.200	0.520	.791	0.981	.443			1.236	.296	0.846	.538
Level of endemism	**2.523**	**.027**	1.570	.166	1.000	.431			2.064	.066	1.201	.314
Altitude	1.861	.143	0.910	.440	0.406	.749			1.825	.150	0.172	.915
Habitat	1.221	.303	0.484	.819	0.194	.978			0.892	.504	0.936	.473
Medicinal use	−1.551	.125	−1.551	.125	−0.540	.590			−0.127	.899	−0.859	.393
Habit	**4.904**	**.001**	**4.904**	**.001**	0.686	.604			**2.693**	**.036**	1.748	.147
Length (cm; literature)	−0.610	.544	**3.088**	**.003**	−0.916	.362			−1.794	.076	−0.223	.824
Width (cm; literature)	0.521	.603	**3.707**	**.000**	−0.654	.515			−0.936	.352	−0.169	.866
NaOH												
Geographical region					0.931	.477	**3.616**	**.003**				
Level of endemism					1.564	.167	1.066	.389				
Altitude					0.799	.498	0.516	.673				
Habitat					1.091	.374	0.993	.435				
Medicinal use					0.243	.808	−0.713	.478				
Habit					0.507	.731	0.739	.568				
Length (cm; literature)					0.580	.563	**2.830**	**.006**				
Width (cm; literature)					−0.432	.667	**3.390**	**.001**				

TABLE 4 (Continued)

	CBM3a		JIM5		JIM7		LM5		LM15		LM18	
Water	**Test stat**	***P* value**	**Test stat**	***P* value**	**Test stat**	***P* value**	**Test stat**	***P* value**	**Test stat**	***P* value**	**Test stat**	***P* value**
Leaf thickness			**0.415**	**.016**	**0.350**	**.046**					0.127	.480
Leaf width			**0.533**	**.001**	**0.452**	**.008**					0.238	.182
Leaf thickness:width			0.147	.414	0.107	.555					0.060	.738
CDTA												
Leaf thickness			**0.367**	**.036**	**0.366**	**.036**	**−0.516**	**.002**			0.098	.587
Leaf width			**0.373**	**.033**	**0.394**	**.023**	**−0.418**	**.016**			0.153	.395
Leaf thickness:width			0.051	.779	0.073	.687	0.268	.132			0.076	.673
NaOH												
Leaf thickness	**0.474**	**.005**							**0.441**	**.010**		
Leaf width	**0.565**	**.001**							**0.507**	**.003**		
Leaf thickness:width	0.176	.328							0.113	.530		

TABLE 4 (Continued)

	LM19		LM20		LM21		LM25		INRA-RU2		CCRC-M170	
Water	**Test stat**	***P* value**	**Test stat**	***P* value**	**Test stat**	***P* value**	**Test stat**	***P* value**	**Test stat**	***P* value**	**Test stat**	***P* value**
Leaf thickness	0.092	.611	**0.375**	**.032**	−0.230	.199					**−0.369**	**.035**
Leaf width	0.219	.221	**0.466**	**.006**	**−0.363**	**.038**					**−0.456**	**.008**
Leaf thickness:width	0.107	.555	0.111	.538	−0.071	.696					−0.144	.423
CDTA												
Leaf thickness	**0.385**	**.027**	**0.385**	**.027**	−0.165	.358			−0.223	.213	−0.160	.375
Leaf width	**0.426**	**.013**	**0.426**	**.013**	−0.216	.228			−0.052	.774	−0.226	.206
Leaf thickness:width	0.080	.657	0.080	.657	−0.001	.995			0.297	.094	−0.110	.542
NaOH												
Leaf thickness					0.042	.816	**0.441**	**.010**				
Leaf width					0.099	.583	**0.500**	**.003**				
Leaf thickness:width					0.071	.693	0.076	.673				

In addition to correlations with the overall polysaccharide composition, the major polysaccharides within each dataset (accounting for a mean of 5% or higher abundance) were individually correlated against the explanatory variables. In total, 7 polysaccharides were analysed from the water extractions, 9 from the CDTA and 4 from the NaOH extracted datasets. Individual polysaccharides were analysed against our explanatory variables, with analyses of variance (ANOVAs) performed to correlate geographical region, level of endemism, altitude, habitat, habit and leaf morphology against the relative abundance of polymers (the normalised dataset of relative signal strength of the individual antibodies), and Student's *t* test was used for differences associated with medicinal use. Leaf length and width (literature) were correlated against each antibody individually using Pearson's product moment correlation.

Finally, because of the promising interaction between leaf morphology (width and length) from the literature, leaf morphology was further investigated with width and thickness (degree of succulence) measured on individual leaves from 33 of the original 93 plant species (the same individuals obtained from Copenhagen). Here, compositional variation and individual polysaccharides were correlated against leaf width and thickness (measured) as previously for leaf width and length (literature).

3 | RESULTS

3.1 | Polysaccharide variation in aloes

The detected polymers represented six major groups of polysaccharides (pectic polysaccharides, xylans, xyloglucans, mannans, cellulose and glucans) as well as arabinogalactan proteins. The polymers were targeted by 27 different monoclonal antibodies (listed in Table S2), and the polysaccharide data are presented in a heatmap format organised according to habit and listed alphabetically within each group (Figure 3). The polysaccharides were extracted in a three-step extraction series. The initial extraction with water targeted soluble un- or loosely bound polysaccharides primarily showed signals for the mannan antibodies, LM21 and CCRC-M170, but sporadic signals were also observed from the pectic polysaccharide antibodies. However, not all species seem to have loosely bound and easily extractable pectic polysaccharides. The second extraction step, CDTA, targeted pectic polysaccharides and some hemicelluloses. Here, signals from pectic polysaccharide binding antibodies INRA-RU2, LM18 and LM19 were found in almost all species, and strong mannan signals were seen again from both LM21 and CCRC-M170. No signals were detected from the mannan antibody LM22 in any of the samples. Finally, the NaOH extractions primarily target hemicelluloses and cellulose. Within the NaOH extractions, the antibody CBM3a, binding to cellulose epitopes, showed consistent signals from all species around the same level of intensity. The same pattern was seen for antibodies LM15 and LM25 binding two different xyloglucan epitopes. Mannan signals were present in the NaOH extraction, although not as strongly as in the water and CDTA extractions.

The polysaccharide composition for each species included in the study was tested using MGLM and the results showed (see Figure 4) that the composition was significant and unique for each species under each extraction solvent (A: Water [Df = 92, Dev = 1776, $P = .002$], B: CDTA [Df = 92, Dev = 3502, $P = .002$] and C: NaOH [Df = 92, Dev = 1866, $P = .002$]).

3.2 | Correlation of polysaccharide profiles with traits

The test for phylogenetic correlation showed $K = .20–.23$ across all extraction methods, values close to zero suggesting random dispersal across the tree and therefore no correspondence with phylogeny (Molina-Venegas & Rodríguez, 2017). The full value of Bloomberg's *K* values and *P* values for every PC for each extraction method has been included in Table S7.

The polysaccharide profiles of all sampled aloes were correlated using MGLM (Tables 3 and 4) to a selected list of variable traits—geographical region, level of endemism, altitude (going from low to high), habitat (soil type and habitat), habit, leaf morphology (leaf width and leaf length obtained from the literature) and medicinal use. See also Table 1 for trait descriptions.

The monoclonal antibodies used to identify the specific polysaccharides are listed in Table S2 along with their target epitopes. Binding was recorded for all antibodies presented in Figure 3 covering 5 polysaccharide groups. A measured leaf thickness was tested for a subset of the species to see if it could be used as a proxy for level of succulence. Leaf thickness was the primary factor associated with the overall variation of the polysaccharide composition, which significantly correlated with the polysaccharide composition for all three extraction solvents (Tables 3 and 4). Within the water extraction, the data on leaf thickness were the only variable that correlated with the polysaccharide composition, however, measured leaf width also correlated with the polysaccharide composition for both the CDTA and NaOH extractions. There were however also unique correlations, with habit also correlating with the polysaccharide composition from the CDTA and geographic region correlated with the polysaccharide composition extracted with NaOH (Table 3).

Major polysaccharides were identified as having a mean average above 5%, which accounted for 3 polysaccharide groups within the water dataset (pectin epitopes were detected by INRA-RU2, JIM5, JIM7, LM18, LM19 and LM20, xyloglucan epitopes detected by LM25 and mannan epitopes detected by LM21 and CCRC-M170), 3 in the CDTA (the same antibodies were binding pectin as in the water fraction, and LM5 and INRA-RU2 were present with strong signals) and 4 within the NaOH (cellulose was detected by CBM3a, xyloglucan epitopes were detected by LM5 and LM25, and mannan epitopes were detected by LM21. Additionally, sporadic pectin epitopes were detected by LM5 and LM6).

Based on the binding patterns seen in the three extraction datasets, the major antibodies (>5% average abundance) were individually analysed. The polysaccharides (from separate extractions) detected by

these antibodies were subsequently correlated against the previously selected variables. For the water extracted polysaccharides, 3 out of the 5 antibodies correlated with leaf thickness (Figure 4a) with pectin epitopes all significantly increasing as leaves became thicker and wider (JIM5, JIM7 and LM20). The opposite correlation was seen for mannan as represented by antibodies LM21 and CCRC-M170. Unlike the overall compositional level, these 2 polysaccharides (LM21 and CCRC-M170) also significantly correlated with leaf width. However, more like the overall compositional level, there were no other correlations between individual polysaccharides and the other explanatory variables.

For the CDTA dataset (Figure 4b), 3 out of 6 presented antibodies showed that only some pectin epitopes (represented by JIM5, JIM7 and LM20) significantly varied with increased leaf thickness. For the other antibodies recognising pectin epitopes (INRA-RU2, LM19 and LM5), the binding strength did not seem to be influenced by the measured thickness of the leaves. INRA-RU2 binds to the backbone of rhamnogalacturonan-I, a pectin region consisting of a considerable number of sidechains and LM19 binds to partially methyl esterified homogalacturonan with the concentration and availability of their epitopes remaining the same irrespective of the available data on measured leaf thickness. Finally, LM5 binding to (1 → 4)-β-D-galactan showed a slight decrease in binding strength as the measured thickness of leaves increased. There is an overlap with the water and CDTA, with 3 of the same antibodies (JIM5, JIM7 and LM20) each significantly varying with both measured leaf width and thickness.

With 4 major polysaccharide groups identified in the NaOH dataset (Figure 4c), only the LM21 recognised polysaccharide was included in both the water and CDTA extractions as well. The other 3 were unique for NaOH. With the overall compositional analysis, leaf thickness and width correlate with 3 out of 4 polysaccharides, which also increased in abundance with the measured leaf thickness and width. Here, all three of these polysaccharides also varied between the geographic regions in 3 out of 4 (Tables 3 and 4).

3.3 | Correlation of polysaccharide profiles with succulence

Correlations were found between literature derived measures of leaf morphology (width and length) and the overall polysaccharide

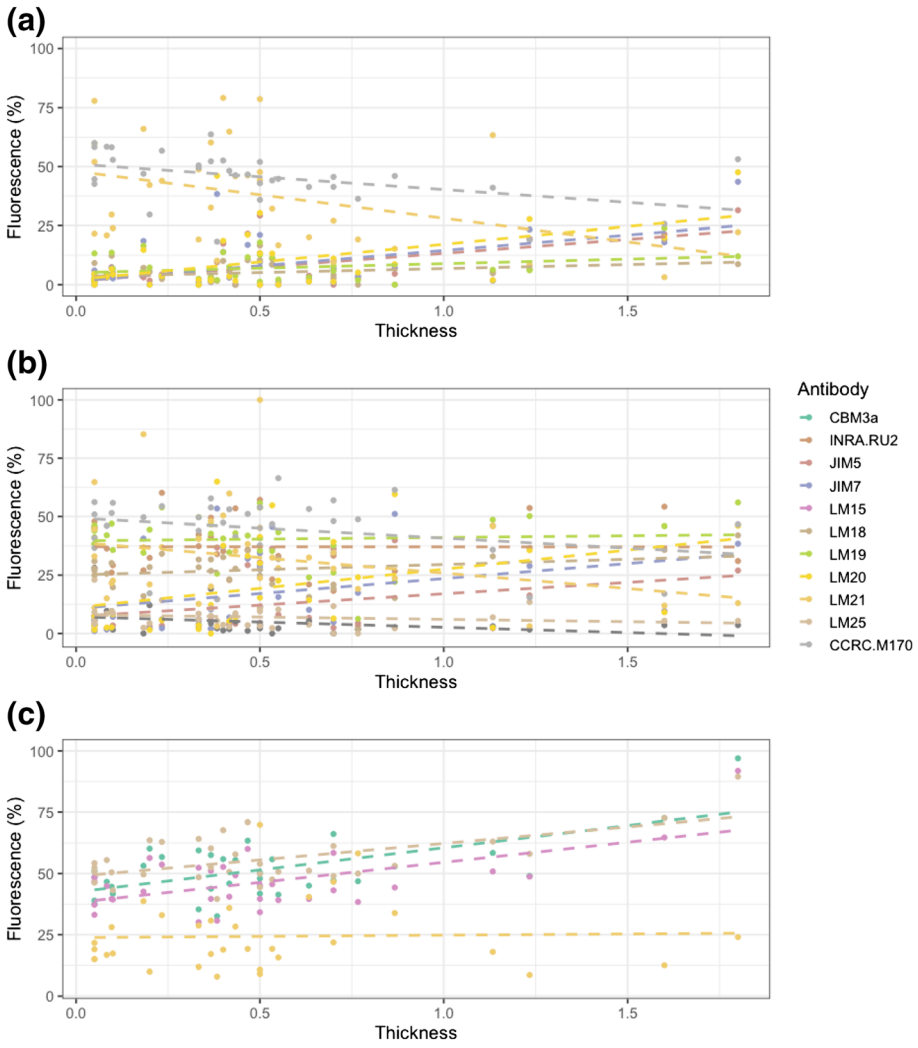

FIGURE 4 Line charts of *Aloe* species showing the correlation between leaf thickness in centimetres and antibody binding strength measures as obtained from the water (a), cyclo-hexane-diamine-tetraacetic acid (CDTA) (b) and NaOH (c) extractions. Data normalised to the strongest fluorescence as a percentage.

composition and individual polysaccharides. However, these metrics can be highly variable between plants, and they are therefore not a good proxy for the plant's level of succulence. Therefore, a subset (33 from 93) of the sampled plants had their leaf width and thickness measured and compared with the polysaccharide composition. Despite smaller sampling numbers, there was a significant correlation between measured leaf width and the overall composition from the water (Table 4), the leaf thickness:width ratio in CDTA data, and all three measures using the NaOH data. As with the leaf morphology taken from the literature, the individual major polysaccharides were correlated against leaf width, leaf thickness and width:thickness (measured). Leaf width (measured) correlated with even more individual polysaccharides using data from each dataset, despite lower sampling numbers (Table 4). Measured leaf thickness was a more accurate measure of leaf succulence and further correlated with 4, 5 and 3 individual polysaccharides from the water, CDTA and NaOH extractions, respectively.

4 | DISCUSSION

4.1 | Polysaccharide patterns correlated with leaf succulence

The polysaccharide profiles of 93 *Aloe* species for this study were produced using the MAPP methodology, and data showed significant species level variation (Figure 3, Tables 4 and S3–S5). However, there was no correlation between polysaccharide composition and phylogeny, suggesting other factors are likely at play. In particular, level of succulence (measured as leaf width and thickness for a subset) seemed predominantly to determine polysaccharide composition rather than relatedness among species. *A. vera* and other commercially used species such as *A. ferox* shared similar polysaccharide profiles with many other species and especially with those having similar growth forms (habit). Based on microarray analyses using 27 unique molecular probes targeting polymer epitopes, the polysaccharide composition was revealed, and it showed that composition was primarily driven by the degree of measured leaf succulence and to a lesser degree by distribution and habit for which a correlation was only observed in the CDTA extraction. Additionally, a correlation was seen for geographical regions in the NaOH extraction. These data indicate that the primary driver of polysaccharide composition of succulent leaf tissue is mostly dependent on leaf morphology but that the environments surrounding the plants also influence the polysaccharide composition of the succulent tissue.

Although most botanical descriptions will include an average leaf length and width for most plant species, these data do not suffice when it comes to succulents. Although no universal measure of succulence exists, we therefore tested both measured leaf thickness and the ratio between measured thickness and width to evaluate if either could be used as a proxy of leaf succulence. By taking specific measurements of leaf morphology, we get more correlations making this a better predictor for polysaccharide composition in aloes despite a lower sampling number. For the water extraction, several epitopes detecting pectic polysaccharides increased with measured leaf succulence, and for the NaOH extraction, several epitopes detecting cellulose and xyloglycans also increased with leaf succulence. The correlation between both measured leaf succulence and habit with polysaccharide composition could be explained by the functional role of polysaccharides as the structural building blocks of any plant tissue (Albersheim et al., 2010). NaOH is known to extract polymers that are part of the core structures of the cell wall (Fry, 1988), which corresponds well with the observed increase in cellulose and xyloglycan signals in NaOH extractions. Cellulose strands are bound together by hemicelluloses to form a scaffold that is then embedded in a pectin rich matrix giving the cell wall both strength and flexibility (Cosgrove, 2005). By comparison, CDTA and water extract the polysaccharides more loosely bound in the cell wall as well as the ones found unattached in the cytoplasm (Fry, 1988). These free or loosely bound polymers are likely to be altered due to seasonal changes and overall well-being of the plant.

Although different taxonomic groups of plants have different cell wall polysaccharide compositions, they also differ within individual plants within the different plant tissues (Fangel et al., 2012; Sørensen et al., 2010). The types and composition of polysaccharide structures present in a given tissue relate to its function in the plant. An example of this being mannan found in cells related to storage (Scheller & Ulvskov, 2010). In a recent study on drought responses measured in the succulent tissue by Ahl, Mravec, et al. (2019), it was also shown that hydrenchyma cells have the ability to fold their cell walls in response to drought. Here, the level of measurable pectin was significantly higher in the CDTA fraction of drought stressed *Aloe helenae* compared with the control group. This indicates that pectin polymers play an important role in the plants drought response as it affects how flexible plant cell walls are.

4.2 | Medicinal use and polysaccharide profiles

The finding that measured leaf succulence is the main determining factor of the polysaccharide composition in this study is in alignment with the broad range of *Aloe* species being used medicinally in the regions where they grow. The medicinal use records include a multitude of uses, which may relate to use of the gel, but it may also relate to the use of aloe exudate for digestive purposes associated with anthraquinones rather than polysaccharides (Grace, 2011). It is therefore difficult to assess if there could be an underlying correlation of medicinal use with habit as well as polysaccharide profile. *A. vera* is one of many short-stemmed clump-forming species (Figure 2) that constituted 55% of the species analysed within this study.

As a globally popular natural product, *A. vera* has been thoroughly investigated over the past decades, and the acetylated mannan polymers found in its succulent leaf tissue have been proposed as the primary bioactive component (Reynolds & Dweck, 1999). Looking at the wide distribution of acetylated mannan as detected by CCRC-M170, it seems likely that *A. vera* is in this respect not unique but rather one

of many species possessing similar polysaccharide constituents (Figure 3). This is also in alignment with the lack of correlation between medicinal use and polysaccharide compositions obtained for this study (Figure 3, Table 3). However, the ability to detect species effects on polysaccharide patterns has implications for authentication and optimisation of aloe products (Ahl et al., 2018; Ahl, Al-Husseini, et al., 2019). However, all *Aloe* species except *A. vera* are included in CITES, which raises concerns about their conservation and underlines the need for developing sustainable farming practices to avoid over-harvesting of wild species.

AUTHOR CONTRIBUTIONS

Louise Isager Ahl designed the study and led the research together with Nina Rønsted and Christopher J. Barnes. Louise Isager Ahl sampled the plants. Louise Isager Ahl conducted the polysaccharide experiments and analysis and produced the heatmaps with advice from Henriette L. Pedersen, Bodil Jørgensen and William G. T. Willats. Louise Isager Ahl designed and measured the leaf succulence trait. Louise Isager Ahl designed and constructed the trait database with Christopher J. Barnes and Olwen M. Grace. Christopher J. Barnes designed and conducted the statistical correlation analysis with input from Louise Isager Ahl. Louise Isager Ahl produced the figures with Christopher J. Barnes. Louise Isager Ahl wrote the manuscript with Olwen M. Grace, Christopher J. Barnes and Nina Rønsted. All authors commented on an earlier version of the manuscript and approved of the final version.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available in the supplementary material of this article.

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