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I. General Introduction

1. Plant cuticles and leaf waxes

Plant cuticles are regarded as the primary interface between the aerial parts of land plants and their environment (Shepherd & Griffiths 2006). Cuticles cover all aboveground parts of terrestrial plants, except stems with secondary growth, and are only interrupted by stomatal pores (Müller & Riederer 2005). Simplistically, we can differentiate two physico-chemically different layers of the plant cuticle, the cutin layer, covering the epidermal cells, and the cuticular wax layer. The cutin layer is a polyester of mainly ω-hydroxy-palmitates and stearates and the cuticular wax consists of lipophilic compounds that are embedded in the wax ester matrix and coating it on the surface (Baker 1982, Holloway 1982, Neinhuis *et al.* 2001). Epicuticular waxes are object of multiple studies to better understand their physiological role and contribution to abiotic and biotic interactions of plants. The general notion is that the plant cuticle serves as a barrier against uncontrolled loss of water. As transpiration control it regulates the gas and nutrient exchange with the environment, concomitantly protecting the plant from intensive irradiation; adhesion of surface water and particles and serving as interface between aerial plant parts and other organisms, i.e. bacteria, fungi and insects (Baker 1982, Müller & Riederer 2005, Pfündel *et al.* 2006, Riederer & Schreiber 2001, Schönherr 1982).

The major components of plant epicuticular waxes were identified as odd *n*-alkanes (C21–C35) and even fatty acids (C20–C24), primary alcohols (C22–C40), and aldehydes (C24–C36). Furthermore, secondary alcohols, ketones and *n*-alkylesters may be present (Baker 1982, Jetter *et al.* 2006). Besides, triterpenoids (Szafranek & Synak 2006), cinnamic acid derivatives (Santos *et al.* 2007) have been detected. The outermost part of the plant´s cuticular wax layer, commonly called epicuticular wax or superficial wax, was shown to be deposited on the cuticular wax embedded in the cutin matrix and is arranged in a broad variety of crystal-like structures as plates, tubes, rodlets, filaments and columns or as an amorphous film (Baker 1982, Barthlott *et al.* 1998). Supposedly, all terrestrial plants form epicuticular waxes and a number of studies revealed that it differs from the cuticular wax underneath not only in its ultrastructure, but also in its chemical composition (Jeffree 2006, Jetter *et al.* 2000). Extraction of plant cuticles with organic solvents, however, does not differentiate between epicuticular and cuticular wax. Solubilized epicuticular wax was shown to self assemble under artificial conditions into crystalloid structures, which were similar in shape and size to those on the plant surface (Neinhuis *et al.* 2001). The formation of a particular ultrastructure

is assumed to depend on the chemical compostion and, sometimes, correlated with a single component dominating the wax mixture (Baker 1982, Jetter *et al.* 2006).

The chemical composition of the plant epicuticular wax generally is characteristic for a given plant species, its developmental stage and the plant organ (Müller & Riederer 2005). Plant cuticular wax properties might be a taxon-specific, i.e. stems of the Euphorbiaceae genus *Macaranga* proved as slippery to non-adapted ants (Markstädter *et al.* 2000), but might also differ between species of the same taxonomic level, or might be similar between species of phylogenetically distant plant taxa (Jeffree 2006). Shifts in the chemical composition of cuticular wax relate to the developmental stage of the studied plant organ (Jetter & Schäffer 2001). Ultrastructure and chemical composition of plant cuticles is assumed to be widely controlled by the genetic program of the respective plant species (Jetter *et al.* 2006).

Environmental factors, such as air humidity, UV-radiation, air pollutants, water and salinity stress, affect the physico-chemical properties of plant cuticles (Shepherd & Griffiths 2006). When exposed to extreme air humidity, plants form lower amounts of wax than the control individuals grown under lower humidity levels, but effects on chemistry only were found to be species-specific (Koch *et al.* 2006). Generally, plants living in arid regions of the world form thicker cuticles than plants from comparably more humid regions; an adaptive mechanism to regulate transpiration, however, could not be verified as extensive cuticle wax layer are also known from plants growing in humid habitats.

Leaf wax composition and density of epicuticular wax crystals determine the hydrophobicity of leaf surfaces, the higher the content of non polar components in the leaf wax and the higher the density of epicuticular wax crystals, the higher the hydrophobicity of leaves (Koch *et al.* 2004). Since the degree of hydrophobicity and the fine structure of the leaf surface determine the leaf wettability, these features also influence the retention time of leaf surface water after rain events and from condensation of mist (Holder 2007).

The chemistry of plant cuticular waxes and their surface characteristics are regarded as important factors that influence biotic interactions (Müller & Riederer 2005). Phytophagous insects have to identify appropriate plants for feeding and oviposition. This also is mediated by physical and chemical characteristics of the plant surface which affect the behaviour of the insect after its landing (Eigenbrode & Espelie 1995, Müller & Riederer 2005). Antifungal and

antibacterial substances are deposited mostly in living plant tissues, but were also found as components of leaf waxes (Talley *et al.* 2002, Valkama *et al.* 2005). Leaf surface properties affecting adhesion, host recognition, niche modification, nutrition and water availability, however, are considered as more relevant in mediating plant-pathogen interactions (Beattie 2002, Leveau 2006, Romantschuk 1992).

There is evidence that, prior to infection of plant tissues, bacterial colonization of leaf surfaces is influenced by hydrophobicity and water repellency of the leaf wax; a reduced density of epicuticular crystals caused higher leaf surface water retaining capacity and lower hydrophobicity as well as increased leaching rates that enhance bacterial attachment and colonization rates (Marcell & Beattie 2002). In terms of improving control, it is of special interest if conidia adhesion and germination of biotrophic fungi can be inhibited by plant wax topography and chemistry; however, the observed interactions allow no unambiguous conclusions (Carver & Gurr 2006).

2. Epiphylls and the phyllosphere

The surface of aerial plant leaves is colonized by epiphytic organisms, so-called epiphylls, and, in congruence to the rhizosphere, the leaf habitat was described as phyllosphere (Ruinen 1956).
Epiphyllous organisms are widely supposed to be non-parasitic. Epiphylls on leaves of flowering plants and ferns are particularly rich in diversity and abundance in the humid tropical regions.
A freshly formed leaf in the understorey of the wet tropics is free of epiphylls, but is colonized very soon by various micro-organisms, such as diazotrophic bacteria and archaea, fungi, yeasts, amoebae and flagellates, followed by algae, cyanobacteria and finally lichens and bryophytes (Ruinen 1961). The complexity of the epiphyll community structure in the wet tropics is high and thus most studies focus either macroscopically visible organisms, usually lichens and bryophytes, or microbial communities of the phyllosphere. Especially in the understorey of the rainforest, the density of leaf surface colonization of phorophyll plants is notable. In this study, some of the sampled leaves were covered up to 80% of the total leaf area.

More than 95% of the epiphyllous bryophyte species are members of the liverworts, most of them of the family Lejeuneaceae and the remaining species belong to a few moss families (Lücking 1997). Some characteristic epiphyllic bryophytes occur more or less exclusively on the surface of leaves and are unknown from other habitats (Gradstein 1997).
Foliicolous bryophytes are well adapted to their living space by a small oppressed corpus that firmly adheres to the leaf surface with fused discs of rhizoid bundles (Gradstein 1997). In contrast to foliicolous lichens, epiphyllous bryophytes can be easily removed from wetted leaves (Winkler 1967). On dried leaves removal is very hard and the rhizoid bundle and young leaflets of the bryophyte will remain on the surface. The mucilage which is produced under leaf tips and under the rhizoid bundles is water soluble, but very adhesive, when dried out and it is mixable with that of other species without loosing its adhesion capacities (Winkler 1967). Dispersal of epiphyllic bryophytes mainly occurs by asexual propagules via water as medium (Coley & Kursar 1996), adhesion and germination of the propagules is assumed to be facilitated by wet surfaces, but water currents on leaves during rain events also can cause detachment of young epiphyllous liverworts (Winkler 1967).

Compared to lichens, epiphyllic bryophytes exhibit higher demands for nutrients and, besides external nutrient sources as from throughfall and rainfall, leachates from the host´s leaves as well as from associated cyanobacteria are assumed to match these demands (Coley & Kursar 1996, Ruinen 1961).

Foliicolous lichens show the highest diversity in the wet tropical regions of the world notwithstanding low endemism (Lücking 1997). Epiphyllous lichens are characterized by short life cycles and fast sexual and vegetative reproduction (Pinokiyo *et al.* 2006). Attachment to surfaces seems to be the most critical moment for establishment of the mycobiont´s spores as for non lichenized fungi and yeasts (Leveau, 2006). After arrival surface adhesion is mediated via hydrophobic interactions, i.e. van der Waal attraction forces, hydrogen bonds. Thus, successful attachment of fungal spores is facilitated on hydrophobic surfaces and is usually hindered on wetted leaf surfaces (Lücking 1998).

It is still unclear to what extent epiphyllation, especially on older leaves, which are often totally overgrown by an epiphyll layer, causes detrimental effects on the host plant, but negative effects are assumed to outweigh the positive (Coley & Kursar 1996). The most obvious disadvantage of epiphyllation for the host plant is a reduction of the photosynthetic

activity. Epiphylls can reduce the life time photosynthesis rates of the colonized plants by 20 to 30% (Coley & Kursar 1996), but heavily lichenized leaves react to increased shading by adaptive mechanisms, i.e. by augmenting the chlorophyll contents in the affected tissues; some even seem to be able to fully compensate the light loss (Pinokiyo *et al.* 2006). Although epiphyllous organisms do not damage the leaf cuticle of their host plant, they might augment the probability of pathogen infections by constant wetting of the leaf surface (Huber & Gillespie 1992).

The phyllosphere community is supposed to play an important role in the nitrogen cycle of tropical wet rainforests, in particular cyanobacteria associated with bryophytes, but since it was shown that bryophytes take up N- sources much faster than their host leaves, nutrients might not be well available to densely epiphylled leaves (Wanek *et al.* 2004). A possible positive effect of epiphylls for the host plant could be the protection from herbivory and pathogens by antibiotic and antiherbivory metabolites of liverworts and lichens (Coley & Kursar 1996).

The main factors driving establishment, growth and development of epiphyll communities are relative air humidity, seasonality of rainfall, temperature and light availability (Coley & Kursar 1996, Lücking 1998, Olarinmoye 1974, Winkler 1967). Bryophytic epiphylls prefer habitats of very high air humidity with no pronounced dry season and particularly thrive well in the understorey of lowland rainforests with low light levels; the coverage and diversity of lichens was shown to be higher on drier sites (Coley *et al.* 1993). On sites matching the physiological demands of both foliicolous lichens and bryophytes, competition for living space and nutrients is regarded as the key factor for epiphyll growth and development (Coley & Kursar 1996, Olarinmoye 1975). Several studies investigated if the establishing and distribution of foliicolous lichens and bryophytes depend on shape and surface microstructure of the host leaves. Both lichens and bryophytes were reported to be able to grow on artificial substrates as plastic and glass but only showed decreased colonization rates on extreme rough leaf surfaces, for example such with dense trichome coverage (Winkler 1967). Epiphyll distribution seems not to be influenced by leaf shapes (Monge-Najera & Blanco 1995) and epiphyll colonization equally developed on smooth and rough artificial surfaces (Coley *et al.* 1993). A study with plastic leaves that explored the influence of drip-tips on lichens reported no differences in lichen abundance and diversity between leaves with and without drip-tips; neither differences were found between natural and artificial leaves (Lücking & Bernecker-

Lücking 2005).

In a recent review on foliicolous lichens it was reported that also typical epiphyll species, which are merely found on plant leaves, seem to grow on every substrate, which matches their ecophysiological requirements and, because of the assimilates of the phycobiont, seem to be largely autochtonous of leaf leachates (Pinokiyo, Singh and Singh 2006). Hence, under equal climatic conditions and on leaves of similar surface topography, similar coverage rates by similar epiphyll communities are to be expected. The species identity of the epiphyll host plant, however, can strongly affect epiphyll colonization rates and diversity; phorophyll species with leaves of high longevity generally are covered slowly by epiphylls and host plants with leaves of short life span are colonized comparably fast (Lücking 1998, Coley *et al.* 1993, Wanek *et al.* 2004). Epiphyll host plants with leaves of high longevity may utilize chemical defense mechanisms in their surface wax to avoid heavy epiphyllation and plants with short lived leaves are expected not to invest in such a mechanism. Up to now, however, no study has been conducted to explore this hypothesis (Coley & Kursar 1996, Coley *et al.* 1993, Olarinmoye 1974).

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II. Leaf wax compounds of tropical understorey plants affect epiphyll community growth

1. Introduction

The foliage of land plants provides a habitat for a broad range of epiphytic organisms, so called epiphylls and, analogously to the rhizosphere, was described as phyllosphere (Ruinen 1956).
Species diversity and abundance of epiphylls is highest in the humid tropical regions and in particular on plant leaves in the rainforest understorey. During its ontogenesis a leaf is colonized by a variety of micro-organisms, such as bacteria, archaea, yeasts, protozoa and fungi, followed by algae and cyanobacteria and finally by lichens and bryophytes (Ruinen 1961). Epiphyllous bryophytes and lichens are the dominant taxa on the phyllosphere of tropical understorey plants, covering 80% and more of the leaf area.

Most of the epiphyllous bryophytes belong to the liverwort family Lejeuneaceae, which exhibits high endemism, whereas foliicolous lichens derive from various taxa of low endemism (Lücking 1997). As adaptation to the phyllosphere, epiphyllous bryophytes generally are small, pale coloured, oppressed and firmly adhered to the substrate by rhizoid discs (Gradstein 1997). To prevent detachment most foliicolous lichens have a crustous habitus, which is strongly attached to the leaf surface (Lücking 1997). Due to the ephemeral character of their living space, both epiphyllous bryophytes (Gradstein 1997) and lichens (Pinokiyo *et al.* 2006) invest in fast reproduction; bryophytes preferentially form asexual propagules and lichens form both generative and vegetative dispersal units.

Climatic conditions as relative air humidity, light availability and seasonality of precipitation are the main factors regulating establishment, growth and development of epiphyll communities (Coley & Kursar 1996, Lücking 1998, Olarinmoye 1974, Winkler 1967). Foliicolous bryophytes favour understorey sites of very high air humidity and low light intensities, lichens in contrast show higher abundance and diversity at drier and more luminous sites (Coley *et al.* 1993).

The overall effect of epiphyll colonization on the host plant, in particular when the extent is large, is supposed to be negative (Coley & Kursar 1996). Epiphyllous organisms do not penetrate the leaf cuticle of their host; however, they might increase the probability of pathogen infections by constant wetting of the leaf surface (Huber & Gillespie 1992). The most evident disadvantage of epiphyllation is the shading of leaves, which reduces life time photosynthetic rates of host plants by estimated 20 to 30% (Coley & Kursar 1996). Nitrogen supply to the host leaf by epiphyllous diazotrophic bacteria and cyanobacteria (Wanek *et al.*

2004) and protection from herbivory and pathogens by bioactive metabolites of liverworts and lichens (Coley & Kursar 1996) are discussed as possible, yet unconfirmed positive effects to the host plant. The degree of epiphyll coverage and diversity could not be related to the size and shape of host plant leaves by previous investigations (Lücking & Bernecker-Lücking 2005, Monge-Najera & Blanco 1995); and epiphyll communities exhibited similar development on artificial substrates and living leaves as well as on smooth and rough surfaces (Coley *et al.* 1993, Lücking & Bernecker-Lücking 2005, Winkler 1967).

We hypothesized that at given climatic conditions and on host plants with similar leaf surface characteristics, epiphyll communities would develop at similar rates. Epiphyll colonization rates and diversity, however, can differ clearly between different host plant species growing at the same site, an effect which is related to the leaf longevity of the phorophyll species (Lücking 1998, Coley *et al.* 1993, Wanek *et al.* 2004). Plant species with long living leaves generally are covered slowly by epiphylls and vice versa phorophyll species with leaves of short longevity are overgrown comparably fast. Epiphyll host plants with long living leaves may suppress extensive epiphyllation by inhibitory chemistry of the surface wax, whereas for plant species with fast growing and short living leaves such a strategy might be too costly (Coley & Kursar 1996, Coley *et al.* 1993, Olarinmoye 1974). Up to now, this hypothesis has not been explored.

Numerous studies have addressed the chemistry and morphology of plant cuticular waxes (Baker 1982, Barthlott *et al.* 1998, Holloway 1982, Jeffree 2006, Jetter *et al.* 2000, Neinhuis *et al.* 2001), as well as the functions of the cuticle for the plant in its environment and the influence of abiotic factors on the physico-chemical properties of the cuticular wax (Koch *et al.* 2006, Müller & Riederer 2005, Riederer & Schreiber 2001, Schönherr 1982, Shepherd & Griffiths 2006).

Some work has been carried out in terms of exploring the influence of the plant wax chemistry on herbivores and pathogenic microorganisms (Eigenbrode & Espelie 1995, Müller & Riederer 2005, Talley *et al.* 2002, Valkama *et al.* 2005). There is, however, still a lack of knowledge about the role of plant wax chemistry in biotic interactions.

Epiphyll–plant leaf associations offer themselves as suitable study model to gain a better understanding about leaf surface interactions in general and about epiphyll-plant interactions in particular. The aim of this study was to examine if inhibited epiphyll colonization on plants with long- lived leaves is mediated by bioactive leaf wax components. We investigated relations of epiphyll community composition and growth on leaves of six selected understorey plant species to leaf wax chemistry of the plants in order to address the following questions:

(1) Do epiphyll colonization rates and patterns differ between leaves of the selected host plant species?
(2) Does leaf longevity influence epiphyll colonization rates?
(3) Does the chemical composition of the leaf wax vary between rainforest understorey plant species?
(4) Does the leaf wax composition change with the leaf age?
(5) Is there a relation between leaf wax chemical composition and average leaf longevity?
(6) Do chemical leaf wax patterns affect epiphyll growing rates?

2. Materials and Methods

2.1. Study Site

Plant material was collected between late February and April 2005 in the Esquinas forest (8° 41.316´N. 83° 12.305´W) near the tropical research station Estacion Tropical La Gamba. The Esquinas forest is part of the Parque Nacional Piedras Blancas, which is situated in the south-east of Costa Rica, bordering to the Pacific Ocean, respectively the Golfo dulce, in the West. The forest's climate is characterized by an average annual precipitation of about 6000 mm and an average annual temperature of 27° C and thus it classifies as tropical wet (Holdridge *et al.* 1971). The Esquinas forest is subjected to a relatively wet season from August to November with average amounts of monthly rainfall of more than 500 mm and a drier period from January to March with a monthly precipitation of less than 250 mm (Weber *et al.* 2001). Topographically, the Esquinas forest is dominated by hills up to 579 m with more or less steep slopes, narrow ridges and deeply cut ravines with only small plain areas at the coast and deep inside the park. The forest near the tropical research station is a patch work of primary and well-developed secondary forest (Weissenhofer 2005), the former dominating with distance to the agriculturally used and deforested flat lands near the village of La Gamba.

2.2. Study plants

Six species of understorey perennial plants, five of them herbaceous, were selected for the study (see fig. 1). The choice was based on their frequent occurrence in the rainforests near the tropical research station and because of existing data on average leaf longevity and on epiphyllation of four of the six selected plant species (Wanek & Pörtl 2005):

Asplundia pittieri (Woodson) Harling (Cyclanthaceae),
Carludovica drudei Mast. (Cyclanthaceae),
Costus laevis Ruiz & Pav. (Costaceae),
Dieffenbachia concinna Croat & Grayum cf. (Araceae),
Pentagonia wendtlandii Hook. (Rubiaceae),
Polybotrya cervina (L.) Kaulf. (Dryopteridaceae)

The identification of the study plants was carried out using published identification keys (Weber *et al.* 2001, Lautsch 2000). All studied plants will be addressed by their generic taxa in the on-going text.

Asplundia pittieri is a small sized palm with slightly two-parted leaves with numerous drip-tips. The collected individuals were up to 60 cm in height and the leaves´ surface area ranged between 200 and 300 cm. The second Cyclanthaceae, *Carludovica drudei*, is much taller in size and forms much bigger leaves than *Asplundia*. Collected individuals were up to 3 m high and a single leaf's area ranged between 0.4 and 0.5 m².
Costus laevis has numerous- up to 30- linear ovate leaves with a drip-tip and, as in all Costaceae, leaves are arranged in a spiral. Collected individuals were between 2 and 3 metres tall and leaf area was approximately 200 cm² on average.
The collected *Dieffenbachia* individuals ranged between 0.5 and 1 m in height and exposed up to 15 glossy white-spotted linear ovate leaves with drip tips. Leaf areas varied between 300 and 600 cm².
Pentagonia wendlandii is a small treelet and reaches heights of 3 m. Leaves are arranged opposite in pairs, shaped obovate and a full- grown leaf can be up to 1 m long and 0.5 m wide, which corresponds to a leaf area of 5000 m².
The fern *Polybotrya cervina* reaches a height of up to 1.5 m and its fronds form a litter trapping rosette. Fronds are single-pinnate and with up to 15 simple alternate leaflets of lanceolate shape and with a leaflet area of up to 100 cm².

Figure 1: Study plants at sites in the Esquinas forest. A: *Asplundia pittieri*, B: heavily epiphylled leaf of *Carludovica drudei* hosting an arboreal eyelash viper (*Bothriechis schlegelii*), C: *Costus laevis*, D: *Dieffenbachia concinna* cf., E: two individuals of *Pentagonia wendtlandii* and F: *Polybotrya cervina*.

2.3. Collection of material, leaf area and epiphyll colonization

2.3.1. Collection of material

Intact leaves of *Asplundia pittieri*, *Carludovica drudei*, *Costus laevis*, *Dieffenbachia concinna*, *Pentagonia wendtlandii* and *Polybotrya cervina* were collected at forest sites near the tropical research station La Gamba. For each sampled plant individual biometric and site characteristics were noted in situ. Sufficient material for extraction of young, full-grown and senescent leaves was then cut off the selected plant. The category "young" leaves comprises such which were obviously not fully developed; "full- grown" leaves were fully developed, but not senescent and were collected from the youngest third of an individual's foliage and "old" leaves were taken from the oldest third. Leaf age categories depended on the average leaf number of each species, e.g. a "young" leaf of *Pentagonia wendtlandii*, which maximally develops 6 pairs of opposite leaves, was in general older than a young leaf of *Costus laevis*,

which is very fast growing species with up to 40 leaves per year.

2.3.2. Epiphylls and leaf area

Epiphyllous coverage, the percentage of the total leaf area covered by epiphylls, of the collected leaves was estimated by overlaying leaves with a transparent grid. Epiphyll quality, the composition of the epiphyllous community, was also estimated. Three categories were chosen to reflect the estimate: (L) leaf covered predominantly by lichens (>66%); (LM) lichens and bryophytes covered the respective leaf in approximately equal quantity, (M) leaf colonized predominantly by bryophytes (>66%). The leaf area of each sample was determined by digitizing and pixel counts with Adobe Photoshop 7.0 (Adobe Systems Incorporated, San Jose, CA, USA).

2.3.3. Average leaf longevity of host plants

Data on leaf longevity were provided by previous studies on the same location, for *Carludovica drudei, Costus laevis*, *Dieffenbachia concinna* cf. and *Pentagonia wendtlandii* (Wanek & Pörtl 2005) and for *Asplundia pittieri* (Sonnleitner *et al.* 2009). Leaf longevity of *Polybotrya cervina* was determined on basis of a survey performed with 12 individuals of the fern. In April 2005 (t_0) 12 replicates on three sites were selected, the ferns were marked, fronds were numbered and tagged with plastic ribbons from the oldest (n = 1) to the youngest. In October 2005 (t_1), fronds of each individual were again counted, new ones were tagged with numbers and died off ones were recorded. The same procedure was carried out in February 2006 (t_2). Average leaf longevity of the fronts was calculated based on data of 10 ferns (2 were damaged) using the following formula:

$$\text{N fronts } (t_0) * N^{-1} \text{ fronts died off } (t_2) * yr^{-1}$$

2.3.4. Epiphyll colonization of host plant leaves

Characteristics of leaf colonization by epiphyllous organisms were surveyed by relating absolute age [yrs.] of collected leaves with the respective epiphyll coverage (relative percentage of leaf area colonized by epiphylls). Epiphyll colonization rates were compared between and among the categories host plant species, leaf age, site of host plant and quality of epiphylls using ANOVA, Kruskal Wallis- test and regression models. Calculations and statistics were conducted with Excel (Microsoft Corporation, Redmond, WA, USA) and Statgraphics Plus 5.0. (Statpoint Technologies, Inc., Warrenton, VI, USA).

2.4. Extraction of cuticles

Immediately after collection, leaves were rinsed with tap water and epiphylls carefully cleaned off from the adaxial leaf surface with a soft kitchen sponge and cotton swabs. Keeping the balance between not damaging the leaf and getting off most of the epiphylls is difficult and presents a problem of this cleaning procedure. Lichens often resisted the removal attempts successfully. Leaves were then rinsed off as efficiently as possible and let dry before extraction.

Leaves of the same individual and of the same age category, either young or full-grown or old leaves of the same plant, were pooled as one sample respectively and extracted with ethanol or hexane. If the sampled plant had small leaves, as for instance *Costus laevis*, several leaves were taken for extraction in succession. Vice versa, due to their oversized leaves, in some instances only one half of the leaf of *Carludovica drudei* and *Pentagonia wendtlandii* were extracted.

Limited solvent capacity caused young leaves to be only extracted with ethanol; only some individuals and *Asplundia pittieri* leaf cuticles were extracted with hexane. Leaf wax extraction was done by rinsing the leaf surface with 100 ml of solvent over the adaxial surface of the respective leaf that was fixed above a large glass bowl. This extraction step was then repeated up to 10 times with the same leaf depending on its resistance to solvent infiltration. The solvent volume had to be readjusted during extraction due to high evaporation rates.

After extraction, bowls with samples were covered with an insect protection grid and left for evaporation at ambience temperature for about 24 hours. Extracts were then dissolved in the respective solvent again, transferred into small glass jars with a Pasteur glass pipette, covered with insect protection grid and let stand for evaporation. One hundred µl of $HgCl_2$ (3 mM) were added to the ethanol extracts to protect against fungal growth (moulds).
For each plant species, an ethanol and a hexane extract of fine cut and epiphyll-free leaf material and of freshly cleaned-off epiphylls were prepared. These extracts were to serve as reference material to the cuticle extracts to facilitate differentiation between cuticular and non cuticular components during chemical analysis of the samples. After 48 hours these extracts were filtered over cotton into small glass jars and the solvent was evaporated described.
Jars with dry samples were subsequently packed in airtight plastic bags with packages of silica gel and stored in a drying oven at 40°C till transport to Vienna.

2.5. Chemical analysis

All chemicals used for the preparation of samples were of p.a. quality. Samples were dried for two weeks at 35°C in a laboratory oven to remove water traces. Then they were stored at –25°C for further use. Dried ethanol extracts were dissolved in methanol, hexane extracts in n-hexane, dissolution was enhanced by ultrasonic bath, and then samples were filtrated over glass wool. For weight determination, samples were dried on a rotary evaporator under reduced pressure in a 35°C thermostated water bath. The dry weight of the extracts was determined to 10^{-1} mg.

2.5.1. Hexane extracts by GC–MS

The dried hexane extracts were dissolved in pyridine: silylation reagent (4:1, v/v) to yield solutions of 2 –4 mg/ml. Silylation reagent was a mixture of BSTFA and TMCS (9: 1, v/v; Pierce, Thermo Fisher Scientific Inc., Rockford, IL, USA). Silylation reaction was performed at room temperature for at least one hour, supported by gentle shaking of the sample. Analysis was performed on a gas chromatograph (Auto System XL; Perkin Elmer, Waltham, Massachusetts, USA) with helium as carrier gas. The column used was a PE-5ms (20m x 0.18mm x 0.18µm) from Perkin Elmer. Compounds were detected by a Turbo Mass quadrupol mass spectrometer (Perkin Elmer, Waltham, Massachusetts, USA). An alkane standard (Fluka, Buchs, Switzerland) was analysed to obtain standard retention times for *n*-alkanes. The alkane standard solution contained even n-alkanes from C10 (Decane) to C40 (Tetracosane).

Sample injection was carried out in the splitless mode, injection volume was 0.2 µl. Initial column temperature was 110 °C for 2 minutes, then the column was heated 4 °C per minute to 260 °C and afterwards 2 °C per minute to 330 °C, finally holding this temperature for 25.5 minutes till the end of the program at 100 minutes. Ionisation was performed in the electron impact mode (70 eV and mass spectra were obtained from m/z = 40 to 620 with a scan duration time of 1 second and an inter scan delay of 0.1 seconds). For analysis and processing Turbomass 4.1.1 (Perkin Elmer, Waltham, Massachusetts, USA) was used.

2.5.2. Ethanol extracts by HPLC–UV

Dry Ethanol extracts were dissolved in MilliQ water supported by sonification and fractionated over Amberlite XAD 1180 (Fluka, Buchs, Switzerland). Five ml plastic syringes coupled with an outlet valve served as columns with a filling lot of approximately 4.5 ml

Amberlite XAD1180. Before use, the resin was washed with 20 ml water, 40 ml acetone and 70 ml water. The water fraction was eluted with 20 ml of water, the second fraction with 20 ml of absolute ethanol and 20 ml Acetone to purge the columns. Ethanol fractions were dried in 100 ml round bottom glass flasks on rotary evaporators under reduced pressure in water baths (35°C), redissolved in absolute ethanol and transferred to 1.5 ml glass vials. Samples then were dried under reduced pressure in a SpeedVac, and after weighing dissolved in methanol (10 mg sample per ml) for HPLC measurement. High performance liquid chromatography (HPLC) measurements were performed on a Dionex Summit System (Dionex, Sunnyvale, California, USA) equipped with a Thermostat Column Compartment TCC-100, a photodiode array detector UVD 340 U, and a Famos autosampler (LC Packings, Amsterdam, Netherlands). The column used was a C12 (150 x 2 mm) Synergi Max, 4 µ, 80 Å (Phenomenex Inc., Torrance, CA, USA).

Injection volume was 25 µl and the elution gradient ran for 120 minutes at an oven temperature of 40°C and a constant solvent flow of 0.2 ml per minute. Separation of extracts was carried out along a linear gradient from 100 % of solvent A (H_2O: CH_3OH: H_3PO4 = 95: 5: 0.5, v/v/v) to 100 % of solvent B (CH_3OH). The solvent mixture remained unchanged for 2 minutes, then linearly increased to 100 % of solvent B within 98 minutes and was maintained at this concentration for further 10 minutes. UV spectra were recorded from 220 - 450 nm with a band wide of 1 nm and a time interval of 10^{-1} seconds. Chromeleon 6.60 (Dionex, Sunnyvale, California, USA) was used for analysis and data processing.

2.6. Data analysis and statistics

2.6.1. GC–MS

Chromatogram integration

Integration was performed from 15–55 minutes. Integration parameters were the same for all chromatograms. Peak smoothing to reduce the noise signal using the Savitzky Golay algorithm and peak purity check was conducted afterwards. Peaks with purity values below 50 % were split if necessary or reduced in area to remove edge- impurities.

Since Turbomass does not allow saving processed data, the peak lists of integrated spectrograms and pictures of all mass spectra of the respective spectrogram were stored in Excel tables.

Peak assignment and MS interpretation

Alkanes were identified based of retention time indices and mass spectra patterns derived from GC/MS chromatograms of the alkane standard. Retention time shifts in the samples due to column cutting also could be corrected by comparison with the alkane standard measurements. Identification of peaks and interpretation of mass spectra and was facilitated by comparison with commercially available libraries, NIST 1.5 (NIST, Gaithersburg, Maryland, USA) and the Wiley 6th ed (John Wiley & sons, Inc., Hoboken, NJ, USA). Acids and alcohols could be identified or specified at least to chain length level. The remaining components could partly be assigned to a chemical class and some remained unidentified.

Statistics

Peak tables were processed before statistical analysis as follows: First, all peaks derived from solvent impurities were removed from the data matrix. Second, peaks which did not occur in any of the samples with at least a relative percentage of 2% were excluded. The sum of components of each sample was normalized to 100% to gain better comparability.
Further, peaks with oleonitrile-like mass spectra (Hanus *et al.* 1999) were skipped; these analytes only occurred in a subset of samples from a distinct time period of sample preparation in Costa Rica.

The data matrix of wax extracts comprises 36 samples with 26 components (variables) and the table of leaf tissue and epiphyll extracts 12 samples and 26 components. Eventually qualitative biometric characters (=factor groups) and quantitative biometric data of samples (see 1.2) were added. Statistical exploration of the sample matrices was performed using Primer 6.1.8 (Primer-E Ltd., Lutton, United Kingdom), Statgraphics Plus 5.0 (Statpoint technologies, Inc., Warrenton, Virginia, USA) and SIMCA-P 11 (Umetrics, Umea, Sweden).
First, multivariate methods were carried out with the two sample sets to test the prediction power of extract compositions for biometric characters. PCA, PLS and SIMPER- analysis and distance resemblance matrix based Cluster- and MDS- analysis were used.
Second, single components were subjected to ANOVA based analyses to test if the relative abundance of compounds correlated with qualitative biometric characters. Correlations between the relative occurrence of a cuticle compound and the leaf age of the sampled leaves, and their epiphyll coverage were tested by regression models.

2.6.2. HPLC–UV

Chromatogram integration and peak assignment

Integration of chromatograms was carried out from 5–120 minutes retention time with the same integration parameters for all samples. UV signal 229 nm was chosen for integration. Some peak areas were manually corrected due to unusual baseline shifts and riders and a peak table comprising the integration parameters was calculated for each sample. Assigning components to chemical classes with an in house spectra library was not possible due to the low degree of specificity and structural information of the obtained UV spectra. Peaks were characterized by retention time and UV spectra by comparing narrow retention time windows of samples. All distinct peaks (n = 163) of the sample set were listed and numbered according to retention time and tagged with their UV- spectra. For each sample relative peak areas were assigned to the respective components in the peak table for further analysis.

Statistics

All analytes that did not occur in any of the samples with a relative percentage of at least 4 % together with those analytes that were not detected in more than 3 samples were removed. In a second step, two data sets were extracted: all ethanol extracted samples and leaf cuticle extracts. In the latter, all components deriving from leaf tissues and from epiphylls were sorted out of the data matrix. For example, carboxylic acids, which were detected in all samples, were excluded from the leaf cuticle data set for example.

After this procedure, the table containing all ethanol extracts comprised 51 samples with 60 components and the table of leaf cuticle extracts 38 samples with 20 components. For better comparability, the sum of compounds of each sample was normalized to hundred percent. Finally qualitative biometric characters (= factor groups) and quantitative biometric data of samples were added. Factor groups comprised type of extract (cuticle, leaf tissue, epiphyll), plant species (*Asplundia*, *Carludovica*, *Costus*, *Dieffenbachia*, *Pentagonia*, *Polybotrya*), leaf age (young, full -grown, old leaves), epiphyll quality (L = lichen dominated, M = moss dominated leaves, LM = moss and lichens in aprox. equal abundance) and accession site (slope, ravine).

Statistical exploration was performed using Primer 6.1.8 (Primer-E Ltd., Lutton, United Kingdom), Statgraphics Plus 5.0 (Statpoint technologies, Inc., Warrenton, Virginia, USA) and SIMCA-P 11 (Umetrics, Umea, Sweden). First, multivariate analyses were carried out to get an overview of groupings and similarities among components and samples and of relations

between biometric data and components of samples. (PCA, PLS and SIMPER- analysis and distance resemblance matrix based Cluster- and MDS- analysis). Second, compounds and metavariables of compound bundles, which seemed to be characteristic for a specific factor group (e.g. old leaves), were tested with ANOVA methods and regression analysis. ANOVA facilitated testing the validity of single components to serve as predictors for qualitative biometric characters. Regression analysis was conducted to test correlations between a single component's relative percentage and quantitative biometric data (epiphyll coverage and leaf age).

3. Results

3.1. Biometry

3.1.1 Leaf longevity of study plants

The six selected species of understorey herbal plants differed among each other in the average leaf longevity in the following order: *Asplundia* (4.34 yrs.) > *Polybotrya* (3.73 yrs.) > *Dieffenbachia* (3.62 yrs.) > *Carludovica* (3.22 yrs.) > *Pentagonia* (2.91 yrs.) > *Costus* (1.56 yrs.). Variation in lifetime of leaves was high for all studied epiphyll host plant species. Means and Scheffe- confidence intervals (P= 0.95) of leaf longevities of the six study plants are shown in figure 2.

Leaves of *Costus laevis* fall off after 1.56 years on average, thus much earlier than leaves of *Asplundia pittieri* (4.34 yrs.), *Dieffenbachia concinna* cf. (3.62 yrs.) and fronds of *Polybotrya cervina* (3.73 yrs.). Average life expectancy of *Pentagonia wendtlandii* leaves was 2.91 and of *Carludovica drudei* leaves 3.22 years, but the respective confidence intervals overlapped with those of the other plants.

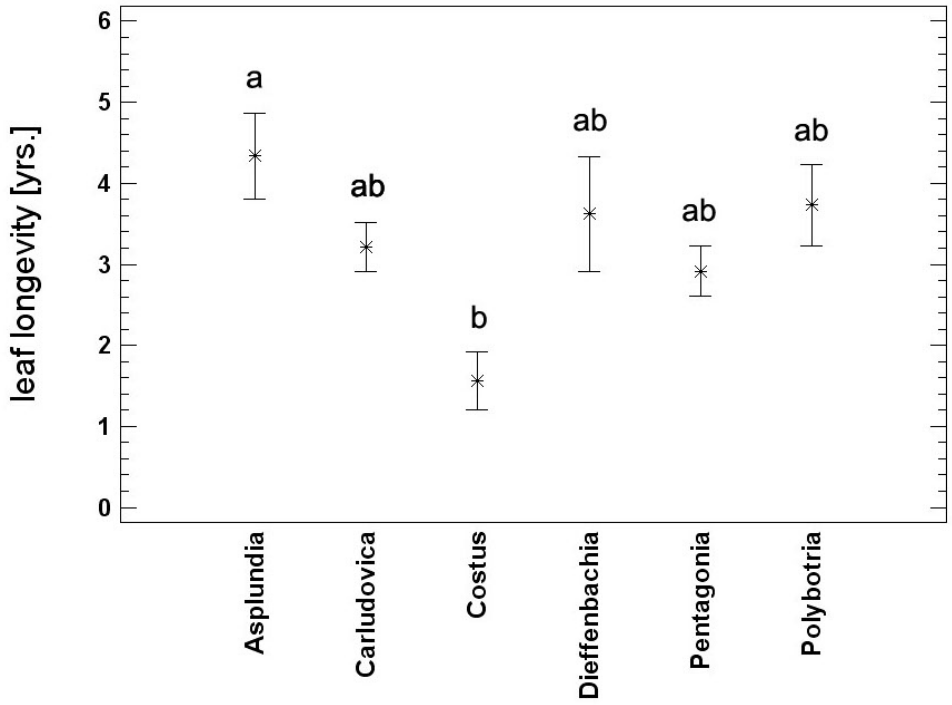


Figure 2: Means of leaf longevity of the six sampled understorey epiphyll host plant species. Leaf lifetime in years is displayed on the y- axis; plant species on the x- axis. Data points labelled with different letters differ from one another at P= 0.95 based on ANOVA based Scheffe test. Error bars indicate standard errors. Leaf longevity for each species was calculated from data of several replicates using the formula: N leaves (t0) * N leaves died off $(t2)^{-1}$ * yr^{-1}. Number of replicates: *Pentagonia* = 6, *Costus* = 8, *Dieffenbachia* = 8, *Carludovica* = 9, *Polybotrya* = 10 and *Asplundia* = 11.

3.1.2. Epiphyll community composition and site of host plant species

Macroscopically, the sampled plant leaves were predominantly colonized by bryophytes, for the most part by liverworts of the Lejeuneaceae family (M. Sonnleitner personal comm., Sonnleitner 2009), by lichens and, on some leaves, also by cyanobacteria (see fig. 3). The latter were determined microscopically as cyanobacteria on basis of heterocyst formation.

Figure 3: Old leaf of *Costus laevis* (left) and *Dieffenbachia concinna* cf. (right). Pictures were photoscanned with 200 dpi immediately after collection. On both leaves liverworts (light green arbuscular structures) and crustous lichens (light grey) can be seen, on the *Dieffenbachia* leaf also some cyanobacteria- colonies are visible (brown- lily round dots).

The studied plant species partially showed a site preference. Most individuals of *Pentagonia* and *Dieffenbachia* were growing in ravines, *Polybotrya* was only found on slopes and *Costus*, *Carludovica* and *Asplundia* were observed on both slope and ravine sites. Lichens showed a tendency to dominate the phyllosphere of plants from slope sites and, conversely, bryophytes dominated leaves of ravine sited plants. Fifty-eight % of the ravine leaves were dominated by bryophytes (coverage contribution > 66%) and 64% of the slope leaves by lichens. Equal colonization was found on 23% of the leaves collected from slopes and 38% from ravine sites. Only 4% of the plant leaves collected from ravines was dominated by lichens and 13% of the leaves from slope sites were covered predominantly by bryophytes.

3.1.3 Epiphyll growth over time

The coverage of the phyllosphere by macroscopically visible epiphylls increased with leaf age for each of the six studied plant species. Plotting all values of epiphyll coverage against the calculated age of the sampled leaves resulted in a R^2 of 0.67 for the exponential regression and of 0.52 for the linear regression, both values indicating a moderately strong relationship between leaf age and epiphyll coverage. Plotting values of calculated leaf age against the epiphyll coverage separately for each host plant species showed that colonization of leaves by epiphylls happened at different time rates depending on the host plant species (see fig. 4). Exponential regressions were chosen for the description of the relation between the age and the epiphyll coverage of the colonized leaves for the six studied plants, since the regression parameters were the most powerful of all tested regression models. The R squared for the exponential regression of all sampled leaves versus their epiphyll coverage ($R^2 = 0.67$) was lower than for the regression curves of the single plant species except for *Asplundia* ($R^2 = 0.64$).

The leaf age when epiphylls would cover 50% of the leaf area was calculated with the equations of the exponential regressions and delivered the following values: 1.7 years for *Costus*, 2.5 years for *Pentagonia* and *Polybotrya*, 2.9 years for *Dieffenbachia*, 3 years for *Carludovica* and 6.1 years for *Asplundia*.

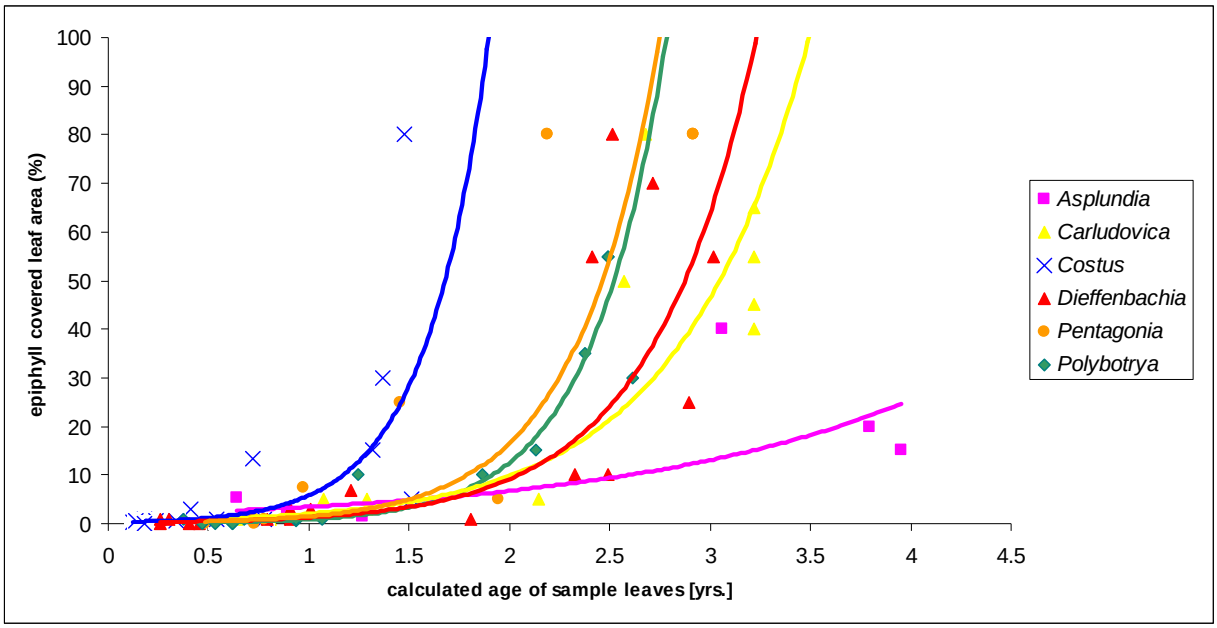


Figure 4: Exponential regression model of epiphyll colonization on the leaves of the six tested understorey plant species. X values: calculated age of sample leaves in years; y – values: relative leaf area colonized by epiphylls. N samples: *Asplundia* = 5, *Carludovica* = 15 , *Costus* = 13 , *Dieffenbachia* = 18, *Pentagonia* = 14, *Polybotrya* = 16. Curve function and R^2: *Asplundia*: $y = 1.79 * e^{0.66 x}$, $R^2 = 0.64$; *Carludovica*: $y = 0.43 * e^{1.56 x}$, $R^2 = 0.90$; *Costus*: $y = 0.2413 * e^{3.17 x}$, $R^2 = 0.73$; *Dieffenbachia*: $y = 0.19 * e^{1.95 x}$, $R^2 = 0.78$; *Pentagonia*: $y = 0.14 * e^{2.39 x}$, $R^2 = 0.79$; *Polybotrya*: $y = 0.06 * e^{2.65 x}$, $R^2 = 0.86$.

Considerable differences of epiphyll colonization rates between the host plant species were found in Student's t- tests of the slopes (see table 1). Leaves of *Asplundia* were colonized by epiphylls significantly slower than the other plants´ leaves. Also the second studied Cyclanthaceae- palm, *Carludovica drudei*, was covered by epiphyllic organisms at considerably slower rates than the other plants except for *Dieffenbachia*. *Costus laevis* leaves were epiphylled faster than leaves of both Cyclanthaceae- species and faster than *Dieffenbachia* leaves at P= 94.4 %.

The correlation of epiphyll cover and leaf age according to the two sampling sites ravine and slope did not differ from each other significantly, such that the probability that the slopes of the two resulting curves are the same was 45 %.
Also a comparison of the three regression curves according to the factor epiphyll community composition (lichen dominated, moss dominated and mixed colonized leaves), showed high similarities among the curves (P > 0.38). Thus the host plant species showed the strongest influence on differences in epiphyll growth over time within the sample set.

Table 1: Comparison of epiphyll colonization rates between host plant species. Values in the table are P- values between the slopes of the six epiphyll coverage curves. P- values equal to or beneath 0.05 mark significant differences between slopes. The test statistic was Student´s t, which was computed using the following formula: $t = (b_1 - b_2) / \sqrt{(S^2_{b1} + S^2_{b2})}$ (b: slope of exponential regressions; S_b: standard error of the slope).

Probability	*Asplundia*	*Carludovica*	*Costus*	*Dieffenbachia*	*Pentagonia*
Carludovica	0.007				
Costus	0.001	0.010			
Dieffenbachia	0.002	0.204	0.056		
Pentagonia	0.001	0.040	0.250	0.320	
Polybotrya	0.000	0.002	0.417	0.080	0.575

3.2. Ethanol extracts

3.2.1 Epiphyll, leaf tissue and leaf wax samples

Extract yields

In average, 58 mg wax /m^2 leaf were obtained, dry mass values, however, showed high variance. Thus, only old leaves of *Pentagonia* were characterized by significantly higher amounts than other samples, 246 mg /m^2 respectively. Old leaves of *Costus*, *Dieffenbachia*,

Pentagonia and *Polybotrya* yielded slightly higher extract amounts than the corresponding young developing and fully developed leaves, but not at a significant level. Leaf tissue extract yields were much higher than wax extract yields, ranging from 3.2 g (*Dieffenbachia*) to 6.5 g (*Carludovica*) dry weight per m² leaf. Extracts of epiphylls, which were collected from leaves, yielded approximately between 100 and 500 mg dry weight per square metre leaf surface.

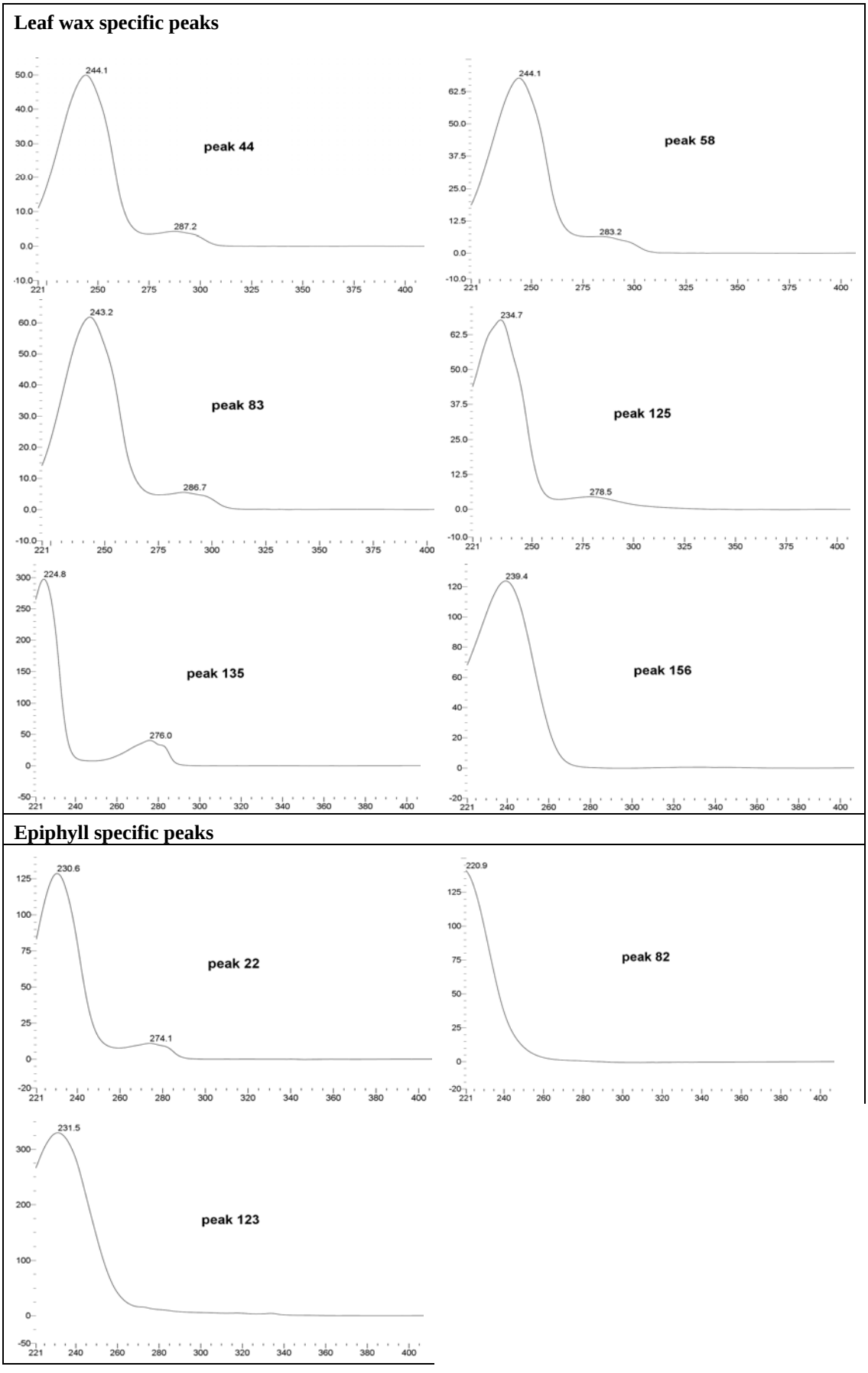
HPLC–UV analyses

In all leaf wax extracts, peak 135 (see fig. 5) was detected and in 38 of 41 wax samples this peak was the one with the greatest relative peak area in HPLC/UV- chromatograms. Besides peak 135, 19 analytes were detected, which frequently occurred in the wax samples, but were missing or occurred only as minor amounts in the leaf tissue or epiphyll extracts. All peaks classified as wax-specific showed either one absorbance maximum between 221 nm and 245 nm or two absorbance maxima, the first also between 221 and 245 nm and the second, always lower than the first, between 270 nm and 290 nm.

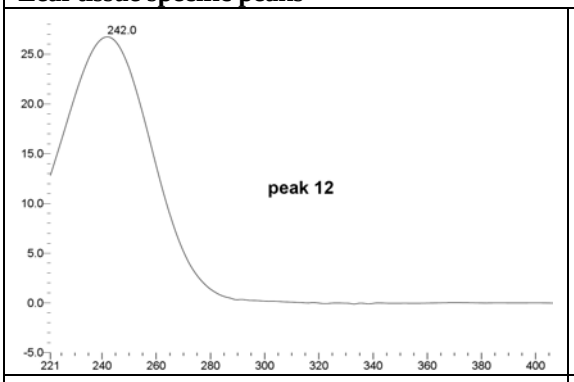
Characteristic epiphyll peaks included peak 22 and 123 (see fig. 5), which occurred at least as traces in all epiphyll extracts. Peak 82 was detected in all epiphyll extracts except that of *Costus* and the peaks 132, 134 and 158 were present in all epiphyll extracts except that of *Carludovica*. These epiphyll peaks were characterized by unspecific UV- spectra with absorbance maxima between 221 and 300 nm.

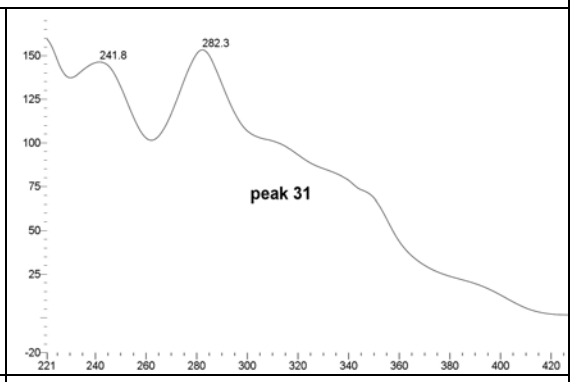
Leaf tissue extracts were even more heterogeneous than epiphyll extracts with no peak occurring in all of the extracts. *Carludovica* and *Dieffenbachia* shared the peaks 12, 154 and 155, a chlorophyll (see fig. 5). Leaf tissue extracts of *Costus,* which also contained peak 12, and *Pentagonia* shared the peaks 31, 33 (see fig. 5), 39, 42 and 53, which all showed absorbance maxima at approximately 240 nm and at 280 nm. *Polybotrya´*s leaf tissue shared the peaks 31, 33 and 154 with other tissue extracts. Some of the leaf tissue specific peaks showed interesting UV- spectra and could be assigned to chemical classes as flavonoids, but more specific identification of leaf tissue compounds would be beyond the scope of this work.

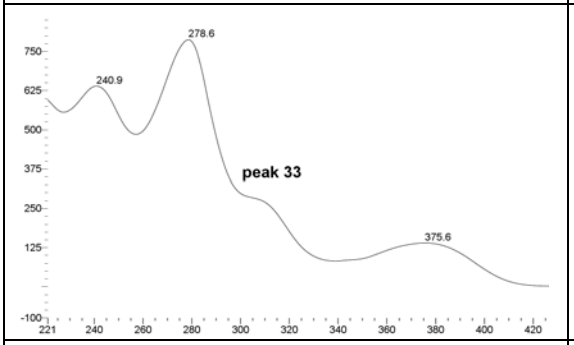
Figure 5: UV- spectra of extract specific peaks. The x- axis of spectrograms shows the wavelength of light in nanometre and the y- axis the respective relative absorbency intensity of a peak. Spectra are tagged with absorbance maxima.

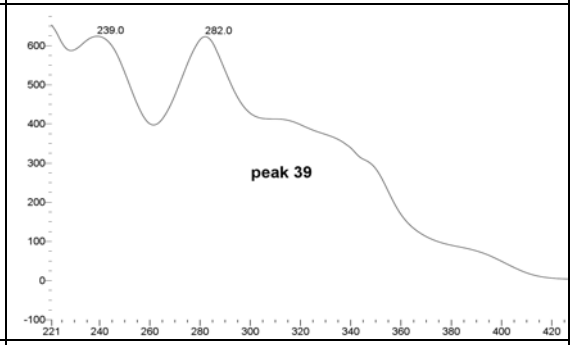


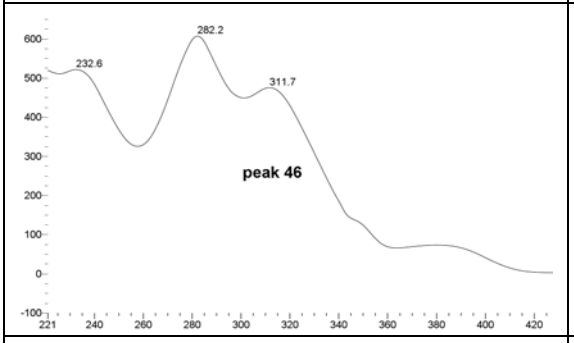
Leaf tissue specific peaks

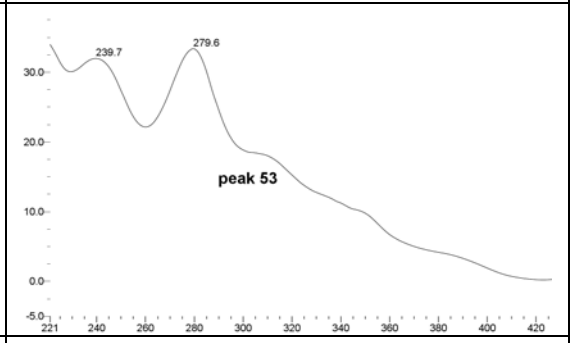


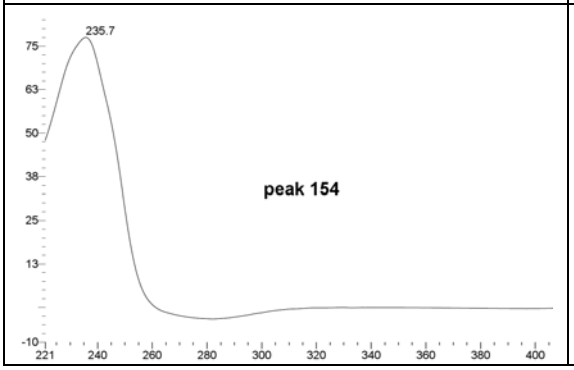


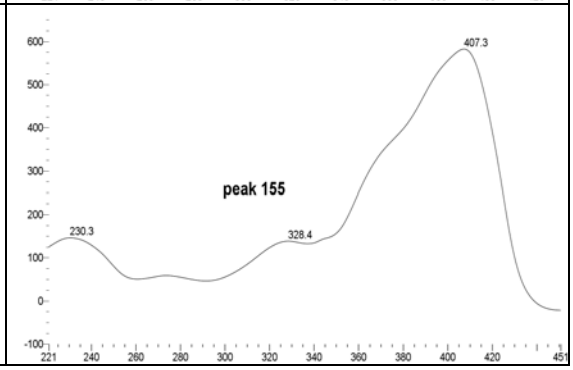












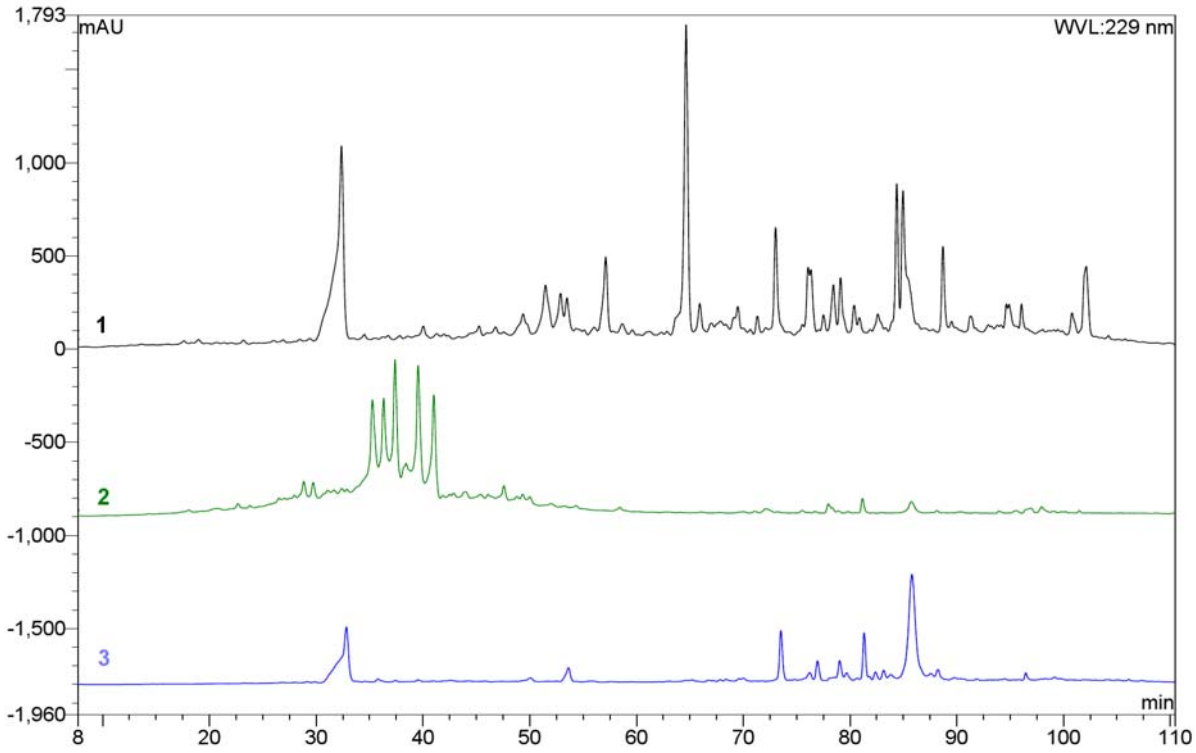


Figure 6: Comparison of HPLC/UV- chromatograms of *Pentagonia wendtlandii* ethanol extracts. 1: extracted epiphylls from *Pentagonia* leaves, 2: leaf tissue extract of *Pentagonia* leaves, 3: wax extract of *Pentagonia* leaves. X-axis: retention time in minutes, y- axis: signal wavelength: 229 nm, mAU.

Similarity percentage analysis (= SIMPER) of compounds showed that leaf tissue, epiphyll and leaf wax extracts represent distinguishable groups, differing from each other by specific peak elution patterns. Between wax and epiphyll extracts dissimilarity accounted for 90.9% with peak 135 contributing 37.8% (wax specific) and peak 22 (epiphyll specific) 14.6%. Between wax and leaf tissue extracts dissimilarity was 90 % with peak 135 contributing 34.6% and other peaks 5% or less. Between epiphyll and leaf tissue extracts dissimilarity was 94.1% with peak 22 contributing 14.4% and peak 82 7.8%. Within group resemblance however was low, making out 62% for leaf wax, 49.2% for epiphyll and merely 11.1% for leaf tissue extracts, indicating considerable differences in compound composition within the three extract groups.

A biplot of the PCA of ethanol extracts illustrates the relationship of samples and detected peaks to each other (see fig. 7). Results of the SIMPER analysis and of univariate statistical tests (Scheffe and Kruskal-Wallis) of single peaks were used to extract metavariables that are also included in the plot as clusters outlining groupings and vectors pointing to group specific peaks. Most of the leaf wax extracts clustered around the intersection of the PCA axes. Four outliers and samples of *Dieffenbachia*, however, were located apart from the intersection (see fig. 7). Epiphyll extracts formed a distinct group apart from leaf tissue and wax extracts. On

the other hand leaf tissue extract samples showed high distance between each other and were interpreted as two weakly related groups.

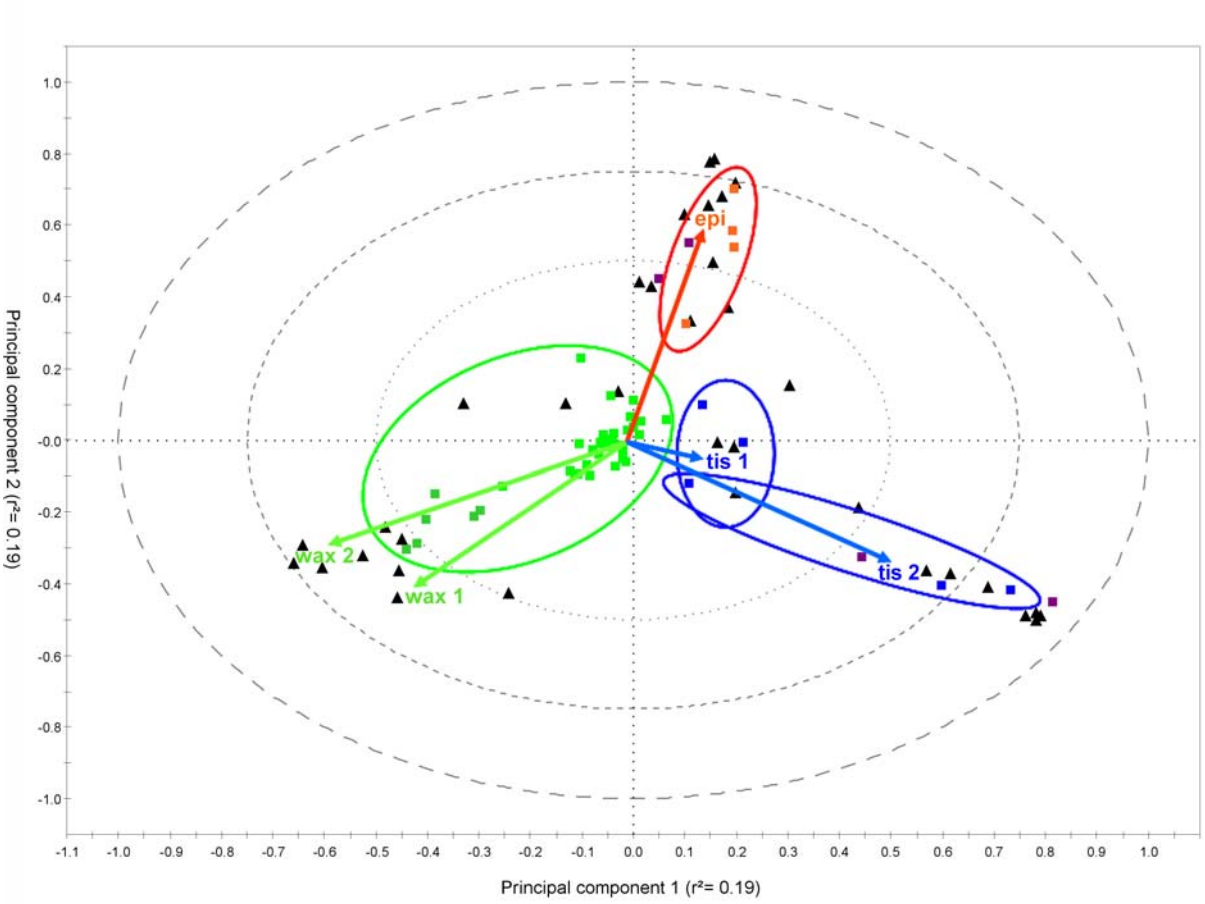


Figure 7: PCA- biplot of HPLC detected compounds (triangles) from ethanol extracted samples (square dots). Model was calculated from relative amounts (% of total peak area, ln+1 transformed) of peaks (n = 60) of 51 extracts; zero values were exchanged with a dummy value of 0.01.
Epiphyll extracts are coloured red, leaf tissue extracts blue, leaf wax extracts green and wax extract outliers violet. Dark green dots mark *Dieffenbachia* wax samples (6 of 10) positioned apart from the rest of wax extracts. The computed extract groups are surrounded by coloured ellipses and contributing peaks and peak metavariables (Kruskal-Wallis and Scheffe Tests, P< 0.05) are plotted as equally coloured vectors: Green vectors pointing to wax extracts are wax 1 (peak 135) and wax 2 (metavariable of peak 44, 58, 83 and 156), epiphyll extracts are tagged by the red vector epi (metavariable of peak 22, 82 and 123) and the two groups of leaf tissue extracts are assigned with the blue vectors tis 1 (metavariable of peak 12, 154 and 155) and tis 2 (= metavariable of peak 31, 33, 39, 42 and 53).

3.2.2 Leaf wax quality

Rarely, peaks were found that occurred exclusively in the leaf wax or in significantly higher amounts than in leaf tissue or epiphyll extracts. Eventually 20 peaks were considered as characteristic for leaf waxes. With multivariate methods, it was not possible to compute any function from the leaf wax components which showed the adequate capacity to divide the wax extract data set according to the associated factor groups plant species, leaf age, epiphyll quality, site or epiphyll coverage. The PCA of the compounds (see fig. 7) showed two main clusters within wax extracts. One comprised six of ten samples of *Dieffenbachia* and the

second cluster the rest of the leaf wax samples.
SIMPER analysis of leaf wax extracts (ln+1 transformed relative percentage of compounds) showed that plant species have low group similarities and very low intergroup dissimilarities. Similarity percentages among samples of one plant species were between 60% for *Dieffenbachia* and 74 % for *Polybotrya* with peak 135 contributing most to species similarity (42 to 60 %). Dissimilarity percentages between plant species were lower with 31.5 to 51 %.

All peaks detected in the ethanolic leaf wax extracts varied considerably in respect to their relative percentages in the HPLC chromatograms. Hence, most of the compounds were not normally distributed. As mentioned above, peak 135 represented the major component in most of the extracts (38 out of 41), but the substance revealed a high variability of occurrence in five of the six investigated plant species (see fig. 8). Only the extracted frond waxes of *Polybotrya* contained reproducible high contents of peak135.
Scheffe– tests showed that the leaf wax of *Dieffenbachia* and *Pentagonia* was distinct from the wax of the other plants (see fig. 9). Peak 44 and 156 were present in significantly higher amounts in the leaf wax of *Dieffenbachia*, peak 125 was detected at higher percentages in wax extracts of *Pentagonia* than in the other plants´ wax extracts. Peak 83 was found to build up waxes of *Dieffenbachia* at significantly higher percentages than waxes of *Carludovica*, *Pentagonia* and *Polybotrya* but not of *Costus*. Furthermore the leaf wax of *Pentagonia* totally lacked the peaks 44, 83 and 156.

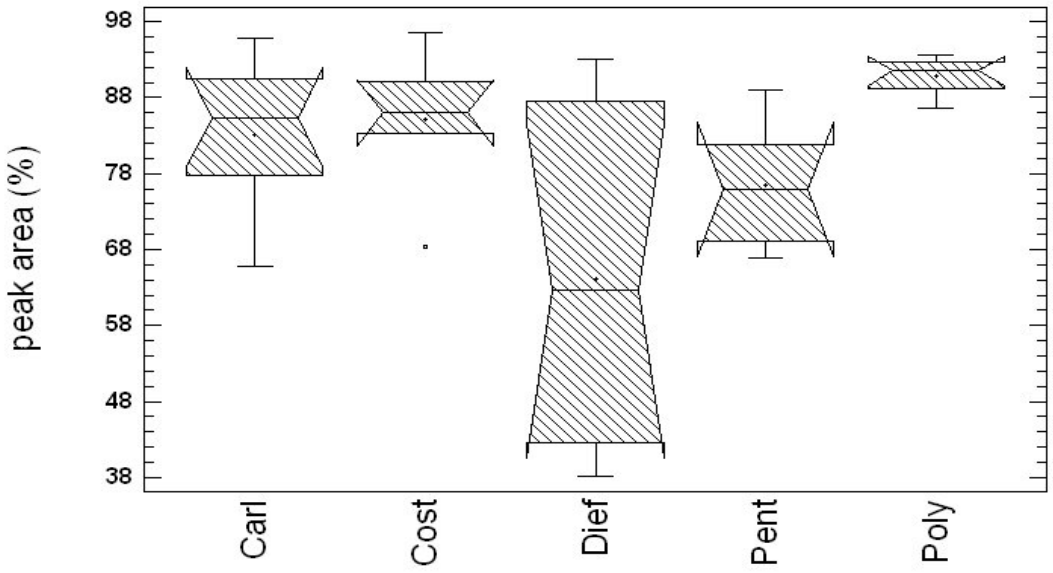


Figure 8: Box and Whisker- plots of the occurrence of peak 135 within the investigated plant species. The x-axis shows the plant species with abbreviated names, the y-axis marks the relative amounts (% total peak area) of peak 135. Boxes indicate 25 and 75 percentile; whiskers mark the 1.5-fold of the interquartile range. Medians are drawn as horizontal lines, means as cross dots within the boxes.

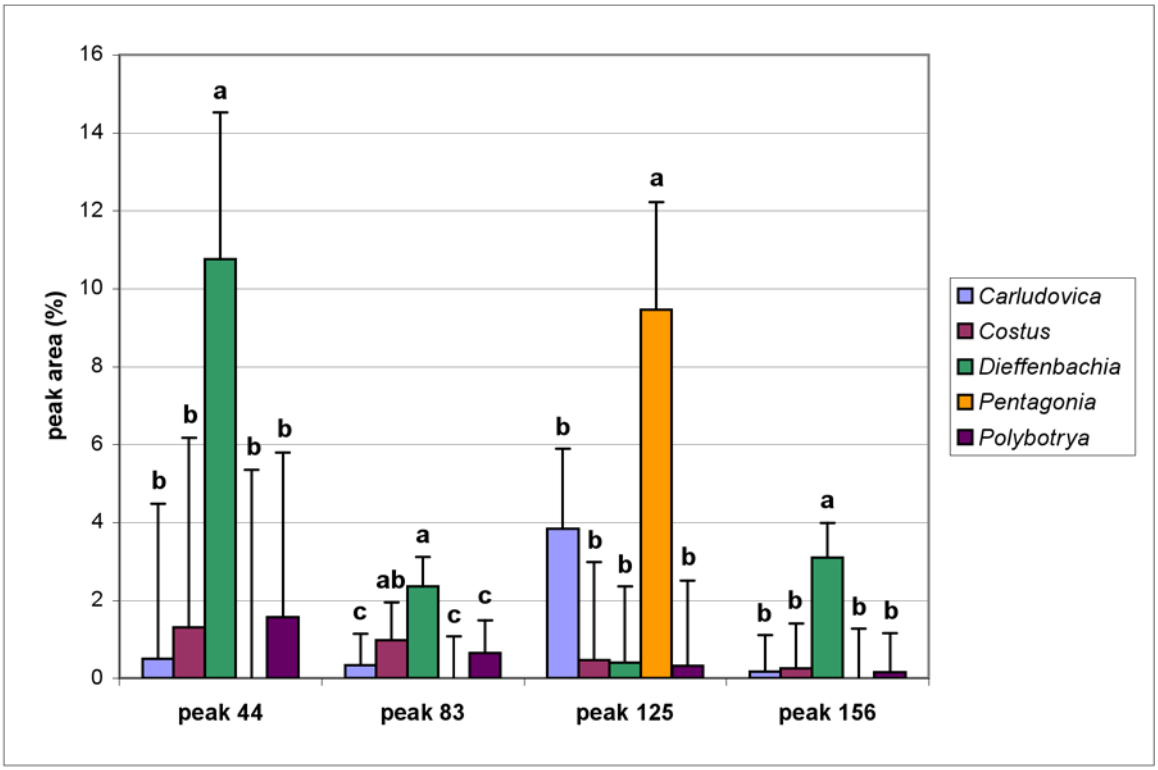


Figure 9: Plant species characteristic leaf wax components. The y- axis marks the relative amounts (% total peak area). Bars figure the mean relative content of a component per plant species related to 20 leaf wax compounds. Error bars indicate Scheffe confidence intervals at P> 0.9; different letters indicate significant differences between samples.

3.2.3 Leaf age effects

Statistically significant relations between leaf wax components and leaf age were evident in *Carludovica* and *Pentagonia*, but no compound reflected those of all investigated species. Peak 129 occurred in the wax extracts of developed *Carludovica* leaves at significantly higher percentages than in those of young and old leaves; peak 135 was present in higher relative amounts in the waxes of young and old leaves than in developed leaves. Whereas younger *Pentagonia* leaves (up to 1.5 years) showed peak 110, older leaves lacked it (see table 2).

Table 2: Relative amounts of leaf wax components (peaks) extracted from young (yg), developed (dl) and old leaves of *Carludovica* (*Carl*) and *Pentagonia* (*Pent*). Values in the table represent means (n = 3) of the relative peak area (% total peak area of HPLC chromatograms); different superior letters indicate significant differences between samples (Bonferroni´s multiple range test: P < 0.05).

sample	peak 129	peak 135	sample	peak 110
*Carl*_yg	0.54 ^{b}	90.93 ^{a}	*Pent* yg	—
Carl dl	6.12 ^{a}	70.87 ^{b}	*Pent* dl	5.33 ^{a}
*Carl*_old	1.48 ^{b}	87.75 ^{a}	*Pent* old	0 ^{b}

3.3. Hexane extracts

3.3.1. Epiphyll, leaf tissue and leaf wax extracts

Extract yields

On average10.5 mg wax per square meter leaf area were obtained by hexane extraction, though variation was high, especially between *Costus laevis* samples (4.6–70.8 mg/m²). The factors plant species, leaf age, site, and epiphyll quality did not affect wax layer load. Leaf wax amounts from *Dieffenbachia sp*. and *Theobroma cacao* (ca.107 mg and 121 mg/m²) that were cultivated in the greenhouse as reference were higher than the samples from the Esquinas forest. Yields of leaf tissue extracts per m² leaf area ranged from 35 mg for the thin leaves of *Asplundia pittieri* to 277 mg for the thick leaves of *Dieffenbachia concinna* cf. Yields of epiphyll extracts from the six host plants´ leaves differed even more from each other.

Chemical composition

The GC – MS detected components of extracts were classified into the following categories: alkanes, alkanols, alkanoic acids, sterols and others (see table 3). The category alkanes comprises long chained alkanes (n = 12) and two alkenes, the category alkanoic acids contains fatty acids and bicarboxylic alkanoic acids and alkanols comprise long chained alkane alcohols. Sterols are represented by four sterolic compounds and the category unidentified contains insecurely identified and unidentified hydrocarbons.

Table 3: Relative amounts of substance classes of leaf wax, leaf tissue and epiphyll extracts. Values in the table represent means and standard deviations of the relative peak area (% total peak area of GC–MS chromatograms). N = 38 (leaf wax), n = 6 (leaf tissue and epiphyll extracts).

extract group	alkanes	alkanoic acids	alkanols	sterols	unidentified
leaf wax	60.9 +/- 31	26.5 +/- 25.6	1.3 +/- 2.1	5.8 +/- 9	5.5 +/- 8.9
leaf tissue	7.7 +/- 4.3	86.5 +/- 5.8	0.5 +/- 0.4	2.9 +/- 2.5	2.4 +/- 2
epiphyll	8.1 +/- 5.7	46.2 +/- 29.3	1.1 +/- 1.3	0.6 +/- 0.4	44.0 +/- 33.7

Significant differences between the extract groups leaf wax (n = 38), leaf tissue (n = 6) and epiphyll (n = 6) were destined for alkanes, alkanoic acids and unidentified compounds (see fig. 10).

The average relative content of alkanes in leaf wax samples accounted for 60.9% and was significantly higher than in epiphyll extracts with 8.1 % and in leaf tissue extracts with 7.7%. Leaf tissue extracts contained very high relative amounts of alkanoic acids, mainly fatty acids,

with 86.5 % relative contribution on average. The relative share of alkanoic acids in epiphyll and leaf wax extracts was significantly lower with 46.2% and 26.5% respectively.
Extracts of epiphylls revealed the highest relative amount of unidentified and insecurely identified components (see table 3) of the extract groups. Mass spectra of some peaks of this category indicated the presence of phenols and terpenes in epiphyll extracts, but more exact identification was beyond the scope of this work
Alkanols and sterols were present in minor quantities particularly in leaf wax extracts, with 1.3 % and 5.8 % respectively. However, the relative amounts of these compounds varied strongly between samples and thus did not contribute a statistically valuable difference between the three extract groups (see table 3).

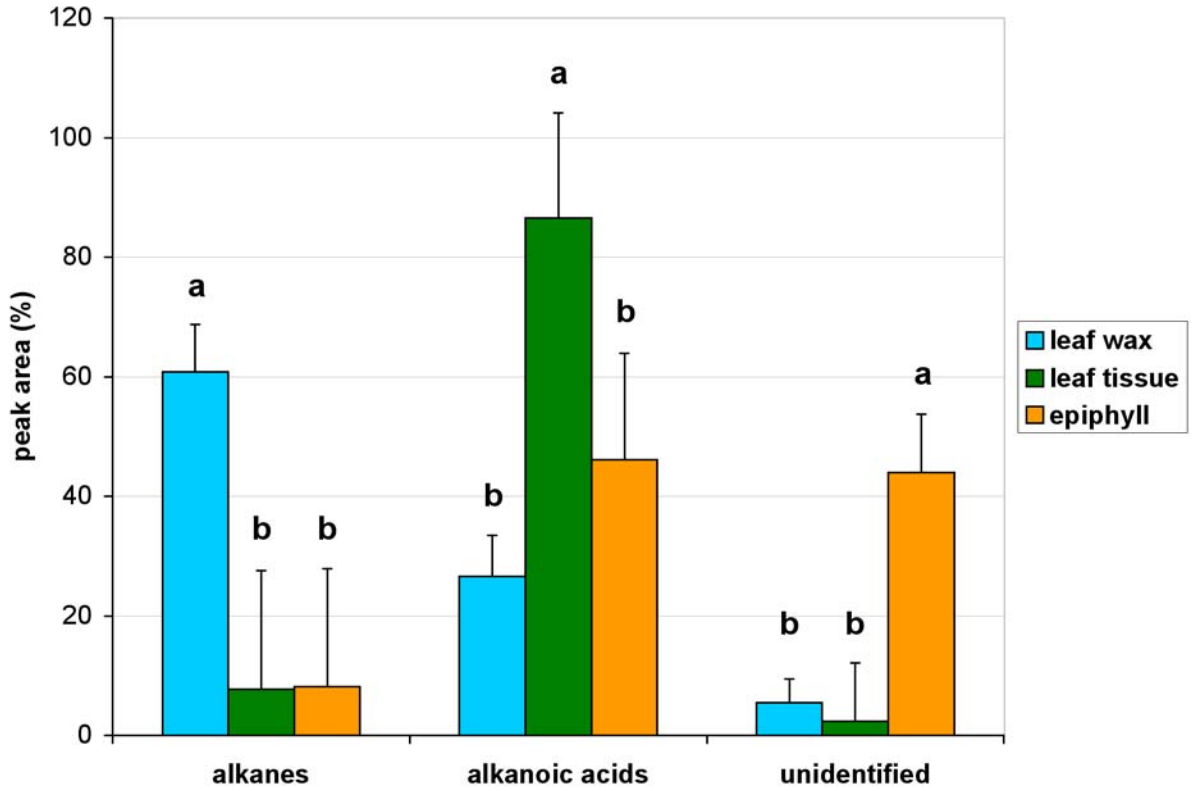


Figure 10: Relative composition of extract groups (x- axis) by the dominant chemical categories. Bars show mean relative percentages of chemical classes per extract group, error bars figure Bonferroni confidence intervals (P > 0.95) and different letters indicate significant differences between extract groups (Bonferroni multiple range test; P < 0.05). Chemical categories: alkanes (= alkanes and alkenes), alkanoic acids (= fatty acids and bicarboxylic alkanoic acids) and unidentified (= unidentified and insecurely identified compounds). Extract groups: leaf wax extracts (N = 38), epiphyll extracts (N = 6) and leaf tissue extracts (N = 6).

3.3.2 Leaf wax variability between plant species

Rough chemical composition of leaf waxes

A multivariate analysis about similarities and dissimilarities between the leaf waxes of the investigated samples (figure 11) revealed that *Pentagonia, Costus and Dieffenbachia* were

similar in the composition of their extracts; *Pentagonia* samples, however, formed a more distinct group than samples of the other two. This was caused by the predominant alkane, C29 in the *Pentagonia* leaf wax and C31 in the extracts the two others. *Polybotrya* samples notably differed from extracts of *Pentagonia, Costus and Dieffenbachia* by the presence of even and odd alkanes in similar amounts and by equal amounts of the C29 and C31 alkane. Even alkanes then dominated in the leaf wax of the Cyclanthaceae *Asplundia* and *Carludovica* (see figure 12).

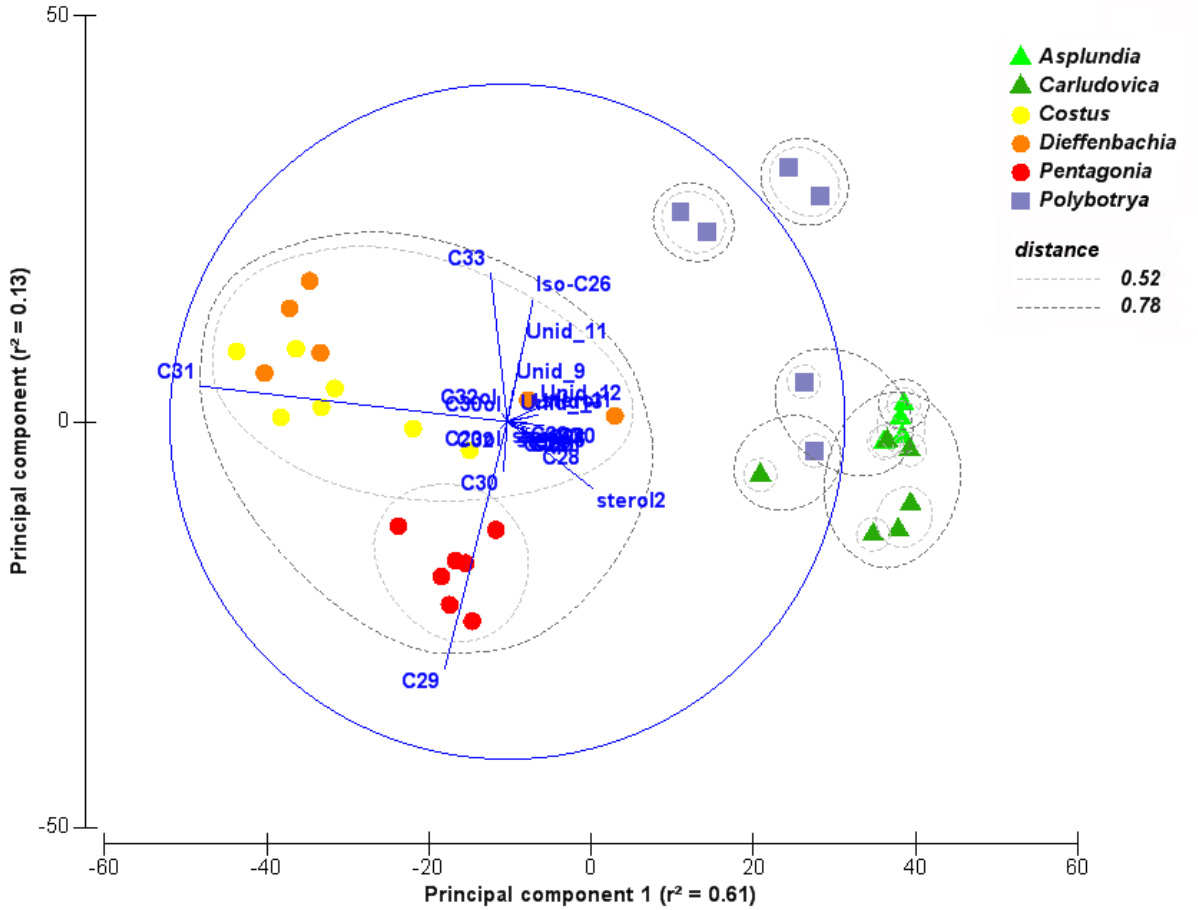


Figure 11: PCA- plot of leaf wax samples (n = 36) overlaid with the distance trajectories (grey dotted lines) of the cluster analysis (group average). The PCA plot and the chord distance resemblance matrix for the cluster analysis were calculated from the relative percentages (% of total peak area) of leaf wax compounds (n = 26).

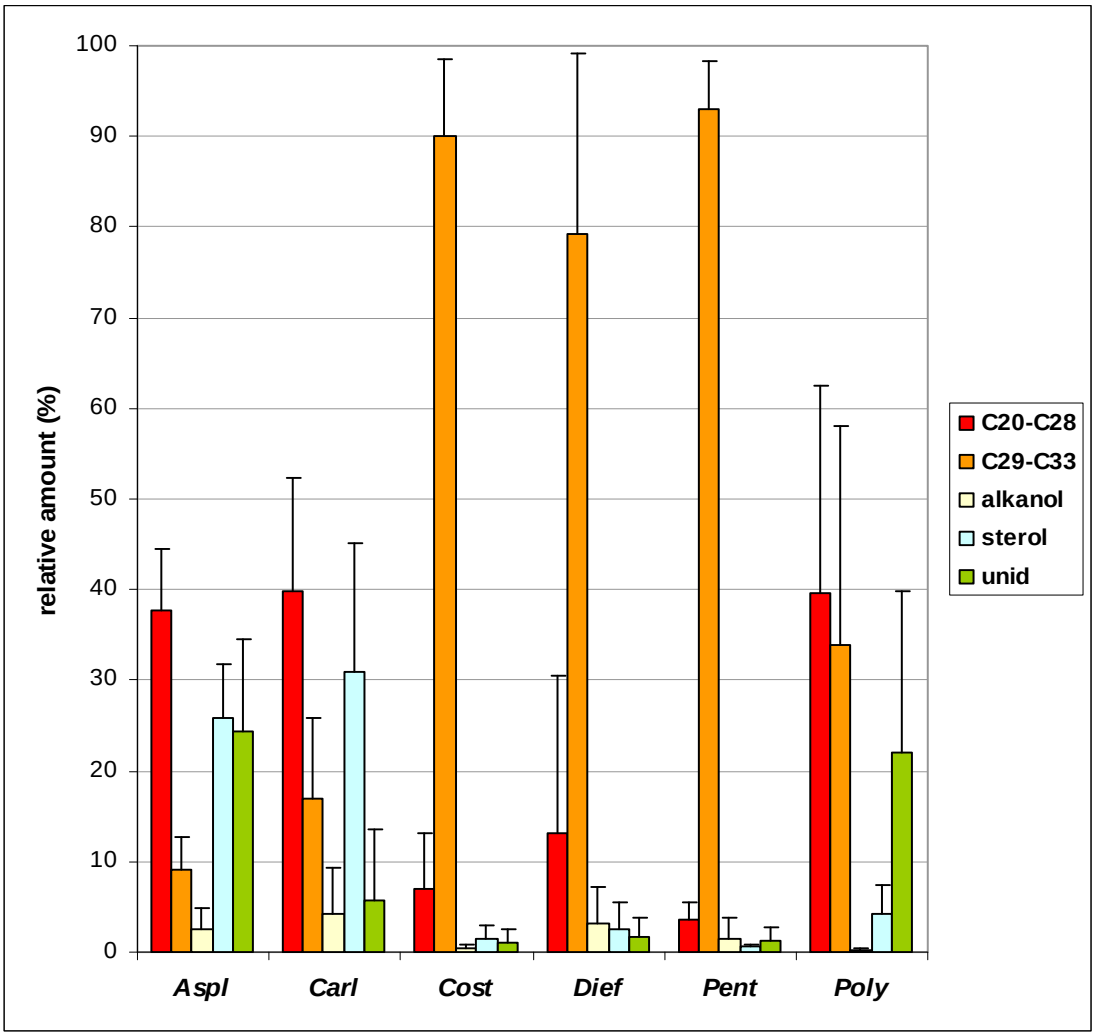


Figure 12: Relative leaf wax composition per plant species. Bars show mean relative percentages of the selected component categories per plant species, error bars indicate standard deviations. Component categories chosen were C20–C28 – alkanes (n = 8), C29–C33 – alkanes (n = 5), alkanols (n = 4), sterols (n = 2) and unidentified compounds (n = 5). Plant species: *Asplundia* (n = 4), *Carludovica* (n = 6), *Costus* (n = 7), *Dieffenbachia* (n = 6), *Pentagonia* (n = 7), *Polybotrya* (n = 6).

Leaf wax compounds in detail

Besides the dominance of odd chained alkanes in the leaf wax of *Costus*, *Dieffenbachia* and *Pentagonia* and, conversely, a shift to even chained alkanes in the wax of *Asplundia* and *Carludovica* (see table 4), also the lacking or high occurrence of some single alkanes turned out being specifically for the investigated plant species.

The leaf surface of *Pentagonia* showed higher shares of C29, C30 and C32 than the other plants. C33 and C32, the longest chained alkanes of all, were not detected in samples of the Cyclanthaceae and C31, which accounted for more than 60% of *Costus*` leaf wax, lacked in extracts of *Asplundia*. Four different alkanols were identified in the studied leaf waxes, all of them however, were occurring in very variable percentages within the sample set and also among samples of each plant species. Octadecanol for examples was found in 22 of the 36 measured leaf wax samples, and *Carludovica* was the only plant which samples all contained

the C18- alcanol, but in a range from 0.6 to 13.5%. Eicosanol was merely occurring in leaf wax of *Pentagonia*, but only in four of the seven samples. Triacontanol (C30) and Dotriacontanol (C32) were found solely on leaves of *Dieffenbachia*; the shorter chained of the two alkanols was determined in two, the longer chained in four of the seven samples (see table 4).

Table 4: Relative contribution of leaf wax compounds and compound categories per plant species. Values represent mean percentages and standard deviations. Thick lettered values mark plant specifically high percentages or lacking of a component or a group of compounds (Bonferroni´s multiple range test: $P < 0.05$).

compound category	***Asplundia***	***Carludovica***	***Costus***	***Dieffenb.***	***Pentagonia***	***Polybotrya***
odd chained alkanes						
C25	3.8 +/- 1.7	3.4 +/- 2.1	0.8 +/- 1.1	1.3 +/- 2.5	0.6 +/- 0.5	3.7 +/- 5.3
C27	4.5 +/- 2.5	5.4 +/- 2.7	1.3 +/- 1.9	1.7 +/- 2.8	0.5 +/- 0.0	3.7 +/- 5.6
C29	5.9 +/- 2.5	11.9 +/- 5.2	16.9 +/- 3.9	10.9 +/- 4.7	**39.2 +/- 5.4**	4.7 +/- 3.9
C31	**0**	2.7 +/- 5.6	**63.4 +/- 10.4**	**58.2 +/- 17.9**	**42.6 +/- 4.3**	10.9 +/- 5.6
C33	**0**	**0**	6.0 +/- 1.8	7.1 +/- 6.6	1.6 +/- 0.5	17.1 +/- 19.3
sum	14.2 +/- 5.5	23.5 +/- 10.5	**88.5** +/- 7.1	**79.2** +/- 17.0	**84.6** +/- 4.6	40.1 +/- 22.4
even chained alkanes						
C22	**5.7 +/- 1.4**	**5.0 +/- 3.5**	0.6 +/- 0.6	0.6 +/- 1.2	0.4 +/- 0.2	1.5 +/- 2.1
C24	9.3 +/- 3.7	8.5 +/- 3.0	1.5 +/- 1.2	2.8 +/- 3.8	0.7 +/- 0.7	6.0 +/- 4.7
C26	3.9 +/- 1.4	5.3 +/- 2.8	1.3 +/- 1.5	2.3 +/- 3.5	0.4 +/- 0.2	5.4 +/- 7.1
C28	4.7 +/- 2.8	9.7 +/- 8.0	1.2 +/- 1.6	2.1 +/- 2.5	0.8 +/- 0.5	5.1 +/- 6.6
C30	3.1 +/- 1,8	2.3 +/- 1.9	2.3 +/- 1.1	2.2 +/- 1.8	**7.2 +/- 1.7**	0.9 +/- 1.4
C32	**0 +/- 0**	**0 +/- 0**	1.3 +/- 0.5	0.9 +/- 0.7	**2.3 +/- 0.8**	0.3 +/- 0.4
sum	26.8 +/- 3.9	30.8 +/- 12.9	8.2 +/- 5.0	10.9 +/- 11.6	11.6 +/- 2.2	19.1 +/- 18.3
alkanols						
Octadecanol	2.6 +/- 2.2	4.3 +/- 4.9	0.4 +/- 0.5	0.3 +/- 0.5	0.5 +/- 0.4	0.1 +/- 0.3
Eicosanol	0	0	0	0	1.1 +/- 1.8	0
Triacontanol	0	0	0	0.5 +/- 0.9	0	0
Dotriacontanol	0	0	0	2.5 +/- 3.2	0	0
sum	2.6 +/- 2.2	4.3 +/- 4.9	0.4 +/- 0.5	3.3 +/- 3.9	1.6 +/- 2.1	0.1 +/- 0.3
sterolics						
Sterolic 1	8.4 +/- 3.3	6.4 +/- 5.3	0.9 +/- 0.5	2.1 +/- 2.5	0.6 +/- 0.3	4.1 +/- 3
Sterolic 2	**17.4 +/- 2.9**	**24.5 +/- 11.1**	0.5 +/- 1.3	0.4 +/- 0.6	0	0.1 +/- 0.3
sum	**25.8 +/- 6**	**30.8 +/- 14.2**	1.4 +/- 1.6	2.5 +/- 3.1	0.6 +/- 0.3	4.2 +/- 3.2
not identified						
NI 1	2.6 +/- 2.1	2.0 +/- 3.9	0	0	0.3 +/- 0.3	0.8 +/- 1.1
NI 3	8.7 +/- 7.1	0	0	0	0	5.3 +/- 12.9
NI 9	0	0	0.1 +/- 0.2	0.3 +/- 0.4	0	3.8 +/- 5.8
NI 11	1.3 +/- 2.6	0	0.1 +/- 0.2	1.1 +/- 1.9	0.1 +/- 0.2	9.5 +/- 12.3
NI 12	11.8 +/- 13.6	3.7 +/- 7.1	1.0 +/- 1.5	0.3 +/- 0.3	0.9 +/- 1.2	3.6 +/- 4.5
sum	24.4 +/- 10.2	5.7 +/- 7.9	1.1 +/- 1.5	1.7 +/- 2.1	1.2 +/- 1.5	22.1 +/- 17.7

Two different sterol–like compounds were identified from mass spectra in the leaf waxes. The samples of *Asplundia* and of *Carludovica*, contained significantly higher percentages of sterolic components than the leaf waxes of the other plants (see table 4). The earliest eluting, Sterolic 1, was found in each of the samples. The leaf wax of *Pentagonia* and of *Costus* showed merely traces of Sterolic 1, whereas in the wax extracts of the other plants it ranged between 2.1% (*Dieffenbachia*) and 8.4 % (*Asplundia*) by average.
Sterolic 2 was detected in all the Cyclanthaceae- samples, but was found only in traces in four single samples of the other plants` leaf waxes.
Another two sterol-like compounds were identified, but only appeared in two, respectively in three single samples and thus were not included in analysis.
Most of the unidentified components were found in leaf tissue and epiphyll samples, but five of them seemed to be typical for the leaf waxes of the surveyed plants. Each of those five compounds showed very high variance of occurrence; only Unid 12 occurred in a valuable number of samples, respectively in 29 of 36 (see table 4).
The unidentified components do probably derive from different chemical classes nonetheless we want to mention that the leaf waxes of *Asplundia* and *Polybotrya* contain a high relative contribution of these compounds compared to the waxes of the other plants.

3.3.3. Changes of leaf wax composition related to leaf age

Four of the six studied plants showed changes in wax composition related to leaf age, *Costus* and *Pentagonia* did not. The wax components from developed leaves were compared with old leaves by PCA and Cluster analysis (see fig. 13) of the compound percentages of the sample set.
Leaf waxes of *Costus, Dieffenbachia* and *Pentagonia* clustered together both in the analysis of developed and old leaves, with *Pentagonia* samples forming a distinct group within this Cluster. Waxes from old *Costus* foliage were highly similar to each other, but those of developed *Costus* leaves form one cluster with *Dieffenbachia* samples. Cyclanthaceae waxes from developed and old leaves always clustered separately from other samples (see fig. 13).

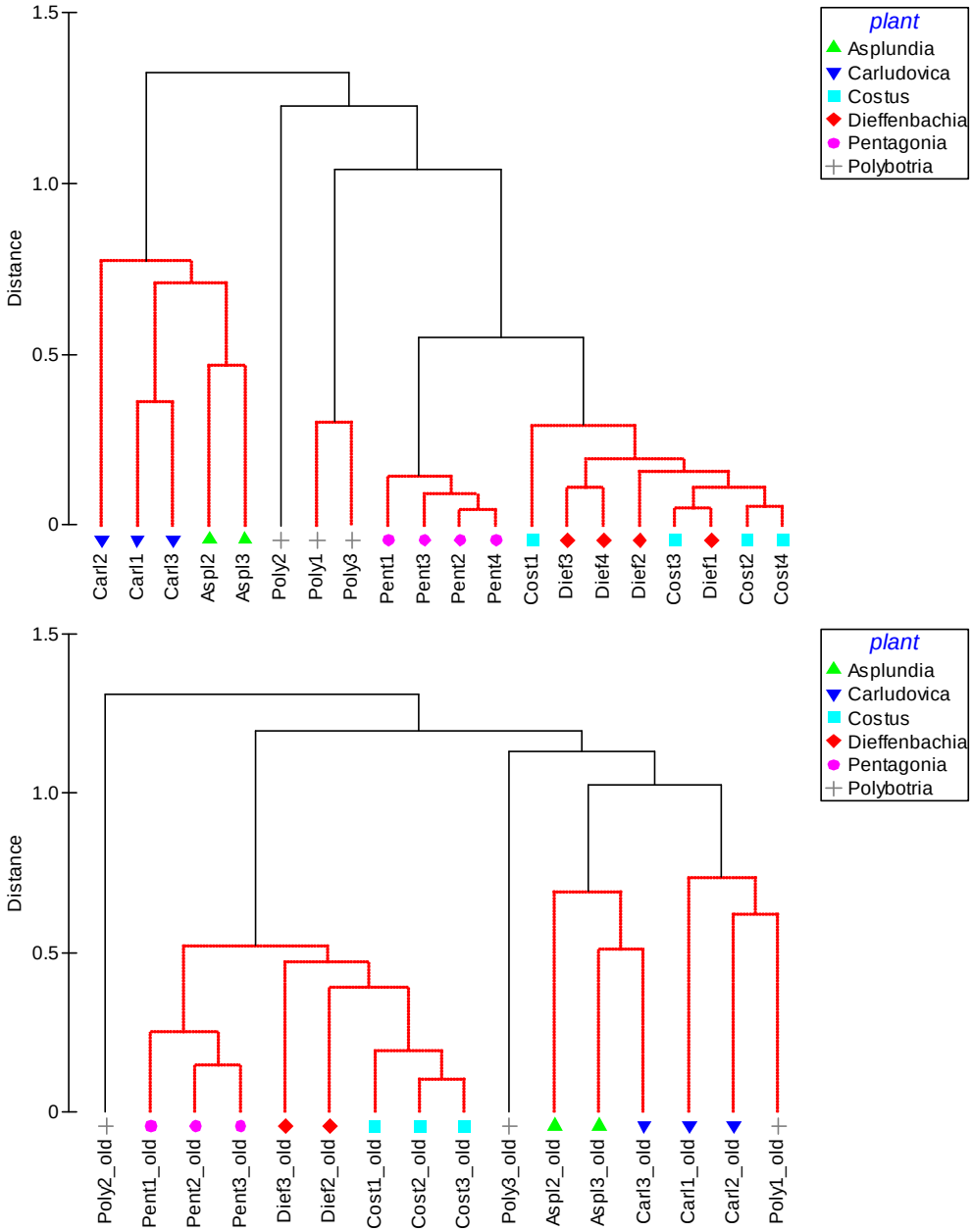


Figure 13: Comparison of leaf waxes from developed (13a, N=20) and old leaves (13b, N=16). Cluster analysis bases on a chord distance resemblance matrix of the relative percentages of 26 compounds.

Table 5: Kruskal Wallis tests of leaf wax analytes (% total leaf area) present in developed and old leaves. All results with a $P < 0.1$ are shown.

plant species	compound	compound class	full- grown leaves	old leaves	P- value
Carludovica	Iso- alkene 1	alkene	4.82	0	0.04
	Untriacontane	alkane	5.37	0	0.04
Dieffenbachia	C20 - C28	Short chained alkanes	2.58	34.49	0.06
	C29 - C33	Long chained alkanes	91.56	54.9	0.06
	Tetracosane	alkane	0.68	7.04	0.06
	Hexacosane	alkane	0.38	6.21	0.06
	Iso- Hexacosane	iso- alkane	0.12	7.01	0.05
	Heptacosane	alkane	0.38	4.21	0.06
	NI 11	unidentified	0.12	2.99	0.05
	Octacosane	alkane	0.52	5.25	0.06
	NI 12	unidentified	0.52	0	0.06
	Sterolic 1	sterol	0.52	5.29	0.06
	Triacontane	alkane	1.05	4.5	0.06
	Sterol 2	sterol	0	1.17	0.03
	Untriacontane	alkane	69.54	35.55	0.06
	Dotriacontane	alkane	1.38	0	0.06
	Tritriacontane	alkane	10.32	0.62	0.06
	Dotriacontanol	alkanol	3.74	0	0.06
Polybotrya	C29 - C33	Long chained alkanes	50.68	17.23	0.05
	NI 1	unidentified	1.63	0	0.04
	Iso- alkene 1	Alkene	1.21	0	0.04
	Docosane	alkane	2.9	0	0.04
	Octacosane	alkane	0.66	9.56	0.05
	Untriacontane	alkane	15.03	6.81	0.05
	Dotriacontane	alkane	0.66	0	0.04
	Tritriacontane	alkane	31.72	2.53	0.05

Dieffenbachia and *Polybotrya* differed between developed and old leaves (see table 5). Both species share a lower percentage of long chained alkanes, in particular of C31 and C33, in old leaves. Developed *Carludovica* leaves contained Iso-alkene1 and Untriacontane; both compounds were not detectable in old leaves. *Pentagonia*, *Costus* and *Asplundia* did not show differences between developed and old leaves in Kruskal Wallis tests.

Table 6: Regression analysis of the calculated leaf age of *Asplundia*- and *Dieffenbachia*- samples versus the respective wax compound percentages. Compound percentages are relative values calculated from 26 leaf wax components. Leaf age of samples in years was calculated using the formula: number of the sampled leaf divided by the total number of the plant´s leaves multiplied with the mean leaf longevity of the respective plant species. A negative correlation coefficient indicates a decreasing component percentage versus an increasing age of the plant leaf.

leaf age [yrs.]	compound percentage	compound class	R	r^2 adjusted for d.f.	P- value
Asplundia	C20 - C28	alkanes	-0.96	0.88	0.04
	NI 3	unidentified	-0.87	0.64	0.13
Dieffenbachia	C20 - C28	alkanes	0.93	0.83	0.01
	C29 - C33	alkanes	-0.87	0.7	0.02
	Tetracosane	alkane	0.88	0.72	0.02
	Hexacosane	alkane	0.87	0.71	0.02
	Octacosane	alkane	0.96	0.9	0.002
	NI 12	unidentified	0.92	0.81	0.01
	Sterol 1	sterol	0.96	0.9	0.002
	Triacontane	alkane	0.89	0.75	0.02
	Sterol 2	sterol	0.96	0.89	0.003
	Untriacontane	alkane	-0.92	0.8	0.01
	Dotriacontane	alkane	-0.9	0.77	0.01

A regression analysis of analyte percentages versus calculated absolute leafage, delivered two correlations for the samples of *Asplundia* (see table 6). The relative share of NI 3 and of short chained alkanes (C20 – C28) decreased with increasing age of *Asplundia´*s leaves. A number of correlations were found in *Dieffenbachia* waxes. Some of them reflected the results of the Kruskal-Wallis tests of compound percentages.

4. Discussion

Bryophytic epiphylls grow better in wetter habitats with no pronounced dry season and lichens coverage and diversity was shown to be higher on generally drier sites (Coley, Kursar and Machado 1993). The site preference and tolerance might be a result of the adaption of the photosynthetic apparatus to work better at lower tissue water contents for foliicolous lichens and at very high water potential for epiphyllous bryophytes (Coley, Kursar 1996). These authors also observed that on leaves at sites which match the ecological demands of both bryophytes and lichens, lichens were always overgrown by bryophytes and never vice versa, but nonetheless lichens will cover comparable leaf areas as bryophytes due to their better colonization abilities.

Studying the composition of ephyllic communities on the collected leaves showed that lichens dominated the leaves from slope sites more often than bryophytes and, vice versa, bryophytic epiphylls generally dominated the phyllospheres from ravine sites. Bryophytes contributed 50% or more to the epiphylled leaf area of plants collected at ravine sites, therefore only 2 of the 45 samples from the ravine were dominated by lichens. Conversely, the epiphyll covered area on sample leaves from slope sites was usually made up by at least 50% lichens. Only 5 of the 39 leaf samples collected at slopes had bryophyte dominated epiphyll communities.

A study on bryophytic epiphyll community composition on understorey plant leaves (Sonnleitner *et al.* 2009), also conducted between February and April 2005 in the Esquinas rain forest showed that bryophyte leaf coverage was significantly higher in the ravine sites than in the slope sites. Microclimatic measurements demonstrated that pronounced drops of relative air humidity occurred during daytime at slope sites, but not at ravine sites; the latter were also characterized by slightly higher average air humidity than the slope sites. The authors of the study concluded that relative air humidity was the principal factor for influencing liverwort growth. Although no microclimatic measurements are available from the exact sampling points of this study, the observed site preference of lichens and bryophytes seems to reflect the generally wet conditions in the Esquinas rain forest and also microclimatic differences among the two chosen sampling sites, most probably differences in relative air humidity.

To test if there were differences in the rates of epiphyll phyllosphere colonization between the sampling sites, the three chosen epiphyll community structures and the studied plant species, regression analysis turned out to be most useful. Comparison of parameters between different regression models showed that exponential functions were most adequate to describe the relation of epiphyll coverage and the calculated age of host leaves. Also, literature data suggest a non linear growth of epiphyllic lichens and liverworts on plant phyllospheres, since most published data refer to a slow initial growth, which is followed by a period of accelerated growth. The establishment of epiphyllic bryophytes and lichens on the phyllosphere might depend on the primary colonization by bacteria, fungi and algae which provide nutrients to the developing epiphylls (Ruinen 1961). Further, adhesion and germination of bryophyte prothallia and lichen propagules is influenced by seasonality of rainfall and air humidity (Winkler 1967) and nutrition by leaching processes (Olarinmoye 1982). After establishment epiphyllic liverworts grow faster (Winkler 1967).

In this study, the colonization rates of macroscopically visible epiphylls on sampled leaves did not differ between the two sites, ravine and slope, and also not between the three chosen epiphyll community compositions (lichen dominated, bryophyte dominated and 1 to 1 colonized). Although leaves of slope sited plants were preferred by lichens and those of ravine sited plants by bryophytes, the studied epiphyll communities on understorey plant leaves seem to possess similar growing rates regardless of the location of the community.

Epiphyll coverage rates differed between the studied host plant species (see fig. 4, table 1). Epiphylls were colonizing *Asplundia* leaf areas slower than the surfaces of all other investigated plant leaves. Also the leaves of the second surveyed Cyclanthaceae palm, *Carludovica drudei*, with clearly bigger and taller leaves than *Asplundia*, were colonized by epiphylls at considerably lower rates than the phyllospheres of *Costus*, *Pentagonia* and *Polybotrya*. By contrast, the flower plant species, and among them, *Costus* with its very short-lived leaves in particular, seemed to facilitate the growth of epiphylls on their leaves. *Dieffenbachia* leaves were colonized by epiphylls at rates between those of the Cyclanthaceae and the fast epiphylled species.
As *Asplundia* leaves are characterized by the highest and *Costus* leaves by the lowest longevity, a relation between leaf longevity and epiphyll coverage rates is possible. However, the expected longevity of the other four plants does not clearly reflect the determined epiphyll colonization rates (compare fig. 2 and 4).

Extraction of leaf surfaces with ethanol yielded the more polar fraction of the leaf wax components. The analyses were aimed at exploring leaf wax extracts separately from epiphyll and leaf tissue derived components. Cleaning of epiphyll host plant leaves, however, was a balance between not damaging leaves and getting off most of the epiphylls. Hence a number of compounds detected in leaf wax extracts originated from the leaf tissue or from epiphylls. Furthermore, the comparison between leaf wax, epiphyll derived and leaf tissue derived metabolites turned out to be difficult, since most detected peaks occurred only in a subset of the samples and showed variable distribution patterns.
However, by excluding compounds of uncertain origin from the data set and by application and combination of different statistical methods a reasonable interpretation of the data set was possible. The leaf waxes, epiphylls and leaf tissue ethanol extracts could be distinguished clearly from each other by their respective analyte composition (see Figure 6). In extracts of epiphylls and of leaf tissues a number of components showed UV- spectra with characteristic chromophores, enabling a tentative assignment to a chemical class. None of the compounds

present in the leaf waxes showed a characteristic chromophore. A chemical characterization of the leaf wax components was impossible, even though speculations about the character of some components are justified. Peak 135 merits special mentioning, as it was the major peak in 38 out of 41 analysed wax extracts. Ethanol-soluble fatty acid esters, triterpenes or alkanols are known as leaf wax elements and thus are possible candidates for peak 135.
Twenty leaf wax-characteristic analytes could be determined. Their UV spectra were similar and unspecific; differentiation was only possible by retention time. Multivariate analyses, however, showed that leaf wax components did not cluster according to the factors "plant species", "leaf age", "site" or "epiphyll quality" because compound amounts varied considerably. One possibility is that irregularities of the extraction method and leaf surface cleaning procedures caused a loss of ethanol soluble components, which is supported by the composition of the epiphyll extract from the *Polybotrya* fronds that contained almost only typical leaf wax compounds. The extracted epiphylls used for these samples were difficult-to-remove lichens; probably, also a considerable amount of leaf wax was cleaned off with the lichens and consequently extracted.
Regardless of the mentioned irregularities, the statistical analysis identified some species-characteristic leaf wax analytes.
The wax of *Dieffenbachia* differed by higher amounts of three analytes (peak 44, 83, and 156) from the other plants´ leaves. In *Pentagonia´*s wax, these substances were absent, but peak 125 was present in larger amounts than in the other plants` surface extracts (see fig. 9).
Leaf age-dependent peaks were detected in *Carludovica* and *Pentagonia* (see table 2). Peak 129 occurred in significantly higher amounts in wax samples of developed leaves of *Carludovica* than in the waxes of young and old leaves. Conversely, peak 135 was found in larger amounts in leaf waxes of old and young leaves than in those of developed leaves.
The leaf wax of *Pentagonia* plants younger than 1.5 years contained considerable portions of peak 110, which was lacking in old leaves.
Since no chemical characterization of the analytes was possible and no bioassay seemed practicable, speculations about possible effects on epiphyll development remain elusive.

On average, hexane extracted leaf waxes amounted to 10.5 mg/m² leaf area, which was a low value compared to literature (Jetter *et al.* 2006) and the mean dry weight of ethanol extracted leaf wax components, which made out 58 mg/m² extracted leaf area by average.
Leaf waxes exhibited unexpected high variability of wax yield per m² extracted leaf area, also between replicates of the same plant species. The variability of the extract dry weights can have several reasons. Growing conditions of a plant, especially the relative air humidity, can

influence the wax quantity on leaves (Baker, 1974). Contaminations also can not be excluded. Leaf waxes of *Theobroma cacao* (~ 121 mg/m²) and a *Dieffenbachia*- species (~ 107 mg/m²) from the greenhouse of the Vienna Ecology Centre yielded 10-times higher wax amounts than samples from the Esquinas rainforest. Plants which grow under conditions of high relative air humidity and low light levels commonly form less pronounced leaf wax layers than plants which grow under conditions of low air humidity and high light intensity (Koch *et al.* 2006). Average relative air humidity in the chamber from where the greenhouse plants were taken, was approximately 65% on average (Thomas Joch, personal information) and thus much lower than at both accession sites in the Esquinas forest (over 90% relative air humidity; Sonnleitner *et al.* 2009).

Three "types" of leaf waxes occurred in the samples:
(1) Type I waxes were characterized by a very high relative percentage of odd-chained alkanes. Odd chained alkanes clearly dominated over even chained alkanes at a relation of approximately 8 to 1. Within the alkane fraction the molecules with more than 28 carbon atoms accounted for 80 to 90%. Leaf cuticles of *Costus*, *Dieffenbachia* and *Pentagonia* belong to this category (see fig. 12).
(2) The two studied Cyclanthaceae palms, *Asplundia* and *Carludovica*, were assigned to the wax type II. Shorter even chained alkanes (C20 to C28) were more prominent than longer odd chained (C29 to C33). On average leaf waxes of *Asplundia* contained 27% of even and 14% of odd chained alkanes, *Carludovica* 31% even versus 24% of odd chained alkanes. Compared with samples of type I and III, the two Cyclanthaceae species lacked Dotriacontane and Tritriacontane. Further, the total percentage of alkanes in the Cyclanthaceae leaf cuticles was significantly lower (< 60%) than in those of *Costus*, *Dieffenbachia* and *Pentagonia,* but compared to the leaf surface of the latter the Cyclanthaceae cuticles were featured by remarkably higher percentages of sterolic compounds and alkanols (see fig. 12).
(3) In the extracts of the fern fronds alkanes with more than 28 carbon atoms occurred at an approximately equal percentage as shorter chained alkanes. With 75% on average the total relative share of alkanes in the hexane extracts of the frond surface was slightly lower than in the leaf wax samples of *Costus*, *Dieffenbachia* and *Pentagonia.* However, the relative content of alkanes with less than 29 carbon atoms was much higher in samples of *Polybotrya* and, with 40% on average, approximately equals the content in the leaf wax of the Cyclanthaceae. Furthermore, the fern´s frond surface extracts contained 20% of unidentifiable components, which is a similar value as for the leaf wax of *Asplundia*, but clearly more than in the extracts of the other study plants.

The predominance of either even or odd chained alkanoic compounds in plant cuticles results from the metabolic pathways, which follow the biosynthesis of the fatty acids (Kolattukudy *et al.* 1976). But it is noteworthy, that in the cuticles of most studied plants odd chained alkanes were dominating over even chained ones (Jetter *et al.* 2006). Untriacontane was the major component of the leaf cuticular extracts of *Costus* and *Dieffenbachia*. *Pentagonia* leaf wax was dominated by nonacosane and untriacontane. Cyclanthaceae palms leaf waxes and the fern *Polybotria* showed a relatively even distribution of alkanes of various chain lengths and lacked components of major occurrence (see table 4).
A number of studies report nonacosane or untriacontane as the major leaf wax constituent (Jetter *et al.* 2006) and, thus, they can not serve as a taxon specific character. The studied plants, which showed nonacosane and untriacontane as the major components in their leaf wax, are only distantly related: *Pentagonia wendtlandii*, member of the Rubiaceae family, belongs to the Dicotyledonae, *Costus laevis* (Costaceae) and *Dieffenbachia concinna* (Araceae) belong to the Monocotyledonae.
Asplundia pittieri and *Carludovica drudei* are both members of the plant family Cyclanthaceae. In this case, the similarity in the chemical wax composition reflects their phylogenetic proximity. Aside from similar alkane patterns, the leaf wax of both species contained high percentages of sterolic compounds. No studies on surface waxes of Cyclanthaceae species have been conducted yet.

It is not clear, if the chemical wax composition of the study plants rather was determined by the respective ontogenetic program or by environmental factors. However, no site related wax component patterns could be found for *Costus*, *Carludovica* and *Asplundia,* which were sampled at both plant accession sites, slope and ravine. The differences of environmental conditions between the two sites may have been too small to cause differences in leaf wax characteristics between plants of the same species (Sonnleitner *et al.* 2009).
So far, only one study on *Brassica oleracea* (Baker 1974) suggested that environmental factors significantly affect the quantity and quality of leaf waxes.
The general notion is that in plants, which grow under variable conditions within the range of their natural habitat, wax properties are controlled rather by genetic programmes than by environmental factors (Jetter *et al.* 2006). As already mentioned above, the amount of wax, which was extracted from the leaves of the studied plants, was low compared to literature data. Most probably, this reflects an adaptation to high air humidity and low light levels in the understorey of the Esquinas rainforest, since thin cuticles would minimize light reflectance and maximize photosynthesis rates and water loss through cuticle transpiration would be

neglectible at very high air humidity levels (Pfündel *et al.* 2006, Riederer & Schreiber 2001).

A study on the adaxial leaf wax composition of *Prunus laurocerasus* (Jetter *et al.* 2000) showed that changes occurred at different developmental stages of the foliage. The percentage of alkanes increased with leaf age and reached its maximum at full leaf development. Senescing leaves were not investigated in this study and, furthermore, *P. laurocerasus* is neither a plant of the humid tropics nor an epiphyll host plant.
A study on tropical rainforest plant leaves and epiphylls showed that not only site conditions but also the life spans of host plant leaves strongly influenced epiphyll growth (Coley, Kursar, Machado, 1993). The authors of the study explain this observation as a selectionary adaptation of plant species to epiphyll colonization and conclude that plants with long-lived leaves would prevent rapid epiphyll colonization by investing in inhibiting chemical or physical cuticle properties. A number of studies postulated such a defence mechanism (Coley & Kursar 1996, Wanek *et al.* 2004), but no study focussed on the relation of the leaf wax chemistry and cryptogam epiphyll growth.

In the present study, chemical differences between the wax of fully developed and senescent leaves could be observed for four of the six studied host plants (see table 5 and 6). *Dieffenbachia* showed the most notable leaf-age-related shifts in the chemical composition. Leaf wax extracts of *Costus* and *Pentagonia*, however, were characterized by a very high content of alkanes, particularly of untriacontane and nonacosane, irrespective of the age of sample leaves. The correlation between epiphyll coverage and leaf age revealed that these two species were colonized by epiphylls faster than the other investigated plant species. This suggests that surface properties of *Costus* and *Pentagonia* leaves somehow favor the epiphyll growth. Site characteristics and epiphyll community composition did not influence epiphyll growth on the studied leaves. Compared to developed leaves, the leaf wax of old *Dieffenbachia* and *Polybotrya* leaves were depleted in long chained alkanes (C29- C33). The percentage of alkanes with more than 28 carbons in the leaf wax decreased from approximately 92% to 35% on average for *Dieffenbachia* and from 51% to 17% on average for *Polybotrya* between fully developed and senescent leaves. Particularly the relative content of untriacontane and tritriacontane was strongly reduced in both plants. *Dieffenbachia* leaf waxes additionally showed a strong increase of shorter chained alkanes (C20- C28) with age and an increase of sterol 1 and sterol 2 (see table 5). Comparing the leaf wax of developed and old *Polybotrya* leaves, the content of shorter chained alkanes increased. Old *Carludovica* leaves lack untriacontane, but show a short chained iso-alkene. *Asplundia,* interestingly,

exhibited the opposite trend: short chained alkanes (C20-C28) decreased slightly with leaf age. *Asplundia´*s leaf waxes were characterised by the lowest relative amount of alkanes and among them by the lowest share of long-chained alkanes of all surveyed plant species regardless of the leaf age. Further, its leaves showed the highest longevity and the lowest rates of epiphyll colonization among the studied plants. Summing up, a trend was evident that high percentages of short chained alkanes (C20-C28) occur in the wax of long living leaves and favour low epiphyll colonization rates. Similarly, the more polar sterols, contribute to leaf age (see table 5 and 6). High percentages of unpolar long chained alkanes (C29-C33), by contrast, correlate with epiphyll colonization and low longevity (*Costus* and *Pentagonia*).

Further studies employing bioassays testing the effect of leaf wax mixtures on germination, survival rates and growth of bryophytes and lichens would provide more stringent evidence if leaf wax chemistry constitutes a decisive factor for epiphyll colonization. Furthermore, leaf chemistry, water repellence (Holder 2007) and epiphyll community development should be assessed simultaneously.

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6. Appendix

6.1. Biometric data of sample leaves

sample	plant	site	leaf age	epiphyll composition	epiphyll coverage (% leaf area)	% leaf area colonized per year	calculated leafage [yrs.]
Aspl1_dev_hexane	Asplundia	slope	developed	L	1.55	1.23	1.26
Aspl2_dev_hexane	Asplundia	ravine	developed	LM	2.775	3.11	0.89
Aspl3_dev_hexane	Asplundia	slope	developed	L	5.25	8.23	0.64
Aspl1_old_hexane	Asplundia	slope	old	L	20	5.27	3.79
Aspl2_old_hexane	Asplundia	ravine	old	M	15	3.79	3.95
Aspl3_old_hexane	Asplundia	slope	old	L	40	13.07	3.06
Carl1_young_ethanol	Carludovica	slope	young	LM	1	1.87	0.54
Carl2_young_ethanol	Carludovica	ravine	young	M	1	1.55	0.64
Carl3_young_ethanol	Carludovica	ravine	young	LM	0.1	0.09	1.07
Carl1_dev_ethanol	Carludovica	slope	developed	LM	5	4.66	1.07
Carl1_dev_hexane	Carludovica	ravine	developed	M	1	1.24	0.80
Carl2_dev_ethanol	Carludovica	ravine	developed	LM	5	3.89	1.29
Carl2_dev_hexane	Carludovica	slope	developed	L	3	2.33	1.29
Carl3_dev_ethanol	Carludovica	ravine	developed	L	5	2.33	2.14
Carl3_dev_hexane	Carludovica	slope	developed	L	1	1.24	0.80
Carl1_old_ethanol	Carludovica	slope	old	LM	80	29.85	2.68
Carl1_old_hexane	Carludovica	ravine	old	M	65	20.21	3.22
Carl2_old_ethanol	Carludovica	ravine	old	LM	50	19.43	2.57
Carl2_old_hexane	Carludovica	slope	old	LM	55	17.10	3.22
Carl3_old_ethanol	Carludovica	ravine	old	LM	40	12.44	3.22
Carl3_old_hexane	Carludovica	slope	old	LM	45	13.99	3.22
Cost1_young_ethanol	Costus	ravine	young	LM	0.18	1.49	0.12
Cost1_young_hexane	Costus	ravine	young	M	0.55	4.00	0.14
Cost2_young_ethanol	Costus	slope	young	LM	1	5.66	0.18
Cost2_young_hexane	Costus	slope	young	M	0.1	0.56	0.18
Cost3_young_ethanol	Costus	slope	young	LM	0.46	1.97	0.23
Cost1_dev_ethanol	Costus	ravine	developed	LM	13.25	18.49	0.72
Cost1_dev_hexane	Costus	ravine	developed	M	3	7.26	0.41
Cost2_dev_hexane	Costus	slope	developed	M	1	1.85	0.54
Cost3_dev_ethanol	Costus	ravine	developed	M	1	1.28	0.78
Cost3_dev_hexane	Costus	slope	developed	L	0.55	1.61	0.34
Cost1_old_hexane	Costus	ravine	old	LM	5	3.30	1.51
Cost2_old_ethanol	Costus	ravine	old	M	80	54.30	1.47
Cost2_old_hexane	Costus	slope	old	M	1	0.67	1.50
Cost3_old_ethanol	Costus	slope	old	LM	30	21.98	1.37
Cost3_old_hexane	Costus	slope	old	L	15	11.40	1.32
Dieff1_young_ethanol	Dieffenbachia	ravine	young	LM	1	3.97	0.25
Dieff4_young_ethanol	Dieffenbachia	slope	young	L	0.1	0.26	0.39
Dieff5_young_ethanol	Dieffenbachia	ravine	young	LM	0.1	0.40	0.25
Dieff6_young_ethanol	Dieffenbachia	ravine	young	M	0.1	0.23	0.44
Dieff1_dev_hexane	Dieffenbachia	ravine	developed	L	6.7	5.71	1.17
Dieff2_dev_ethanol	Dieffenbachia	ravine	developed	LM	1	0.57	1.76
Dieff2_dev_hexane	Dieffenbachia	ravine	developed	M	1	3.41	0.29
Dieff3_dev_hexane	Dieffenbachia	ravine	developed	M	3	3.41	0.88
Dieff4_dev_ethanol	Dieffenbachia	slope	developed	L	3	3.07	0.98
Dieff5_dev_ethanol	Dieffenbachia	ravine	developed	LM	1	1.14	0.88
Dieff6_dev_ethanol	Dieffenbachia	ravine	developed	M	1	1.30	0.77

Dieff1_old_hexane	Dieffenbachia	ravine	old	LM	55	18.73	2.94
Dieff2_old_hexane	Dieffenbachia	ravine	old	LM	80	32.70	2.45
Dieff3_old_ethanol	Dieffenbachia	ravine	old	M	70	26.49	2.64
Dieff3_old_hexane	Dieffenbachia	ravine	old	LM	25	8.87	2.82
Dieff4_old_ethanol	Dieffenbachia	slope	old	L	55	23.42	2.35
Dieff5_old_ethanol	Dieffenbachia	ravine	old	LM	10	4.42	2.26
Dieff6_old_ethanol	Dieffenbachia	ravine	old	M	10	4.13	2.42
Pent1_young_ethanol	Pentagonia	slope	young	LM	0.1	0.14	0.70
Pent2_young_ethanol	Pentagonia	ravine	young	M	1	1.35	0.74
Pent3_young_ethanol	Pentagonia	ravine	young	M	1	1.43	0.70
Pent1_dev_ethanol	Pentagonia	ravine	developed	M	1	1.43	0.70
Pent1_dev_hexane	Pentagonia	ravine	developed	LM	0.1	0.22	0.46
Pent2_dev_ethanol	Pentagonia	ravine	developed	M	5	2.69	1.86
Pent2_dev_hexane	Pentagonia	ravine	developed	M	1	1.43	0.70
Pent3_dev_ethanol	Pentagonia	ravine	developed	M	25	17.94	1.39
Pent3_dev_hexane	Pentagonia	ravine	developed	M	7.5	8.07	0.93
Pent1_old_hexane	Pentagonia	ravine	old	M	80	28.70	2.79
Pent2_old_ethanol	Pentagonia	ravine	old	M	80	28.70	2.79
Pent2_old_hexane	Pentagonia	ravine	old	M	80	38.26	2.09
Pent3_old_ethanol	Pentagonia	ravine	old	M	80	38.26	2.09
Pent3_old_hexane	Pentagonia	ravine	old	M	80	28.70	2.79
Poly1_young_ethanol	Polybotrya	slope	young	L	0.1	0.16	0.62
Poly1_young_hexane	Polybotrya	slope	young	L	0.1	0.21	0.47
Poly2_young_ethanol	Polybotrya	slope	young	L	0.1	0.19	0.53
Poly3_young_ethanol	Polybotrya	slope	young	L	0.1	0.16	0.62
Poly1_dev_ethanol	Polybotrya	slope	developed	L	0.55	0.59	0.93
Poly1_dev_hexane	Polybotrya	slope	developed	L	1	1.07	0.93
Poly2_dev_ethanol	Polybotrya	slope	developed	L	1	0.94	1.07
Poly2_dev_hexane	Polybotrya	slope	developed	M	1	1.47	0.68
Poly3_dev_ethanol	Polybotrya	slope	developed	L	10	8.04	1.24
Poly3_dev_hexane	Polybotrya	slope	developed	M	1	2.68	0.37
Poly1_old_ethanol	Polybotrya	slope	old	L	55	22.10	2.49
Poly1_old_hexane	Polybotrya	slope	old	L	10	5.36	1.87
Poly2_old_ethanol	Polybotrya	slope	old	L	15	7.03	2.13
Poly2_old_hexane	Polybotrya	slope	old	L	35	14.73	2.38
Poly3_old_ethanol	Polybotrya	slope	old	L	55	22.10	2.49
Poly3_old_hexane	Polybotrya	slope	old	L	30	11.48	2.61

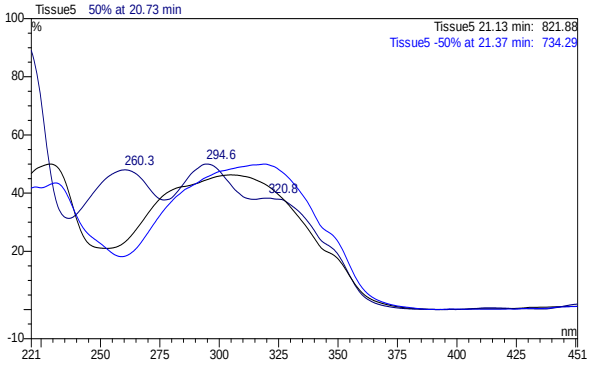
6.2. Sample dry weights

sample	plant	extract	solvent	dry weight (mg per m² leaf area)
Carl1_dev_ethanol	Carludovica	leaf wax	ethanol	65.28
Carl1_old_ethanol	Carludovica	leaf wax	ethanol	71.03
Carl1_young_ethanol	Carludovica	leaf wax	ethanol	17.60
Carl2_dev_ethanol	Carludovica	leaf wax	ethanol	33.06
Carl2_old_ethanol	Carludovica	leaf wax	ethanol	47.01
Carl2_young_ethanol	Carludovica	leaf wax	ethanol	25.51
Carl3_dev_ethanol	Carludovica	leaf wax	ethanol	56.37
Carl3_old_ethanol	Carludovica	leaf wax	ethanol	56.51
Carl3_young_ethanol	Carludovica	leaf wax	ethanol	23.78
Cost1_dev_ethanol	Costus	leaf wax	ethanol	44.74
Cost1_young_ethanol	Costus	leaf wax	ethanol	55.01
Cost2_old_ethanol	Costus	leaf wax	ethanol	142.04
Cost2_young_ethanol	Costus	leaf wax	ethanol	28.23
Cost3_dev_ethanol	Costus	leaf wax	ethanol	36.38
Cost3_old_ethanol	Costus	leaf wax	ethanol	77.49
Cost3_young_ethanol	Costus	leaf wax	ethanol	23.92
Dieff1_young_ethanol	Dieffenbachia	leaf wax	ethanol	39.31
Dieff2_dev_ethanol	Dieffenbachia	leaf wax	ethanol	35.49
Dieff3_old_ethanol	Dieffenbachia	leaf wax	ethanol	74.40
Dieff4_dev_ethanol	Dieffenbachia	leaf wax	ethanol	24.70
Dieff4_young_ethanol	Dieffenbachia	leaf wax	ethanol	55.69
Dieff5_dev_ethanol	Dieffenbachia	leaf wax	ethanol	22.22
Dieff5_old_ethanol	Dieffenbachia	leaf wax	ethanol	63.43
Dieff5_young_ethanol	Dieffenbachia	leaf wax	ethanol	39.91
Dieff6_dev_ethanol	Dieffenbachia	leaf wax	ethanol	42.25
Dieff6_old_ethanol	Dieffenbachia	leaf wax	ethanol	57.37
Dieff6_young_ethanol	Dieffenbachia	leaf wax	ethanol	48.64
Epi_Carl_ethanol	Carludovica	epiphyll	ethanol	97.53
Epi_Cos_ethanol	Costus	epiphyll	ethanol	310.71
Epi_Dief_ethanol	Dieffenbachia	epiphyll	ethanol	353.76
Epi_Pent_ethanol	Pentagonia	epiphyll	ethanol	482.61
Epi_Poly_ethanol	Polybotrya	epiphyll	ethanol	84.55
Leaftiss_Carl_ethanol	Carludovica	leaf tissue	ethanol	6532
Leaftiss_Costus_ethanol	Costus	leaf tissue	ethanol	3179
Leaftiss_Dief_ethanol	Dieffenbachia	leaf tissue	ethanol	3212
Leaftiss_Pent_ethanol	Pentagonia	leaf tissue	ethanol	5586
Leaftiss_Poly_ethanol	Polybotria	leaf tissue	ethanol	5406
Pent1_dev_ethanol	Pentagonia	leaf wax	ethanol	46.46
Pent1_young_ethanol	Pentagonia	leaf wax	ethanol	24.39
Pent2_old_ethanol	Pentagonia	leaf wax	ethanol	360.35
Pent3_dev_ethanol	Pentagonia	leaf wax	ethanol	43.12
Pent3_old_ethanol	Pentagonia	leaf wax	ethanol	131.59
Pent3_young_ethanol	Pentagonia	leaf wax	ethanol	25.53
Poly1_dev_ethanol	Polybotrya	leaf wax	ethanol	17.13
Poly1_old_ethanol	Polybotrya	leaf wax	ethanol	93.24
Poly1_young_ethanol	Polybotrya	leaf wax	ethanol	59.54
Poly2_dev_ethanol	Polybotrya	leaf wax	ethanol	18.69
Poly2_old_ethanol	Polybotrya	leaf wax	ethanol	77.48
Poly2_young_ethanol	Polybotrya	leaf wax	ethanol	29.48
Poly3_dev_ethanol	Polybotrya	leaf wax	ethanol	40.64

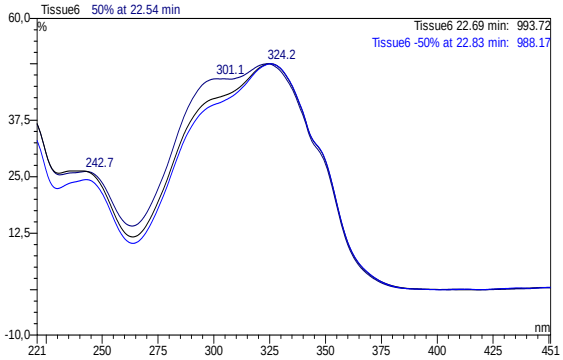
Poly3_old_ethanol	Polybotrya	leaf wax	ethanol	94.02
Aspl1_dev_hexane	Asplundia	leaf wax	hexane	4.79
Aspl2_dev_hexane	Asplundia	leaf wax	hexane	6.33
Aspl2_old_hexane	Asplundia	leaf wax	hexane	7.48
Aspl3_dev_hexane	Asplundia	leaf wax	hexane	4.37
Aspl3_old_hexane	Asplundia	leaf wax	hexane	4.57
Carl1_dev_hexane	Carludovica	leaf wax	hexane	0.89
Carl1_old_hexane	Carludovica	leaf wax	hexane	1.96
Carl2_dev_hexane	Carludovica	leaf wax	hexane	2.80
Carl2_old_hexane	Carludovica	leaf wax	hexane	3.73
Carl3_dev_hexane	Carludovica	leaf wax	hexane	2.59
Carl3_old_hexane	Carludovica	leaf wax	hexane	5.21
Cost1_dev_hexane	Costus	leaf wax	hexane	70.78
Cost1_old_hexane	Costus	leaf wax	hexane	4.55
Cost2_dev_hexane	Costus	leaf wax	hexane	9.78
Cost2_old_hexane	Costus	leaf wax	hexane	60.37
Cost3_dev_hexane	Costus	leaf wax	hexane	7.21
Cost3_old_hexane	Costus	leaf wax	hexane	6.78
Cost4_dev_hexane	Costus	leaf wax	hexane	16.33
Dieff1_dev_hexane	Dieffenbachia	leaf wax	hexane	6.52
Dieff1_old_hexane	Dieffenbachia	leaf wax	hexane	4.20
Dieff2_dev_hexane	Dieffenbachia	leaf wax	hexane	2.32
Dieff2_old_hexane	Dieffenbachia	leaf wax	hexane	7.98
Dieff3_dev_hexane	Dieffenbachia	leaf wax	hexane	5.60
Dieff3_old_hexane	Dieffenbachia	leaf wax	hexane	12.89
Dieff4_dev_hexane	Dieffenbachia	leaf wax	hexane	18.18
Epi_Aspl_hexane	epiphyll	epiphyll	hexane	17.08
Epi_Carl_hexane	epiphyll	epiphyll	hexane	6.20
Epi_Cos_hexane	epiphyll	epiphyll	hexane	22.75
Epi_Dief_hexane	epiphyll	epiphyll	hexane	27.56
Epi_Pent_hexane	epiphyll	epiphyll	hexane	560.00
Epi_Poly_hexane	epiphyll	epiphyll	hexane	7.14
Leaftiss_Aspl_hexane	Asplundia	leaf tissue	hexane	35.42
Leaftiss_Carl_hexane	Carludovica	leaf tissue	hexane	67.50
Leaftiss_Costus_hexane	Costus	leaf tissue	hexane	n.a.
Leaftiss_Dief_hexane	Dieffenbachia	leaf tissue	hexane	277.01
Leaftiss_Pent_hexane	Pentagonia	leaf tissue	hexane	189.15
Leaftiss_Poly_hexane	Polybotria	leaf tissue	hexane	42.03
Pent1_dev_hexane	Pentagonia	leaf wax	hexane	2.52
Pent1_old_hexane	Pentagonia	leaf wax	hexane	6.37
Pent2_dev_hexane	Pentagonia	leaf wax	hexane	2.30
Pent2_old_hexane	Pentagonia	leaf wax	hexane	16.76
Pent3_dev_hexane	Pentagonia	leaf wax	hexane	2.84
Pent3_old_hexane	Pentagonia	leaf wax	hexane	4.49
Pent4_dev_hexane	Pentagonia	leaf wax	hexane	8.08
Poly1_dev_hexane	Polybotrya	leaf wax	hexane	1.71
Poly1_old_hexane	Polybotrya	leaf wax	hexane	18.87
Poly2_dev_hexane	Polybotrya	leaf wax	hexane	9.03
Poly2_old_hexane	Polybotrya	leaf wax	hexane	33.18
Poly3_dev_hexane	Polybotrya	leaf wax	hexane	5.89
Poly3_old_hexane	Polybotrya	leaf wax	hexane	7.20

6.3. UV – spectra of peaks from ethanol extracted samples

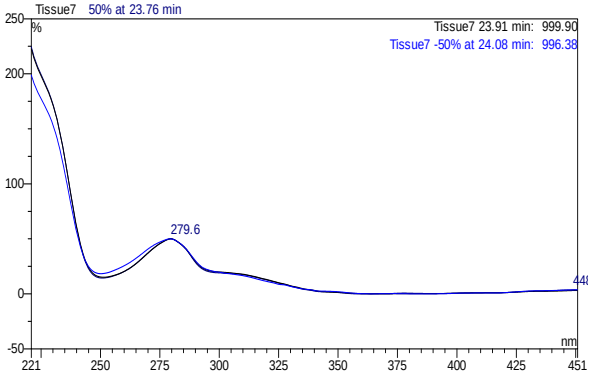
21.1 min.: peak 8



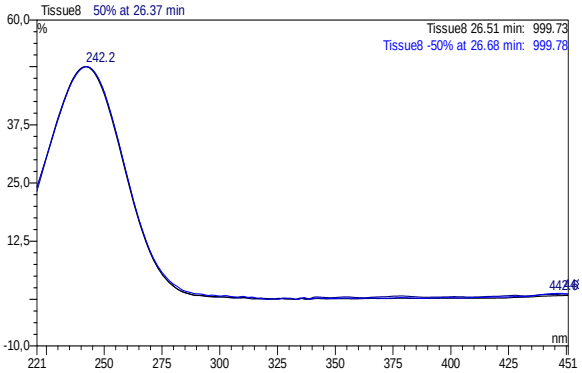
22.5 min.: peak 9



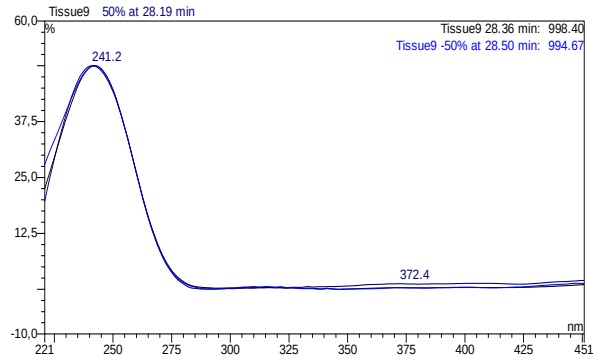
23.9 min.: peak 11



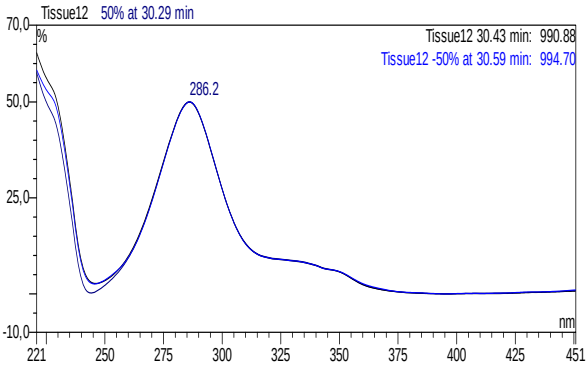
26.1 min.: peak 12



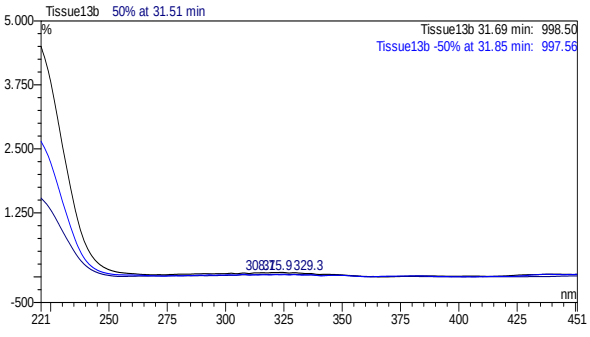
28.4 min.: peak 14



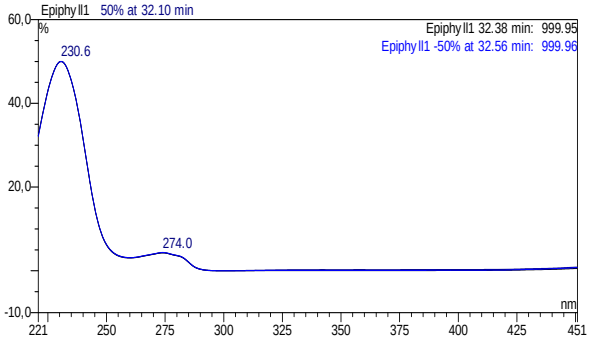
30.5 min.: peak 19



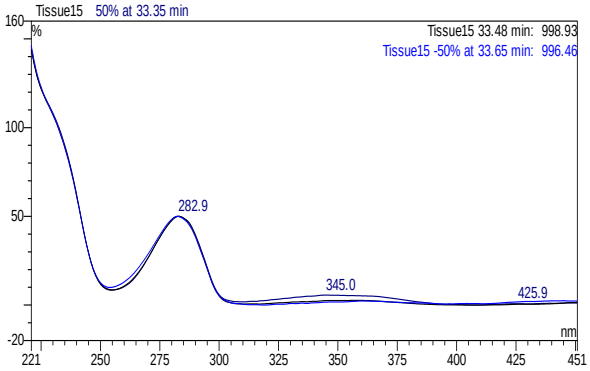
31.7 min.: peak 21



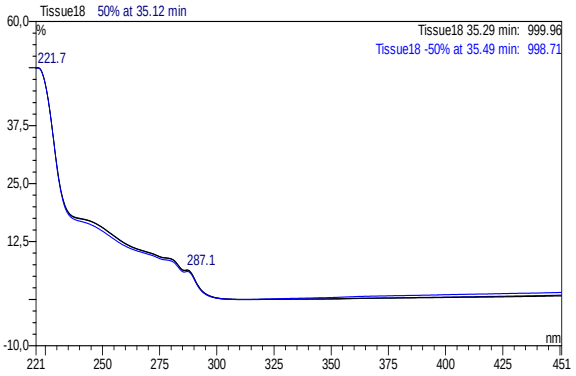
32.4 min.: peak 22



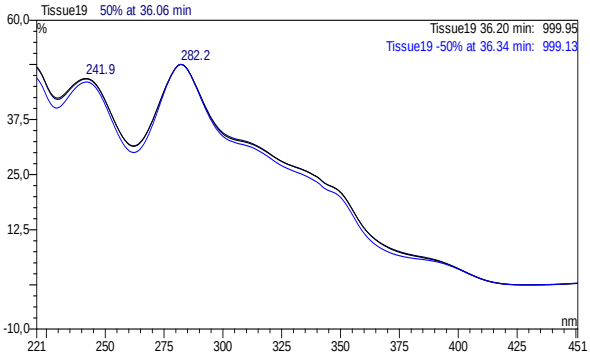
33.5 min.: peak 25



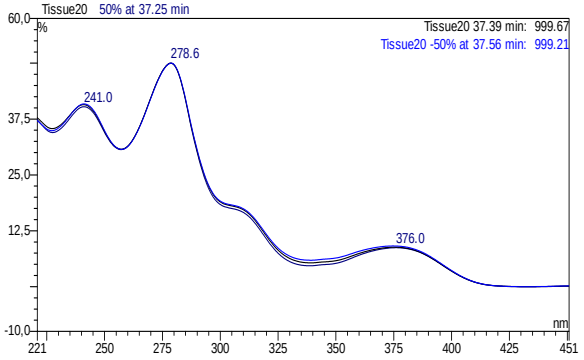
35.3 min.: peak 30



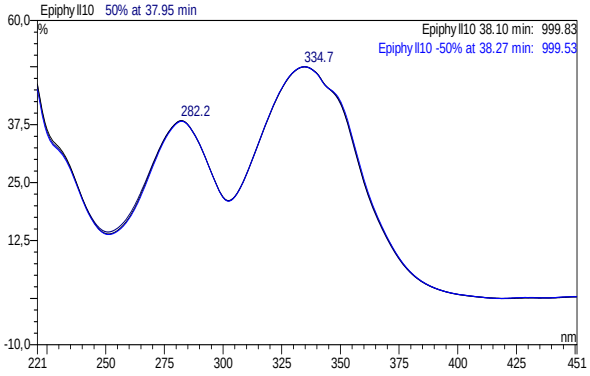
36.2 min.: peak 31



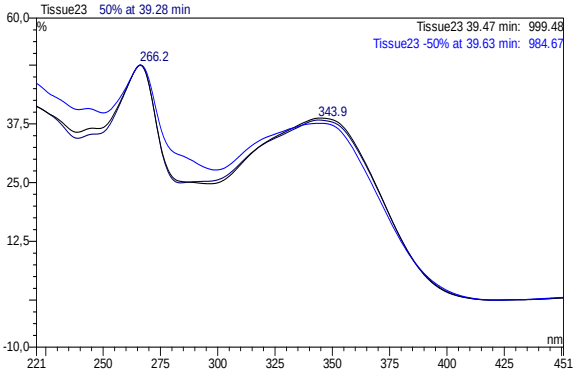
37.3 min.: peak 33



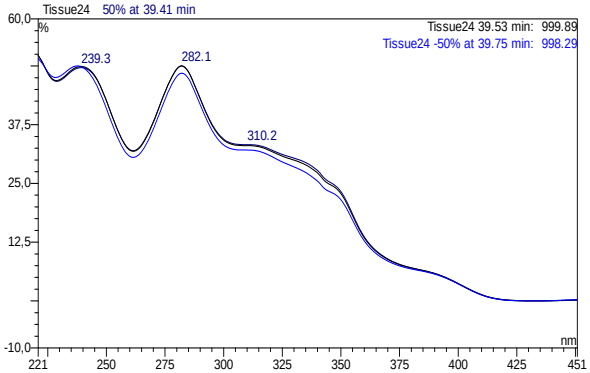
38.1 min.: peak 35



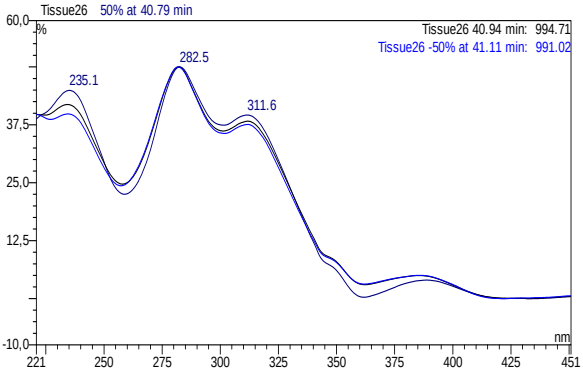
39.4 min.: peak 38



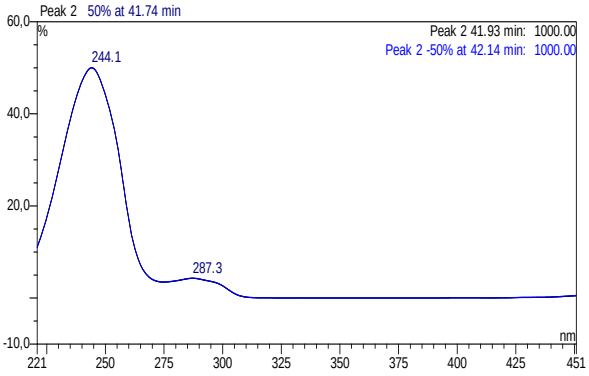
39.5 min.: peak 39



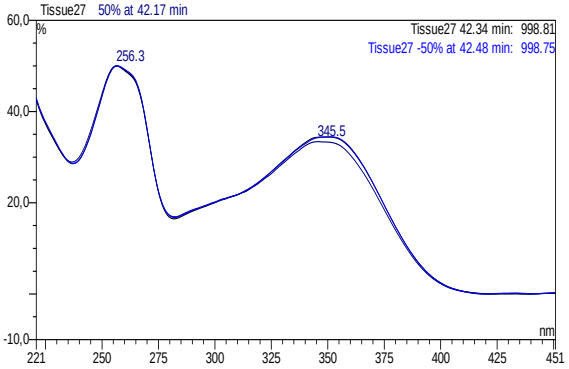
41 min.: peak 42



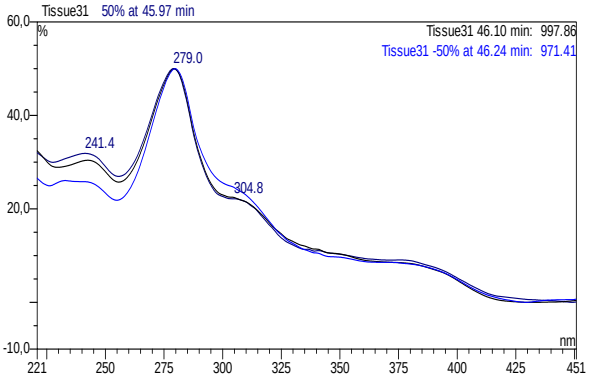
41.8 min.: peak 44



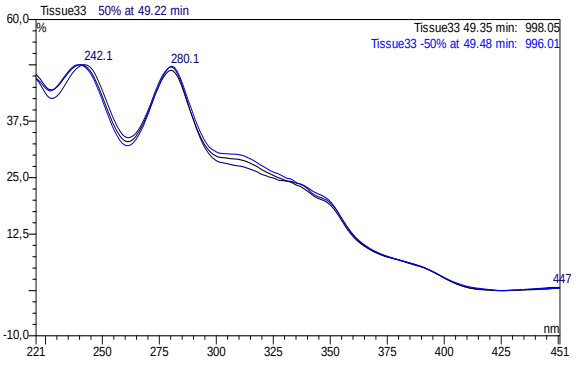
42.3 min.: peak 45



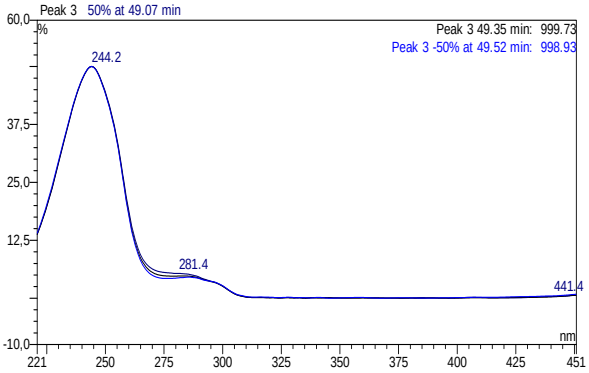
46 min.: peak 53



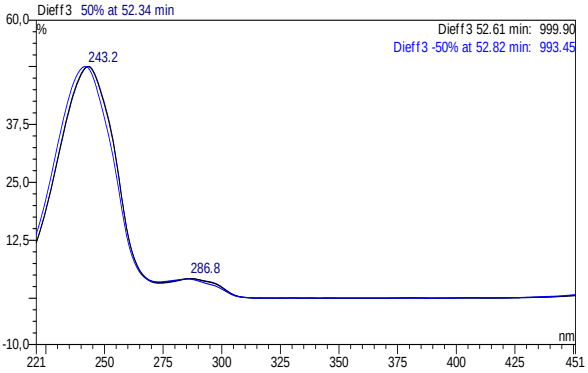
49.3 min.: peak 57



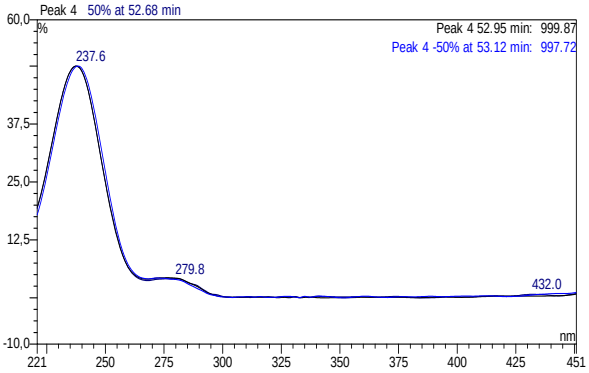
49.6 min.: peak 58



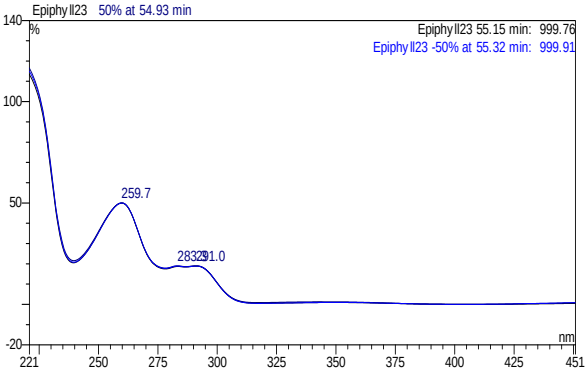
52.6 min.: peak 65



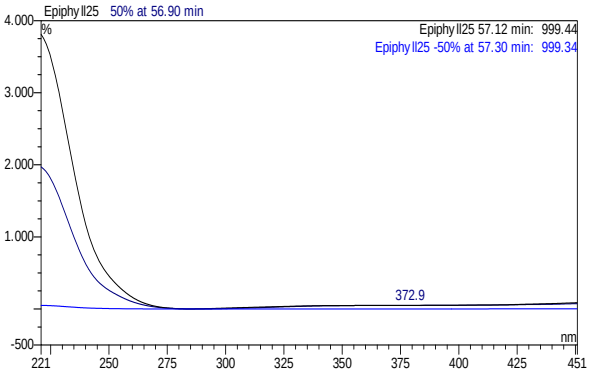
53.2 min.: peak 67



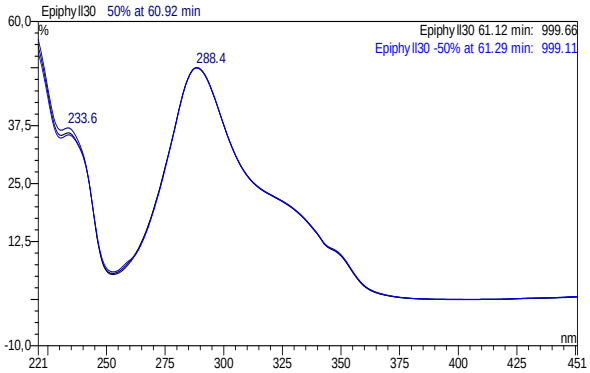
55.2 min.: peak 69



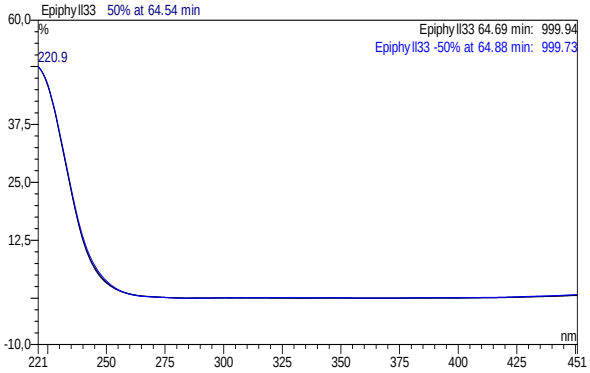
57.2 min.: peak 71



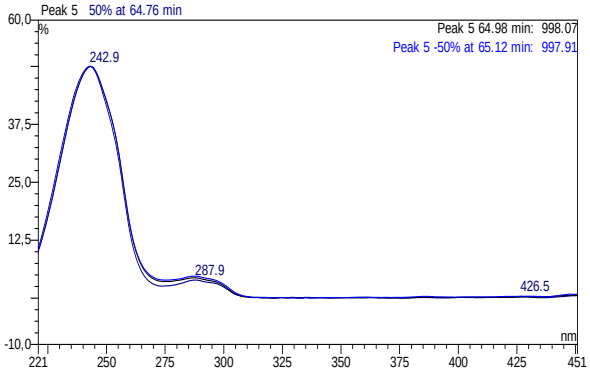
61.1 min.: peak 78



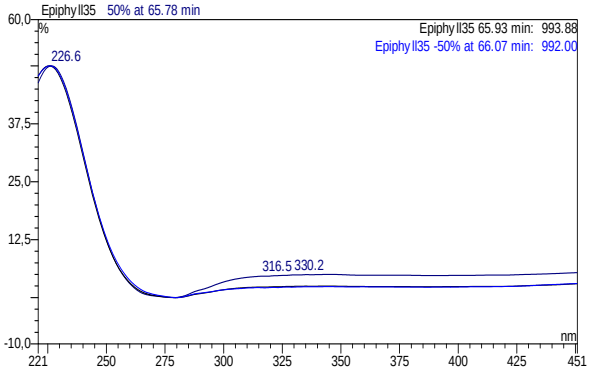
64.6 min.: peak 82



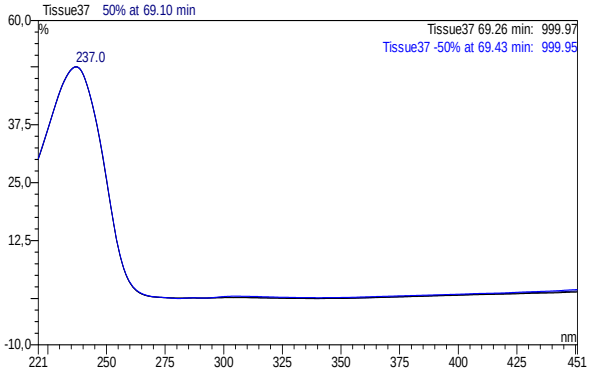
64.7 min.: peak 83



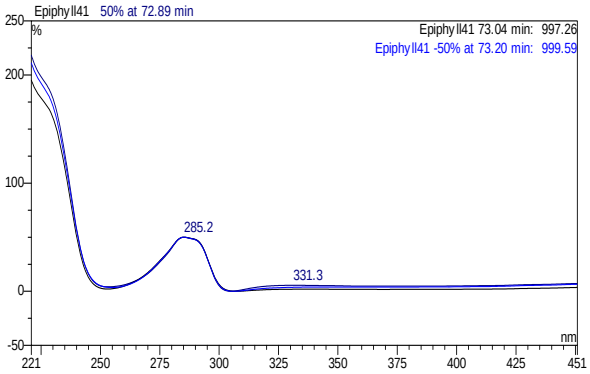
66 min.: peak 85



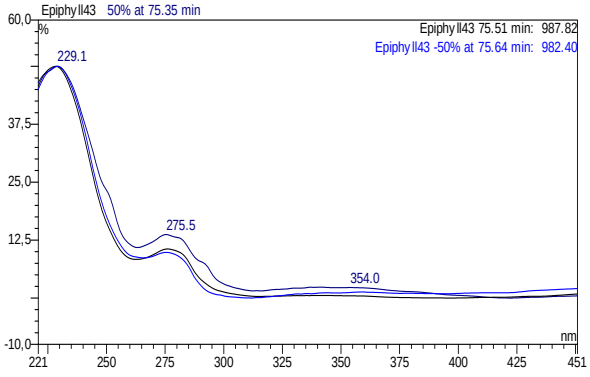
69.3 min.: peak 91



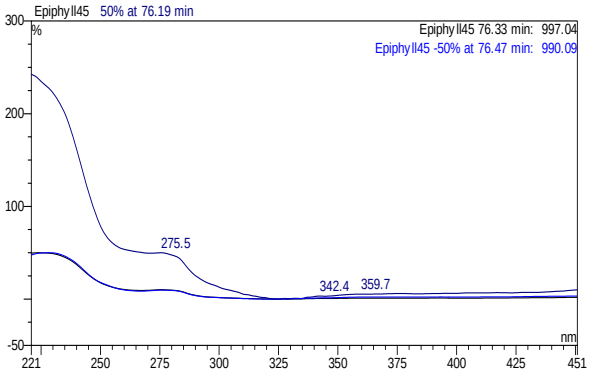
73 min.: peak 98



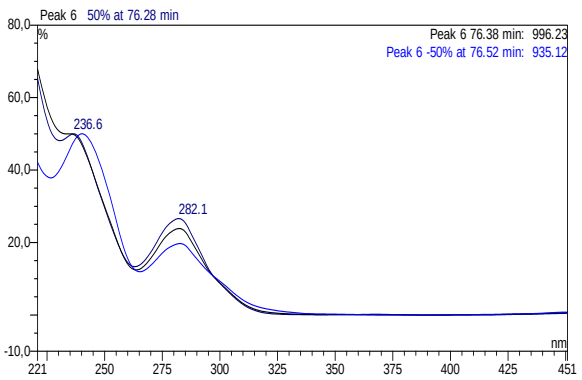
75.6 min.: peak 104



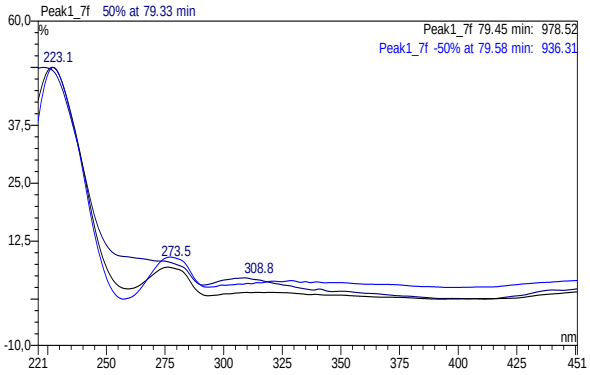
76.3 min.: peak 107



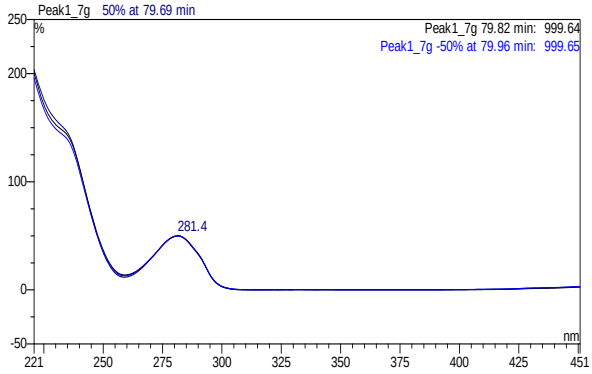
76.7 min.: peak 110



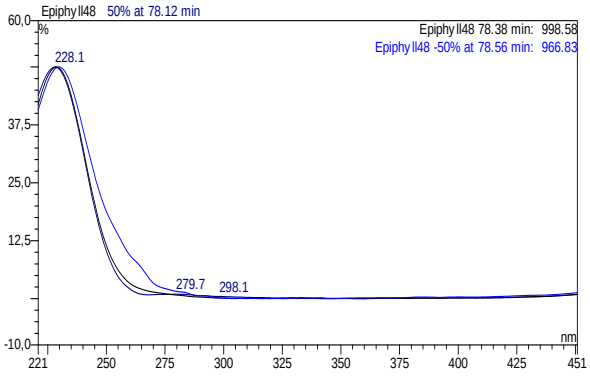
77.6 min.: peak 114



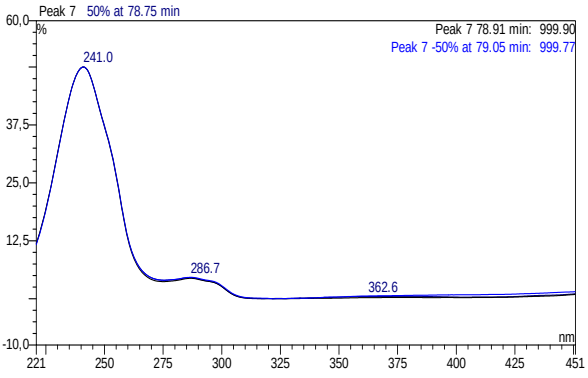
77.9 min.: peak 115



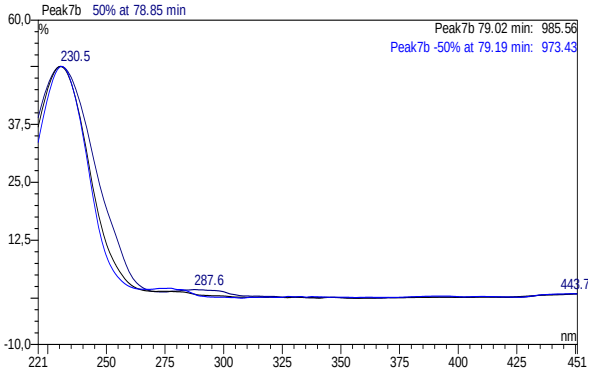
78.4 min.: peak 117



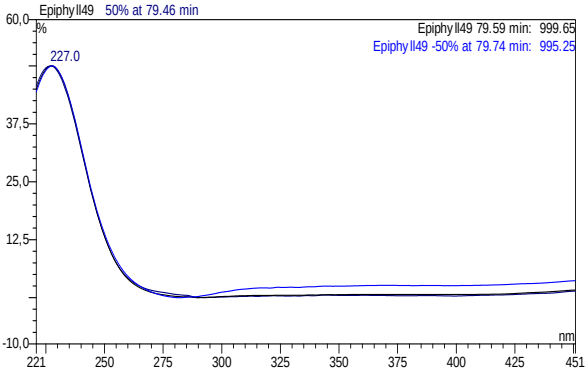
78.8 min.: peak 118



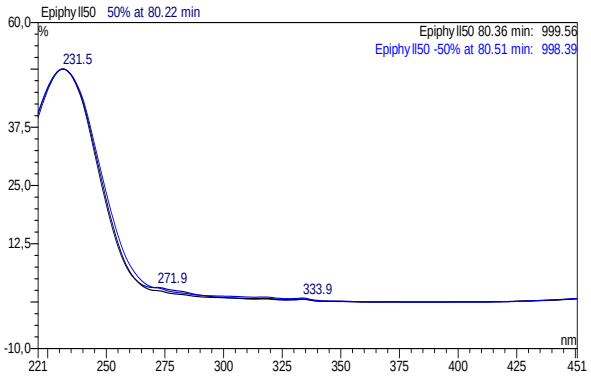
79.1 min.: peak 119



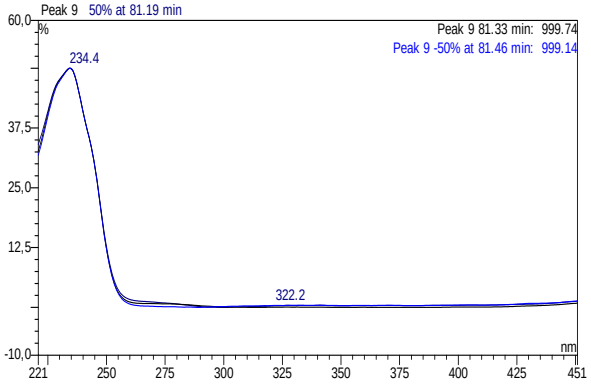
79.6 min.: peak 122



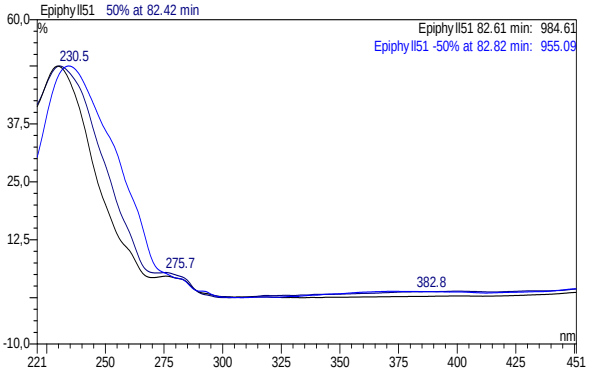
80.3 min.: peak 123



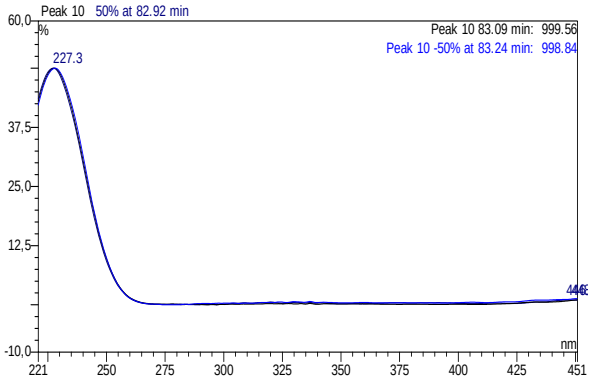
81 min.: peak 125



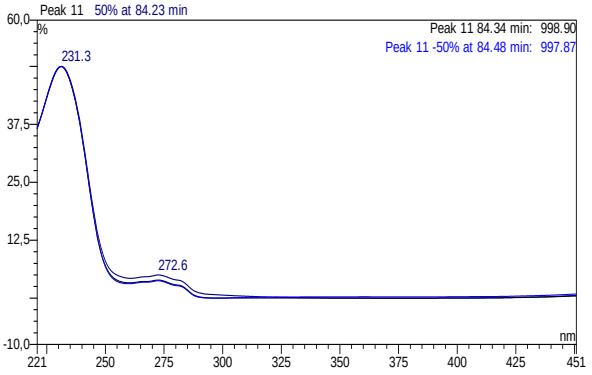
82.5 min.: peak 127



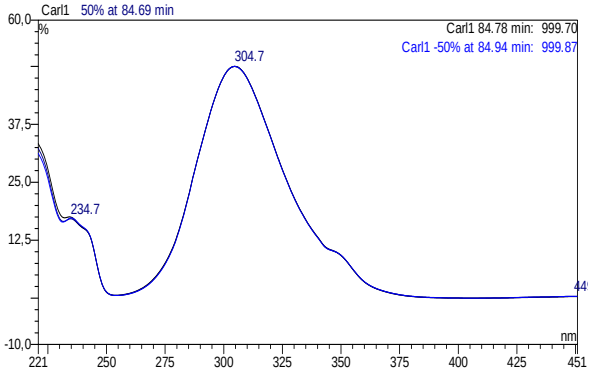
83.1 min.: peak 129



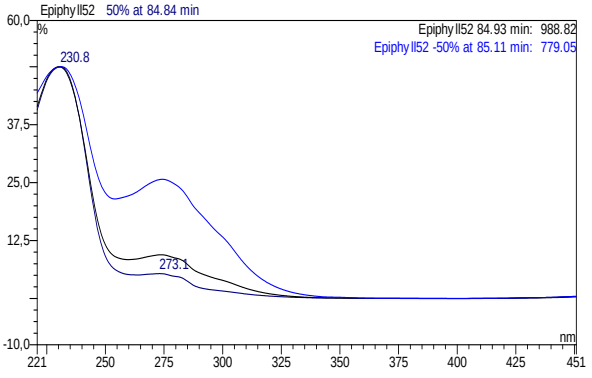
84.5 min.: peak 132



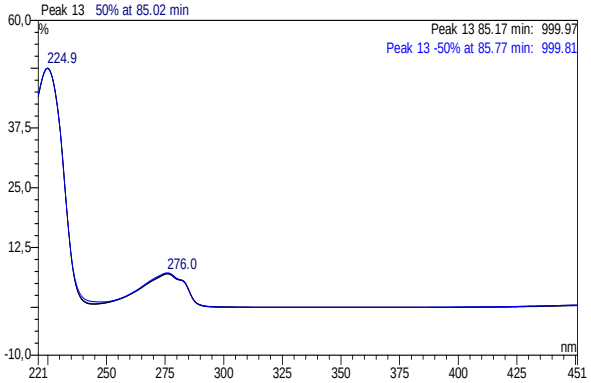
84.8 min.: peak 133



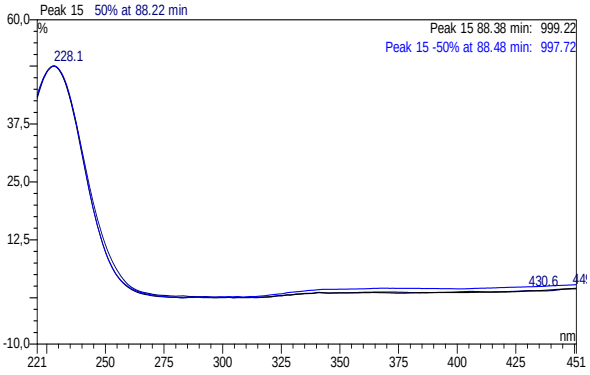
84.9 min.: peak 134



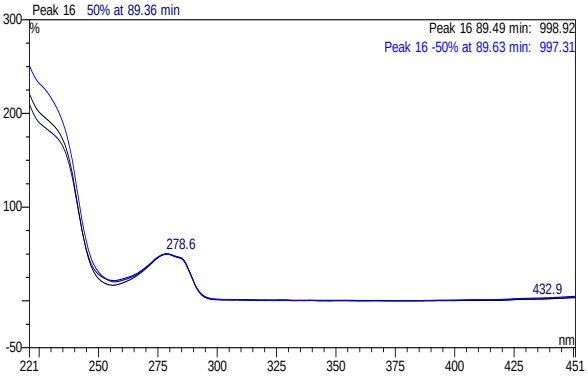
85.7 min.: peak 135



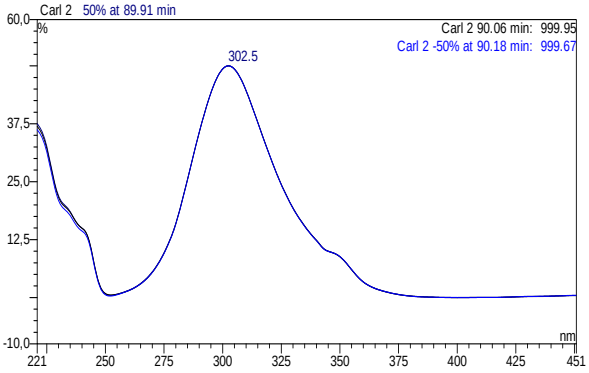
88.4 min.: peak 139



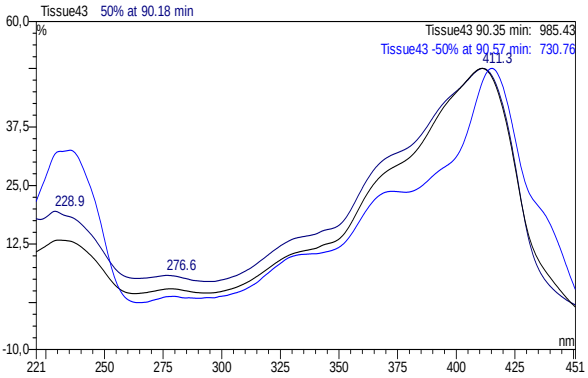
89.5 min.: peak 142



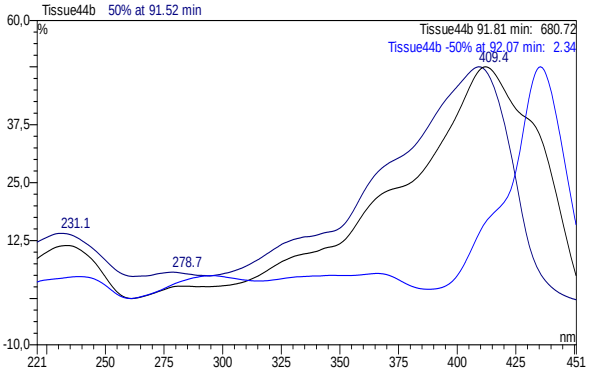
90.1 min.: peak 143



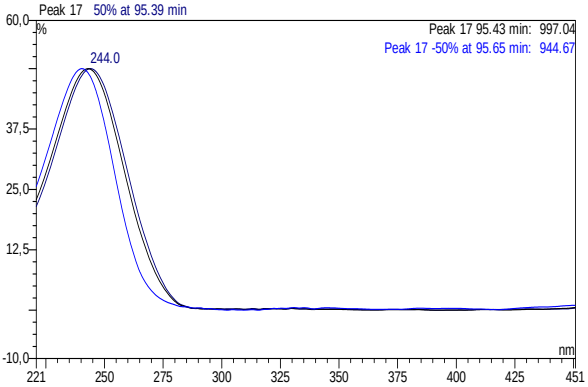
90.4 min.: peak 144



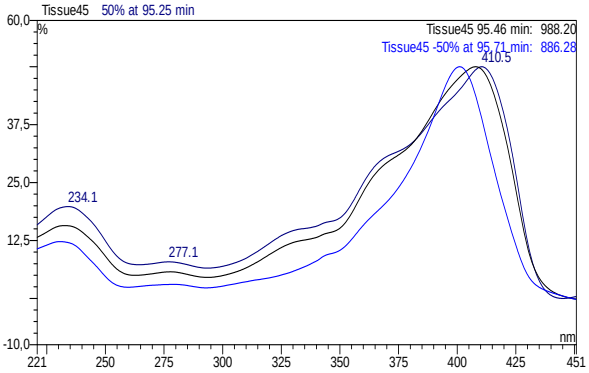
91.7 min.: peak 147



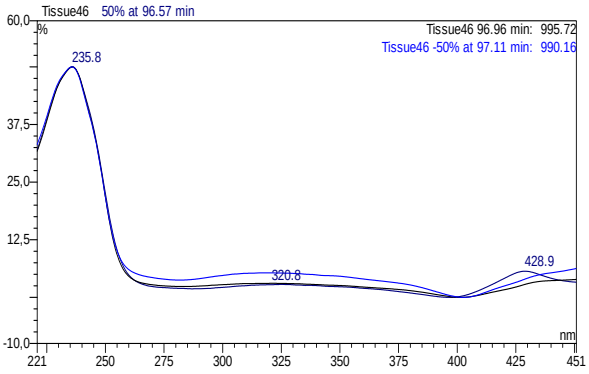
95.3 min.: peak 151



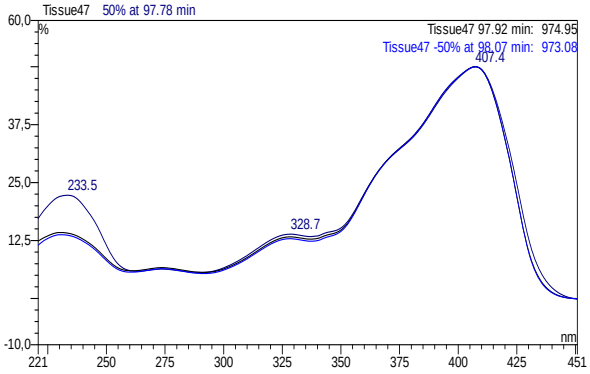
95.5 min.: peak 152



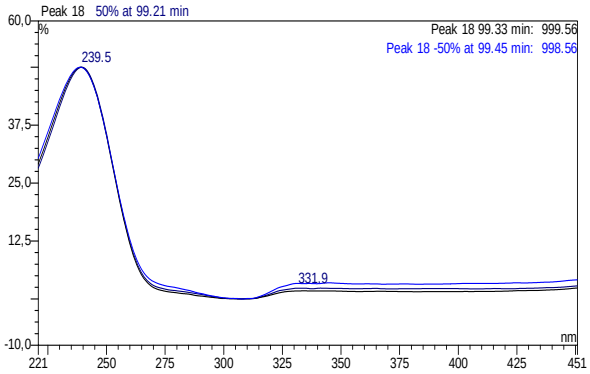
97 min.: peak 154



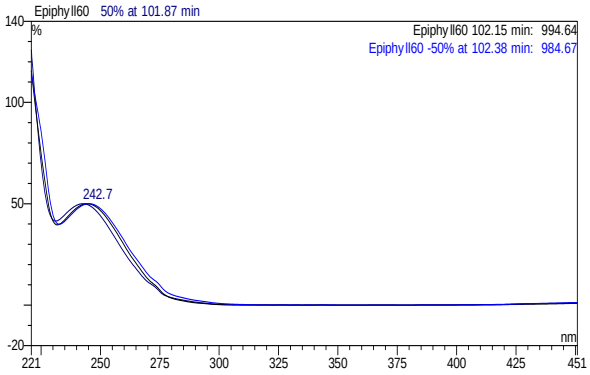
97.9 min.: peak 155



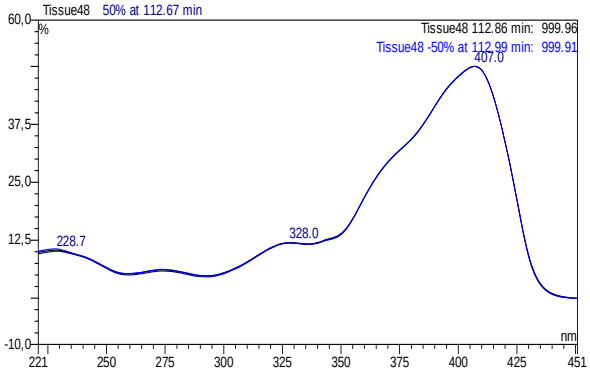
99.2 min.: peak 156



102.2 min.: peak 158

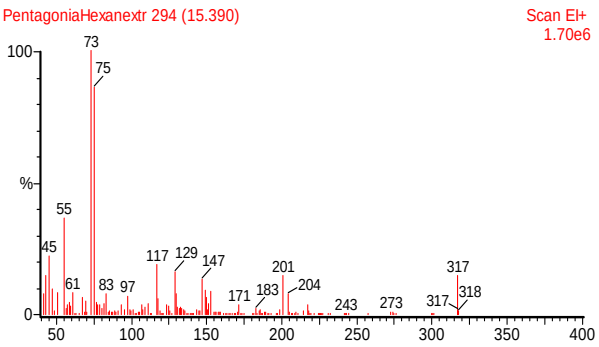


112.8 min.: peak 163

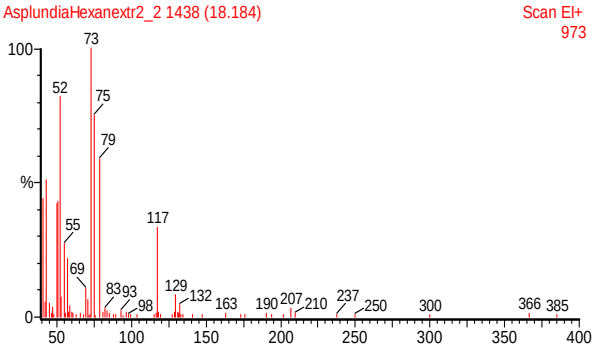


6.4. MS – spectra of peaks from hexane extracted samples

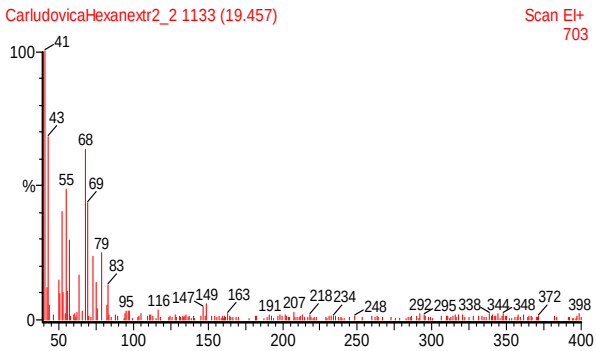
17.7 min.: dicarboxylic acid



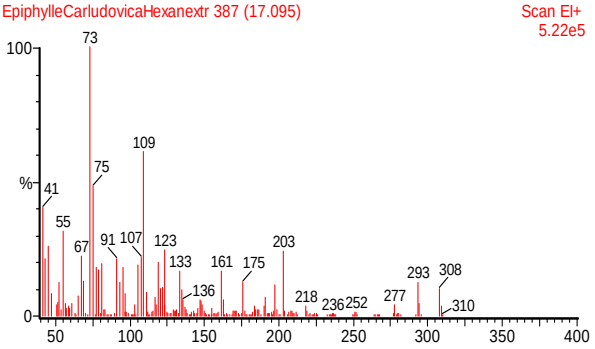
19.0 min.: myristic acid



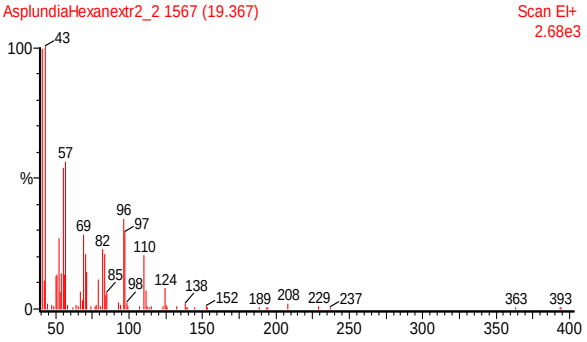
19.2 min.: unidentified 1



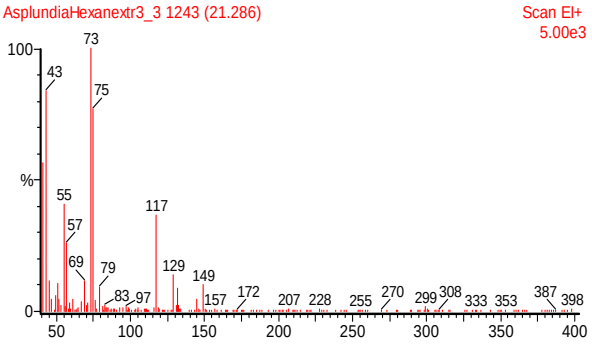
19.5 min.: unidentified 2



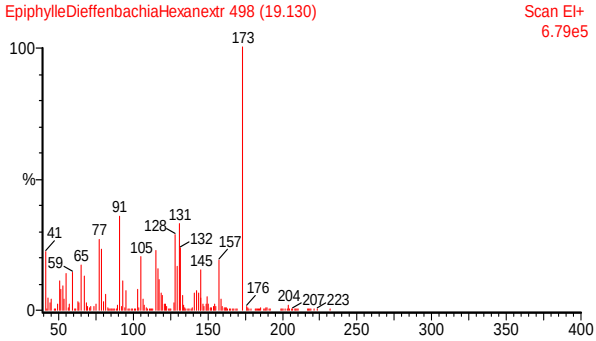
20.1 min.: unidentified 3



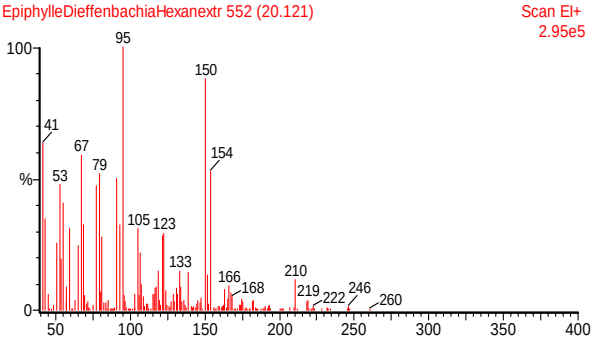
21.25 min.: pentadecanoic acid



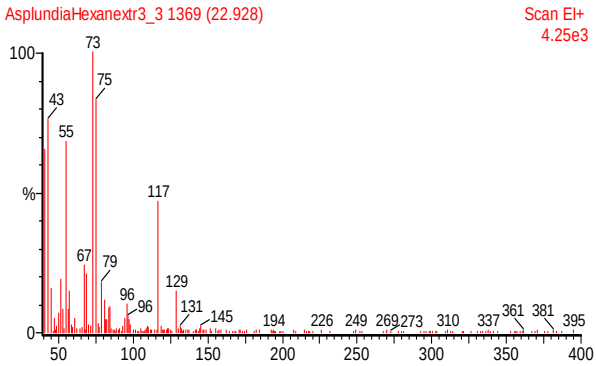
21.5 min.: cyclic1



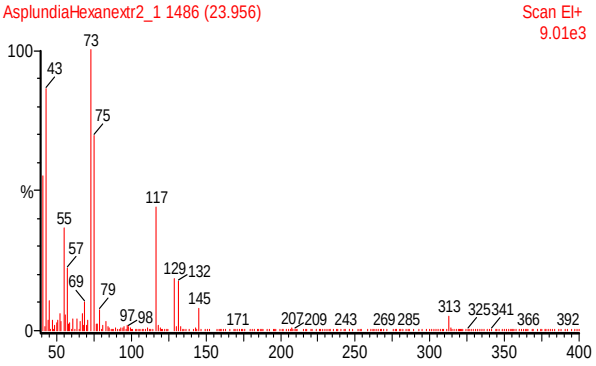
22.5 min.: unidentified 4



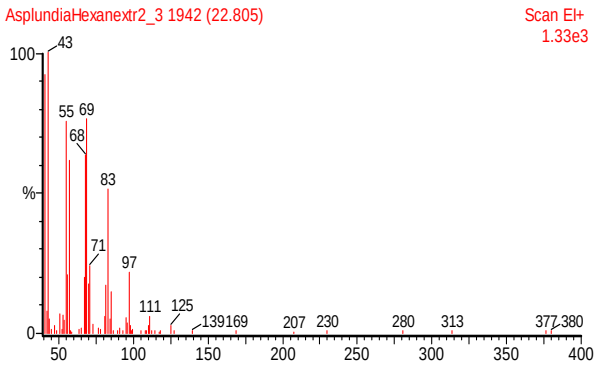
22.9 min.: palmitoleic acid



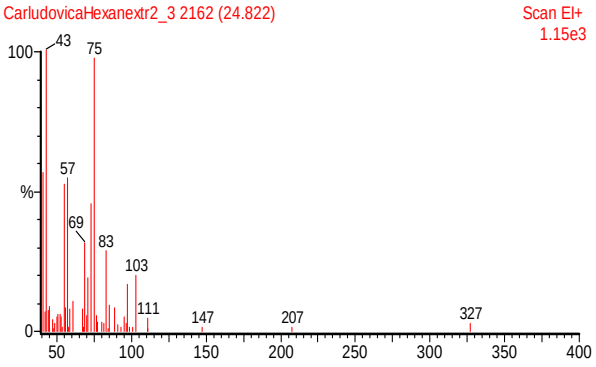
23.6 min.: palmitic acid



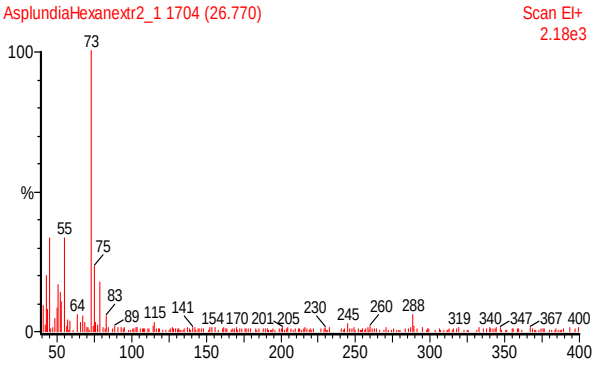
24.0 min.: iso-alkene1



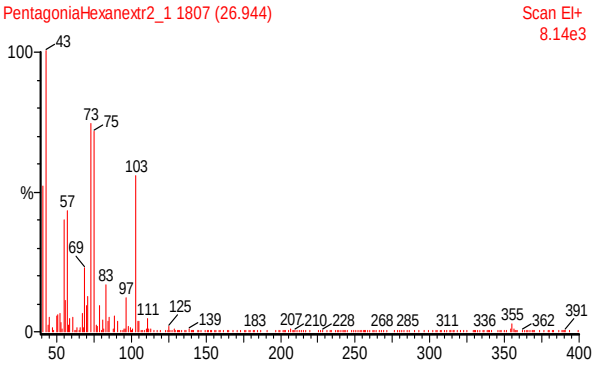
25.9 min.: octadecanol



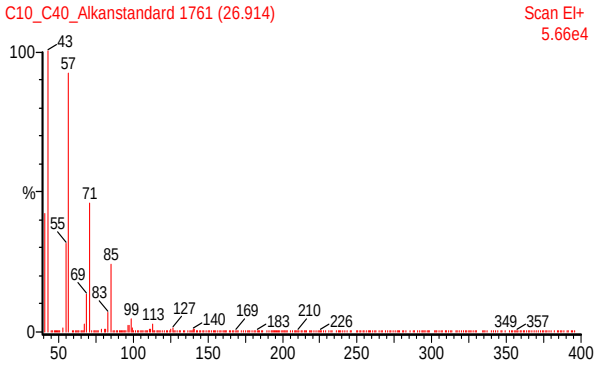
26.4 min.: unidentified 5



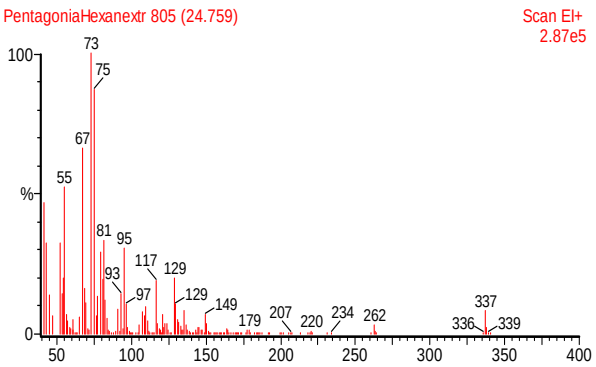
26.6 min.: eicosanol



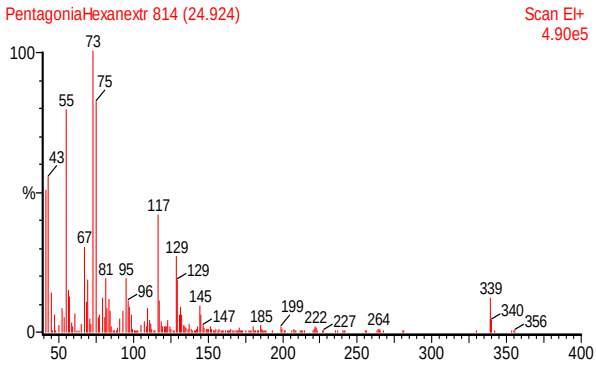
26.9 min.: docosane



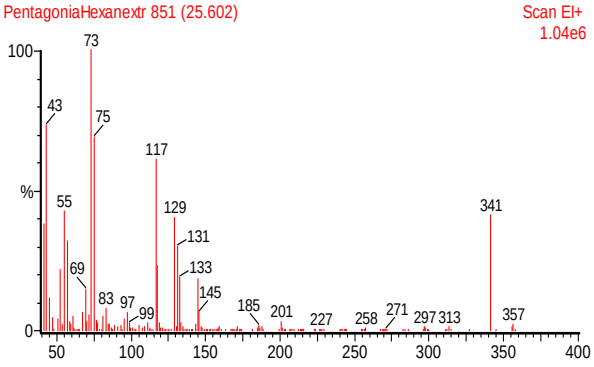
27.1 min.: linoleic acid



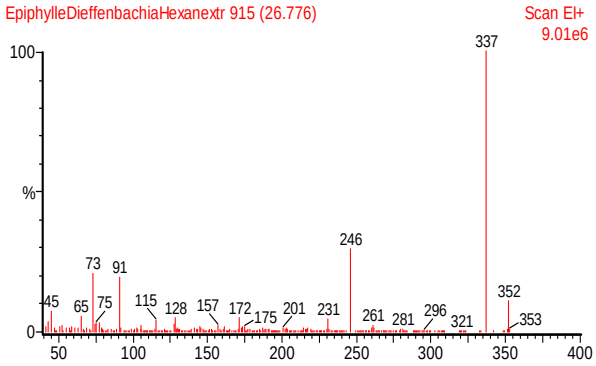
27.2 min.: oleic acid



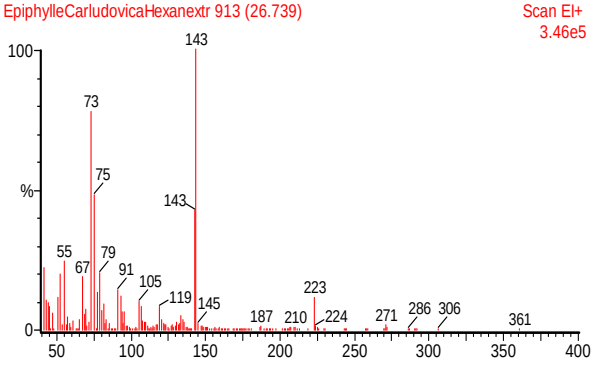
27.8 min.: stearic acid



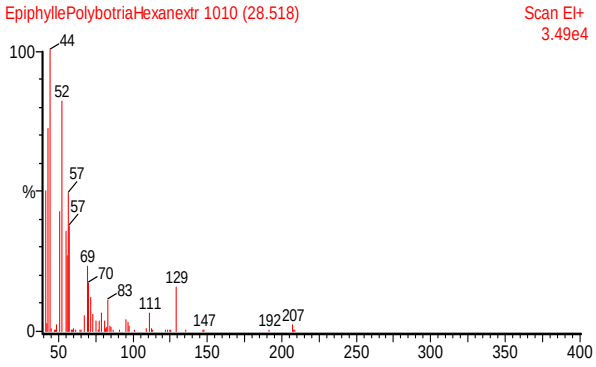
29.1 min.: unidentified 6



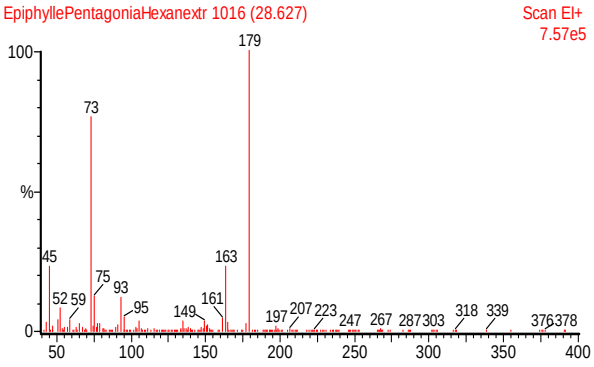
29.2 min.: unidentified 7



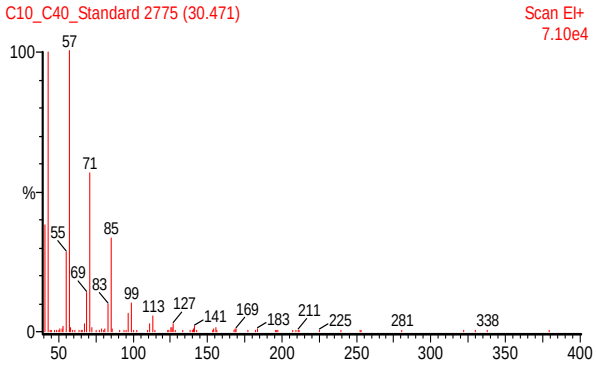
30.8 min.: unidentified 8



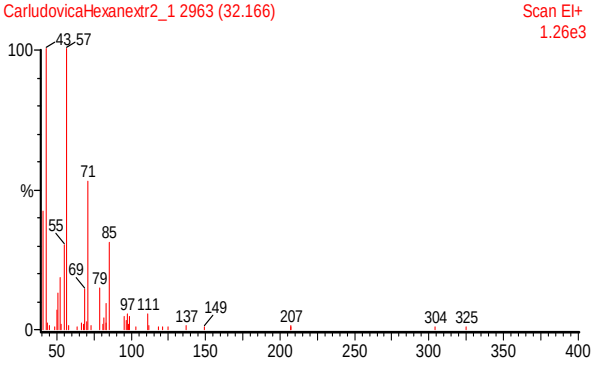
30.9 min.: phenolic 1



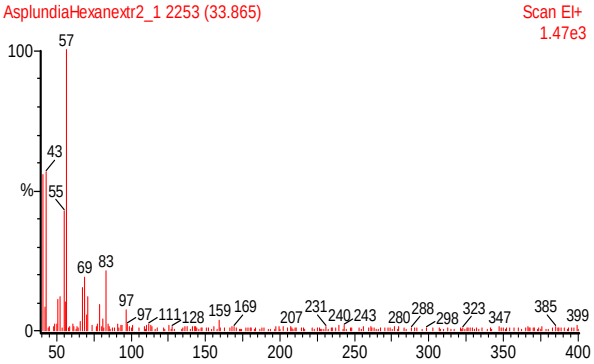
31.0 min.: tetracosane



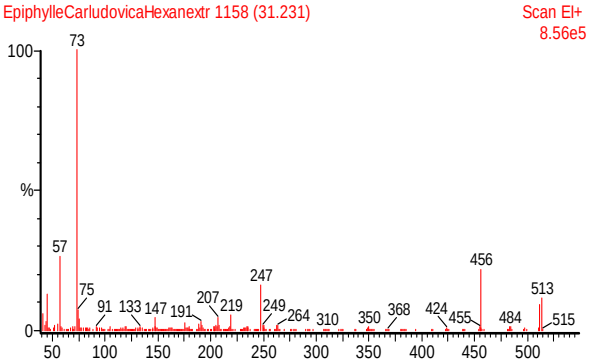
33.0 min.: pentacosane



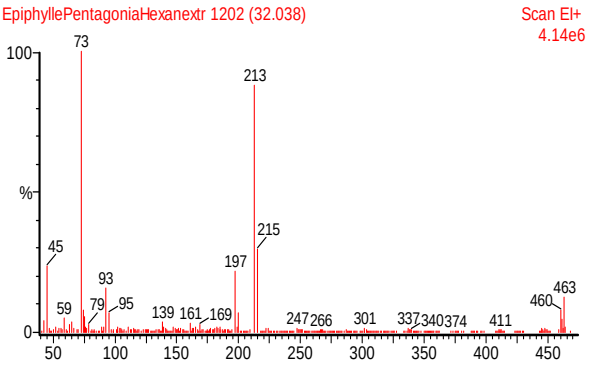
33.4 min.: unidentified 9



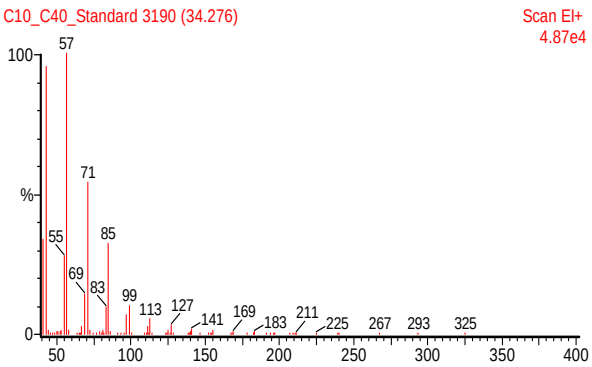
33.6 min.: phenolic 2



34.2 min.: phenolic 3



34.8 min.: hexacosane



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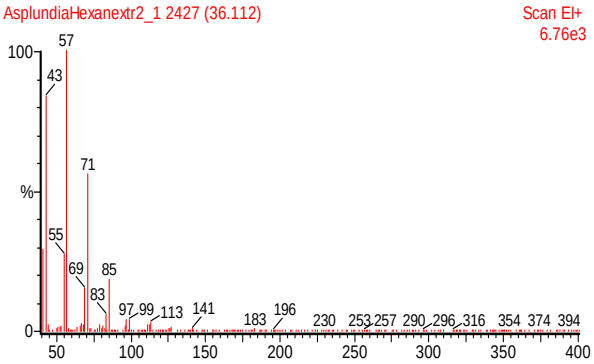
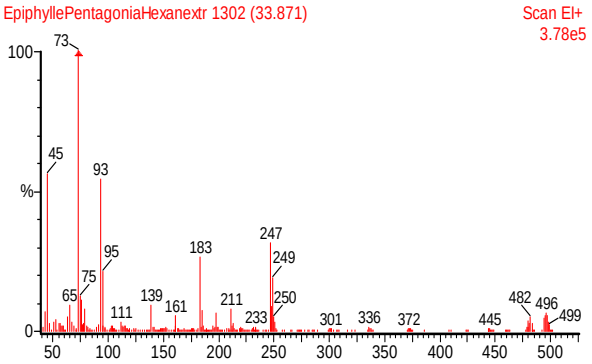
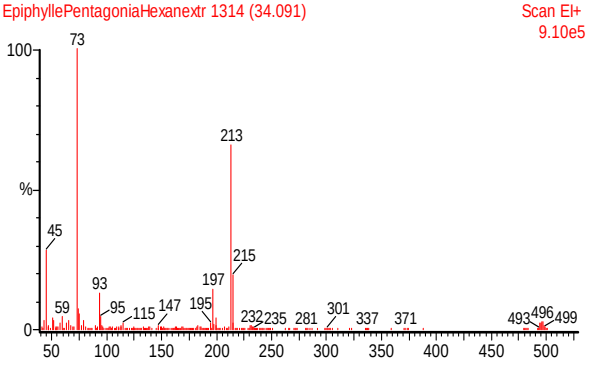


36.1 min.: unidentified 10



36.3 min.: phenolic 4



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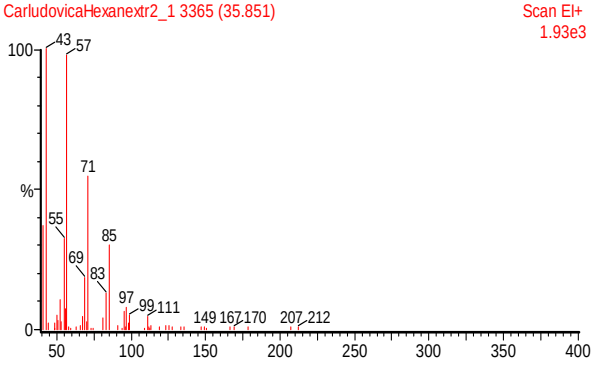
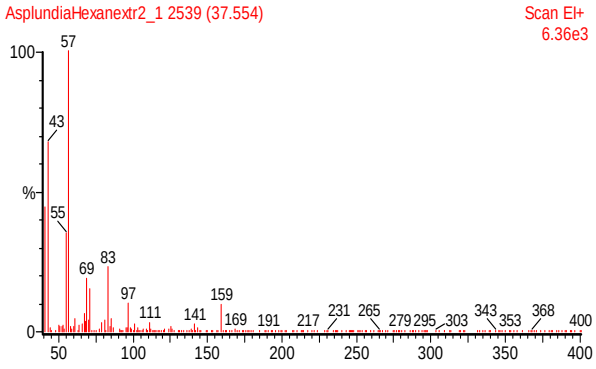
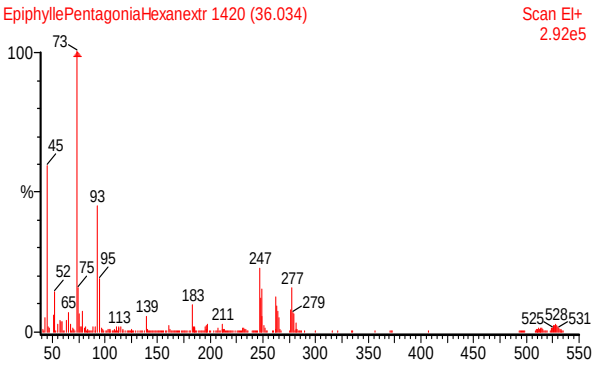


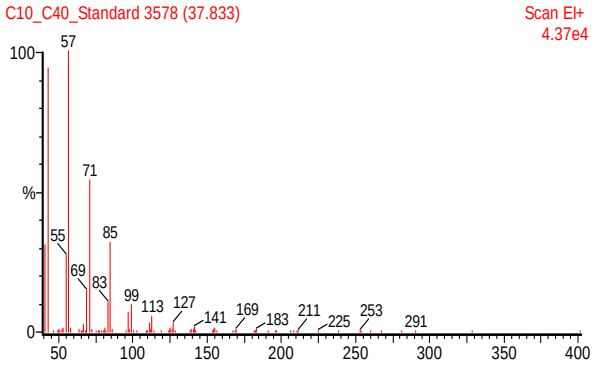
37.1 min.: unidentified 11



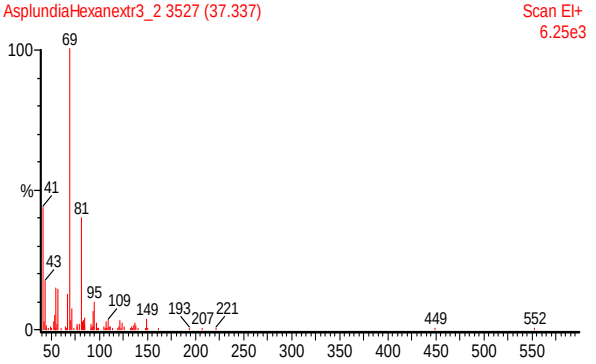
38.2 min.: phenolic 5



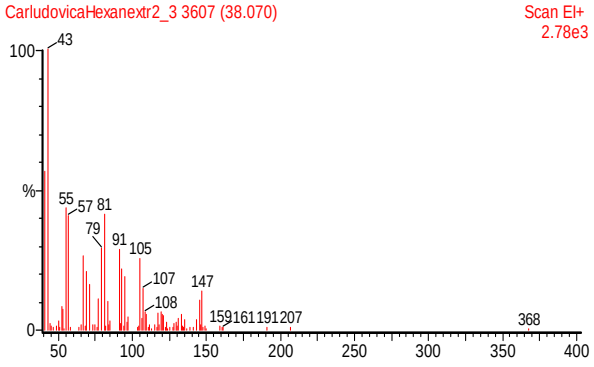
38.3 min.: octacosane



38.3 min.: unidentified 12



39.1 min.: sterolic 1



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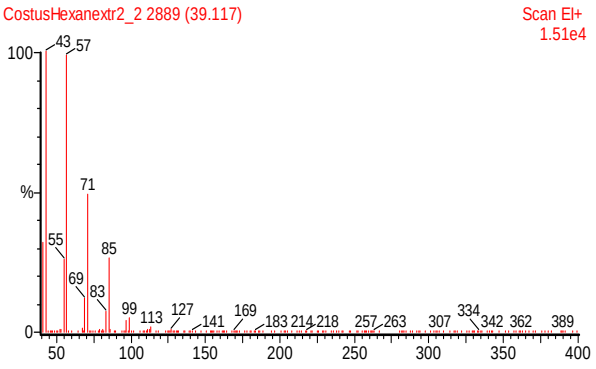
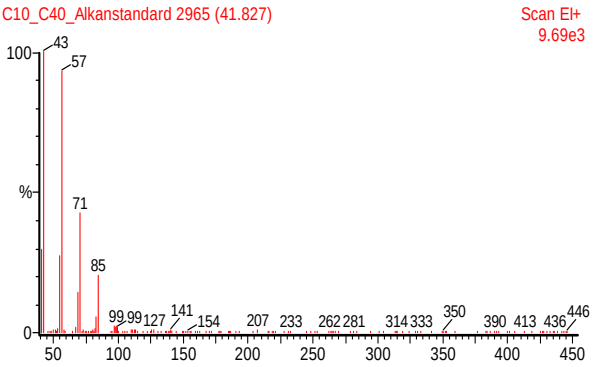
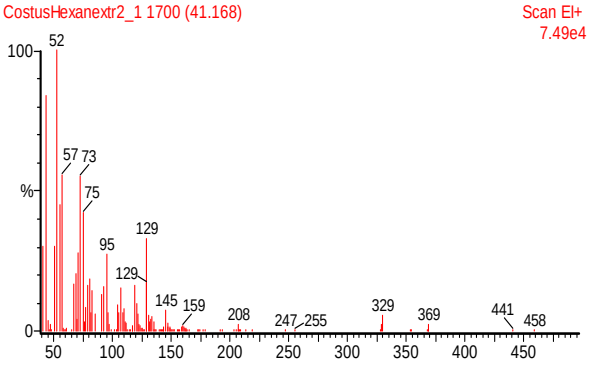


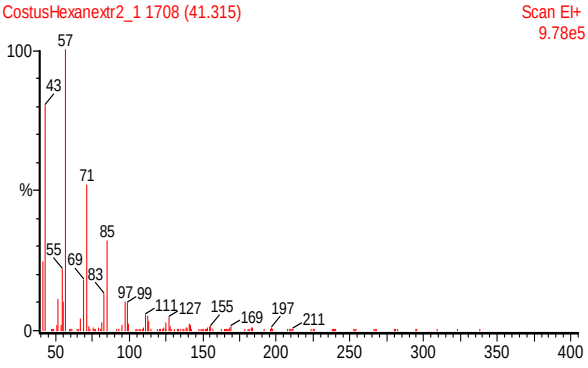
41.8 min.: triacontane



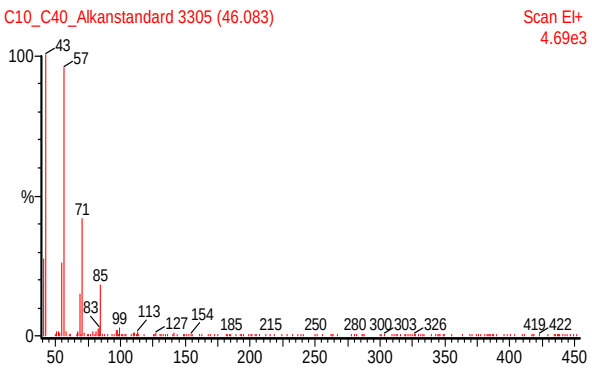
43.9 min.: sterolic 2



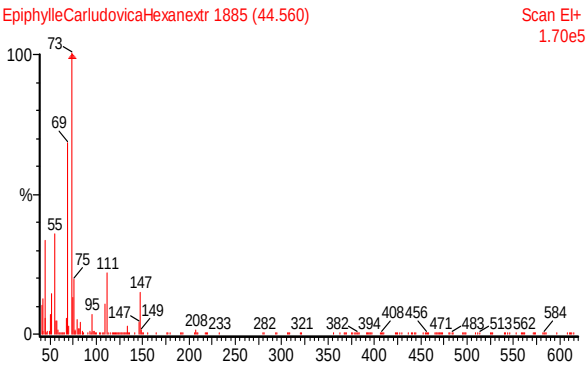
44.0 min.: untriacontane



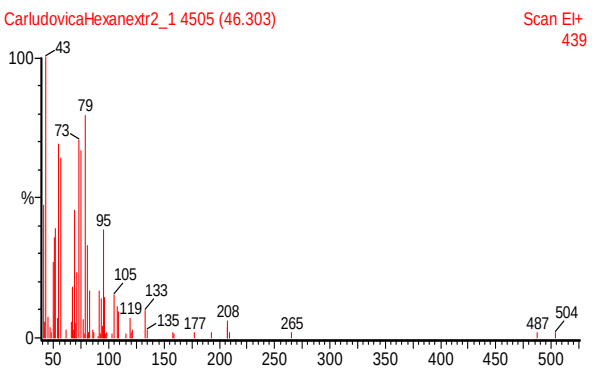
46.1 min.: dotriacontane



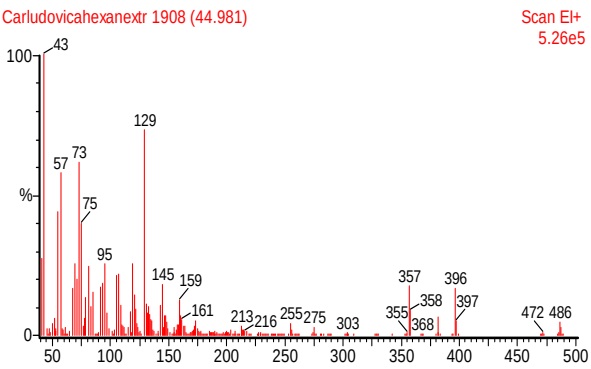
47.4 min.: unidentified 13



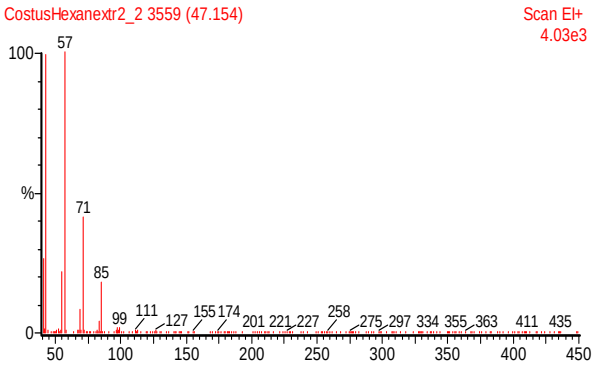
47.6 min.: sterolic 3



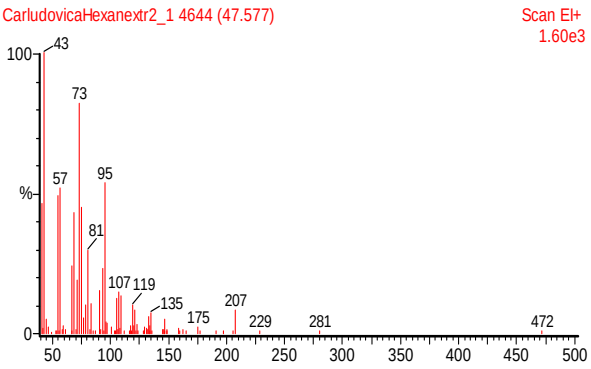
47.7 min.: sterolic 4



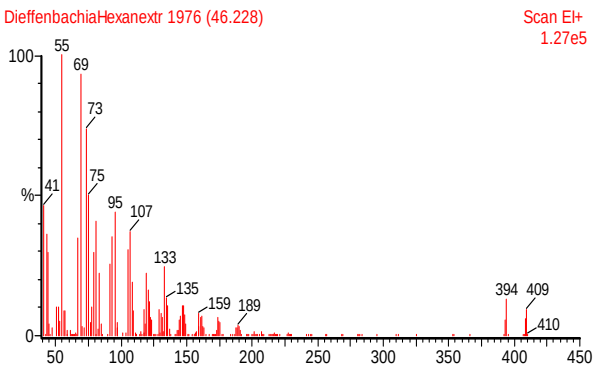
48.2 min.: tritriacontane



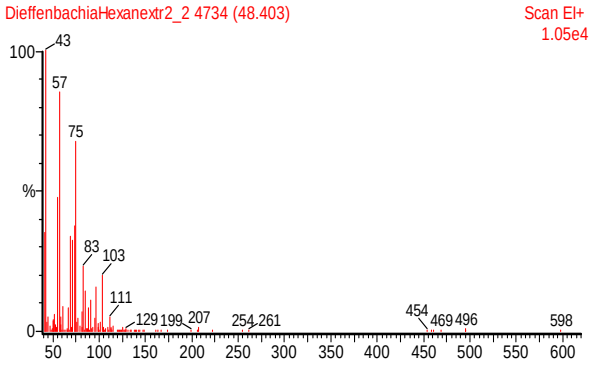
48.9 min.: sterolic 5



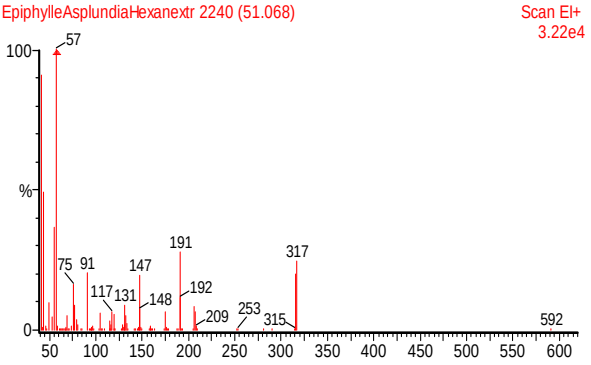
49.0 min.: terpenoic 1



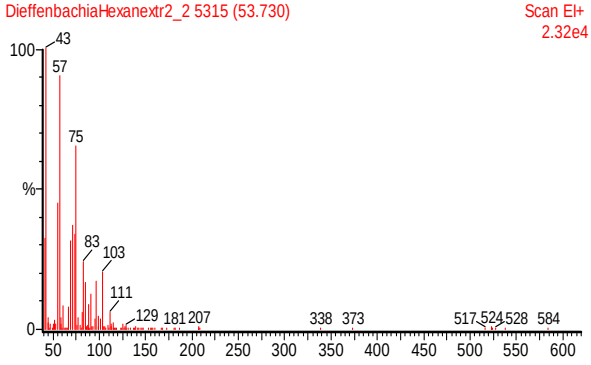
49.8 min.: triacontanol



53.9 min.: unidentified 14



54.6 min.: dotriacontanol



Zusammenfassung

Die Blattoberflächen von terrestrischen Samenpflanzen und Farnen werden von hoch angepassten Organismen, den so genannten Epiphyllen besiedelt. Analog zur Rhizosphäre wurde das Blatt als Mikrohabitat charakterisiert und Phyllosphäre genannt. Besonders in tropischen Regenwäldern findet sich auf den Blättern vieler Pflanzen ein üppiger Bewuchs an Epiphyllen aus unterschiedlichsten taxonomischen Gruppen wie Bakterien, Cyanobakterien, Algen, Pilzen, Flechten und Moosen, ja sogar kleinen Farnen. Klimatische Bedingungen, insbesondere relative Luftfeuchtigkeit, die Jahresniederschlagsverteilung und diurnale Temperaturschwankungen sind die Hauptfaktoren, die die Zusammensetzung und die Wachstumsraten der Epiphyllengemeinschaft beeinflussen.
Epiphylle Organismen dringen nicht durch die Blattoberfläche ins Gewebe ihrer Trägerpflanzen ein und werden daher nicht als pflanzenschädigend angesehen.
Da jedoch Flechten und Moose bisweilen die gesamte adaxiale Blattseite tropischer Unterwuchspflanzen überwachsen, reduzieren sie die Photosyntheserate der Trägerpflanze empfindlich. Zudem trocknet die Oberfläche stark epiphyllierter Blätter durch die Wasserhaltekapazität der Besiedler kaum ab, wodurch die Wahrscheinlichkeit einer Infektion durch Pathogene erhöht ist. Als mögliche positive Effekte des Epiphyllenbewuchses für die Trägerpflanze werden diskutiert, ob Inhaltsstoffe von epiphyllen Moosen und Flechten Herbivoren und pathogene Organismen abwehren oder ob Stickstoffmetaboliten, die von auf den Blättern lebenden diazotrophen Bakterien und Cyanobakterien produziert werden, für die Trägerpflanze verfügbar sind. Es wird derzeit jedoch davon ausgegangen, dass sich starke Epiphyllierung insgesamt negativ auf die Trägerpflanze auswirkt.
In vorangegangenen Studien konnte gezeigt werden, dass weder die Form, noch die Größe und Oberflächentextur der Wirtspflanzenblätter die Besiedelungsrate und Diversität der Epiphyllen beeinflussen. Dennoch werden am gleichen Standort manche Pflanzenarten deutlich schneller als andere von epiphyllen Moosen und Flechten besiedelt. Es konnte gezeigt werden, dass Pflanzenarten deren Blätter langlebig sind, meist beträchtlich langsamer von Epiphyllen bewachsen werden als solche deren Blätter eine vergleichbar kurze Lebensdauer aufweisen.
In der vorliegenden Arbeit wurde untersucht, ob die chemische Zusammensetzung der Blattcuticula die Geschwindigkeit der Blattbesiedelung durch die Epiphyllen- gemeinschaft beeinflusst.
Als Studienpflanzen wurden sechs Pflanzenarten ausgewählt, die häufig im Unterwuchs des

Esquinas Regenwalds nahe der Tropenstation La Gamba vorkommen: *Asplundia pittieri*, *Carludovica drudei*, *Costus laevis*, *Dieffenbachia concinna* cf., *Pentagonia wendtlandii* und *Polybotrya cervina*. An Standorten des Schluchtwalds und des Hangwalds wurden die biometrischen Daten der Pflanzen erhoben und voll entwickelte sowie seneszente Blätter gesammelt. Für jedes Individuum wurden der Epiphyllierungsgrad und die Zusammensetzung der Epiphyllengemeinschaft bestimmt und die mittlere Blattlebensdauer jeder Pflanzenart ermittelt. Nach Entfernen der Epiphyllen wurde die adaxiale Blattseite mit Ethanol oder mit Hexan überspült, um Blattwachsextrakte zu gewinnen. Außerdem wurden Blattextrakte und Extrakte von Epiphyllen hergestellt. Am Department für chemische Ökologie und Ökosystemforschung der Universität Wien wurden die Extrakte mittels HPLC-UV- und GC-MS- Messung aufgetrennt und anschließend ihre chemische Zusammensetzung anhand der erhaltenen UV- und MS- Spektren charakterisiert.

Die Zusammensetzung der Epiphyllengemeinschaft unterschied sich zwischen den zwei beprobten Standorten, jedoch nicht die Blattbesiedelungsrate. Flechten dominierten die Epiphyllengemeinschaft auf den Blättern der Wirtspflanzen vom Hangregenwald, epiphylle Moose hingegen dominierten auf den Blättern von Schluchtwaldpflanzen.
Die Epiphyllenbesiedelungsraten unterschieden sich zwischen den untersuchten Trägerpflanzenspezies. Die Blattoberflächen von *Costus laevis* wurden am schnellsten und jene von *Asplundia pittieri* am langsamsten von epiphyllen Flechten und Moosen überwachsen. Weiters konnte eine Korrelation zwischen der Blattlebensdauer der Trägerpflanzen und der Epiphyllenkolonisierungsrate festgestellt werden. Besonders deutlich war die Korrelation für *C. laevis* und *A. pittieri*; die mittlere Blattlebensdauer von *C. laevis* ist mit 1.6 Jahren die kürzeste und jene von *A. pittieri* mit 4.3 Jahren die längste.
Langkettige Alkane repräsentierten die Hauptkomponenten der mit Hexan extrahierten Blattwachsproben. Außerdem enthielten die Proben Alkanole, Sterole und nicht identifizierbare Substanzen. Die chemische Zusammensetzung der Blattwachse unterschied sich zwischen den Versuchspflanzenarten.
Es konnten drei Blattwachstypen innerhalb der untersuchten Pflanzen festgestellt werden: Ungeradkettige Alkane, insbesondere C29 und C31, dominierten die Wachsextrakte von *C. laevis*, *D. concinna* cf. und *P. wendlandii*. In den Proben von *A. pittieri* und *C. drudei* überwogen geradkettige Alkane und auch Sterole den Anteil der ungeradkettigen Alkane. Und das Wachs der Farnwedel von P. cervina enthielt gerad- und ungeradkettige Alkane zu ungefähr gleichen Teilen, neben beträchtlichen Mengen an unidentifizierbaren Substanzen. Wir fanden außerdem Unterschiede im Blattwachschemismus zwischen seneszenten und

fertig entwickelten Blättern bei *A. pittieri*, *C. drudei*, *D. concinna* cf., und *P. cervina,* aber nicht bei *C. laevis* und *P. wendlandii.* Besonders auffällig war, dass das Wachs alter Blätter von *D. concinna* cf. und *P. cervina* deutlich weniger langkettige Alkane als das Wachs ausgewachsener Blätter enthielt.

Ein Vergleich der Epiphyllenbesiedelungsraten mit der pflanzenspezifischen Blattwachszusammensetzung und den blattalterabhängigen Verschiebungen in der Wachschemie lieferte Hinweise darauf, dass die chemische Zusammensetzung der Blattcutikula Einfluss auf das Wachstum der Epiphylle hat. Ein hoher Anteil an kurzkettigen Alkanen im Blattwachs, beziehungsweise ein mit der Zeit abnehmender Gehalt an langekettigen Alkanen scheint das Epiphyllenwachstum zu vermindern.

Abstract

A great diversity of non-parasitic epiphytic organisms, so called epiphylls, has adapted to colonize the leaf surface of plants. In the wet tropics, epiphylls, particularly bryophytes and lichens, may cover up to eighty percent and more of the adaxial side of leaves and were shown to significantly reduce photosynthesis rates of their host. Although there is also evidence for host plant benefits of epipyllic organisms, the overall epiphyllation impact generally is assumed to be detrimental for the plant.

Previous studies report that the foliage of plant species with leaves of high longevity is covered by epiphylls at significantly slower rates than the foliage of plants with leaves of a short life span. Further it was shown that shape, size and texture of leaves does not affect epiphyll growth.

This study explored if the chemical composition of the leaf cuticle affects epiphyll coverage dynamics.

Fully developed and old leaves of six species of understorey plants, *Asplundia pittieri*, *Carludovica drudei*, *Costus laevis*, *Dieffenbachia concinna* cf., *Pentagonia wendtlandii* and *Polybotrya cervina,* were collected in the humid tropical rainforest of Piedras Blancas National Park, Costa Rica. Average leaf longevity, epiphyll coverage and community composition were determined for each species. After removal of epiphylls, the adaxial leaf wax was extracted with ethanol and hexane and the chemical composition of the extracts was characterized by HPLC-UV and GC-MS.

Site characteristics mainly influenced the ratio of lichen to bryophyte coverage on leaves, with lichens dominating epiphyll communities at slope sites and bryophytes dominating at ravine sites, but epiphyll growing rates did not vary between the two sampling sites. Rates of epiphyll colonization were shown to be host plant species specific. Macroscopically visible epiphylls were the fastest to colonize *Costus laevis* leaves and the slowest to colonize *Asplundia pittieri* foliage. Leaf longevity and epiphyll coverage rates correlated within the plants studied, especially in case of *C. laevis* and *A. pittieri*, the latter forming the longest living foliage (4.3 yrs.) and the former the shortest living leaves (1.6 yrs.).

Longchained alkanes represented the major analytes in the hexane-soluble leaf wax, apart from alkanols, sterols and unidentifiable compounds. The chemical composition of the extracted leaf cuticles was shown to be plant species specific.

Furthermore, shifts in the composition of the foliar wax between fully developed and old leaves were observed for *A. pittieri*, *C. drudei*, *D. concinna* cf., and *P. cervina,* but not for *C.*

laevis and *P. wendlandii.* A comparison of species-specific wax composition and leaf age-related shifts of cuticle components with epiphyll colonization rates provides support for the notion that the epicuticular wax chemistry affects epiphyll growth on leaves.

Keywords: Leaf cuticle, leaf wax, epiphylls, phyllosphere, leaf longevity, tropical understorey plants

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Curriculum vitae

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January 2010	BSc in biology, inscription in master study plant sciences
September 2004	Start of the master thesis at the Department of Chemical Ecology and Ecosystem Research, University of Vienna, master subject: Phytochemistry and plant physiology
September 2002	Recognition of first diploma degree in biology from the University of Graz by the University of Vienna. Inscription in second study phase of biology, study branch botany (A 438) at the University of Vienna
June 2002	First diploma degree in biology (B 437) at the University of Graz
October 1999	Inscription in diploma study biology (B 437) at the University of Graz
June 1998	Graduation at BG Tanzenberg, Maria Saal