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„Chemodiversity of *Clusia minor* (Clusiaceae)"

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1 Abstract

The species of the neotropical woody plants in the genus *Clusia* (Clusiaceae) are primarily researched for their photosynthetic strategies. However, *Clusia* spp. are also remarkable sources of peculiar and bioactive specialized plant metabolites (SPM). The members of this genus appear to predominantly produce compounds derived from the phenlypropanoid biosynthetic pathway, in combination with terpenoid and the polyketide pathways. *Clusia* is noted particularly for the most abundant occurrence of benzophenone derivatives in plants. The aim of the present study was to further enhance the knowledge on the SPM chemodiversity of this genus. For this purpose, root extracts of *C. minor* were chosen for a detailed phytochemical analysis, applying standard chromatographic techniques for separation and isolation. Structure identification of isolated compounds was achieved by MS and NMR data. Of the five isolated compounds, three of them could be structurally characterized. They comprised two substances, which are related to tocotrienols (*Z*-δ-garcinoic acid and δ-tocotrienol), and a further one relating to benzophenones, respectively. The latter is a hitherto undescribed compound with a peculiar 5-membered cycle, for which the name minorone was designated. In addition, current knowledge on the SPM chemodiversity of *Clusia* spp. is summarized and discussed extensively.

1.1 Keywords

Clusia minor, specialized plant metabolites; tocotrienol derivatives, benzophenone derivatives

2 Introduction

The neotropical genus *Clusia* comprises some estimated 400 species (Gustaffson et al., 2007). The countries with the most abundance and diversity of species are Colombia, Ecuador, Brazil and Venezuela (do Nascimento et al., 2019). There they occur in many different habitats for which they appear to be well adapted. Of these adaptations, the photosynthetic peculiarities (primary metabolism) are well understood (Lüttge, 2007). Far less is known about the chemodiverstiy of specialized plant metabolites (SPM; secondary plant metabolism), not only in respect to structural variation, but also concerning their possible function in an ecological context. Due to its crucial role, primary metabolism is highly conserved. On the other hand, SPM may be involved in various functions, in mediation of interactions with the environment, such as coping with stress factors, as defense compounds or involved in symbiotic interactions. Taken together, structural diversity, biosynthesis, specific accumulation, and function, are the key features of SPM chemodiversity.

The roots of *Clusia* are a very poorly researched organs in terms of SPM, with just three studies focusing on *C. paralicola* (G. Mariz) and *C. eugenioides* (Planch & Linden) (Gonzalez et al., 1993; Seo et al., 1999; Delle Monache et al., 2002). Regarding *C. minor* (L.), only few reports exist: Four of these focus on terpenoids and alkanes of the leaves (Medina et al., 2006; Mangas Marin et al., 2008a; Mangas & Leyva, 2018; Mangas et al., 2019), one report on benzophenones from the fruits (Mangas Marin et al., 2008b), and another one on terpenoids from the fruits (Gonzalez et al., 1993), respectively. In view of the number of species in this genus, there is little known about SPM composition. Equally, there is not much information about the biosynthetic background of known SPM of *Clusia* species. In continuation of ongoing studies, this work aims at adding more information of root constituents of *C. minor* to the already described compounds from leaves and stems (Schinnerl, pers. comm.). So far, only a few phenolic compounds have been isolated from *C. minor,* although one might expect the occurrence of a series of phenolics, mainly benzophenones, like known from other *Clusia* spp. (Baggett et al., 2005). As chemical data are quite scattered in literature, another aim was to compile relevant information towards a better understanding of the SPM chemodiverstiy in *Clusia*.

2.1 Background of *Clusia*

Clusia is a genus of plants sometimes referred with the common names of autograph tree, pitch apple, copey, cupey, scotch attorney, porcelain flower (name shared with the genus *Hoya*) or balsam apple (name shared with *Momordica balsamina* (L.)). It is characterized by a wide set of unusual and interesting adaptations which caught the attention of researchers during the years and is the study subject of this thesis.

2.1.1 Higher taxonomical classification

Clusiaceae, to which *Clusia* belongs to, is a family of flowering dicotyledonous plants of the order Malpighiales. Within its order, the Clusiaceae family is part of a suborder clade, called Clusoids, along with the other four families: Bonnetiaceae (**Figure 1A**), Calophyllaceae (**Figure 1B**), Hypericaceae (**Figure 1C**) and Podostemaceae (**Figure 1D**) (Ruhfel et al., 2011).

Figure 1: Flowers of: A) *Bonnetia sessilis* (Bonnetaceae), B) *Calophyllum inophyllum* (Calophyllaceae), C) *Hypericum calycinum* (Hypericaeae), D) *Mourera fluviatilis* (Podostemataceae). (Source: Alexey Yakovlev, Forest & Kim Starr, John Harrison, Armida Madngisa)

Both, the families and their order, possess a high diversity in its morphological structure, making them impossible to classify via morphological studies but thanks to the advent of more advance DNA techniques and phylogenetic analyses, the taxonomy of these groups was better elucidated and integrated in the latest versions of the APG (III & IV) (Gustaffson et al., 2002; Stevens, 2001–2022). Clusiaceae, depending on the literature, possess around 750–1200 species divided in 13–40 genera (Christenhusz & Byng, 2016; Raj et al., 2022). The large size of the interval of species' number is, again, due to the refinement of phylogenetic classification, with genera being moved in and out of this family, within the Clusoids clade and with new species being discovered in the meantime.

Clusiaceae share some common features. They are solely tropical, evergreen, flowering, dicotyledonous plants with a duct system that carries a milky sap through all the plant (Gustaffson et al., 2002; Ruhfel et al., 2011). These plants have a large employment of the lipophilic biosynthetic pathways that produces a plethora of bioactive, exotic compounds, many of these belonging almost uniquely to this family or subordered taxa (Vieira & Kijjoa, 2005, Baggett et al., 2005). Some peculiar morphological characteristics that belong only to some members of the family are: the rewarding of pollinators via resin production, the presence of both C3 and CAM photosyntheses, and the presence of all woody forms (tree, shrub, liana) some of which show primary hemi-epiphytic growth forms. (Stoffer & Lindeman, 1966; Gustaffson & Bittrich, 2002, Lüttge, 2006, Stevens 2007).

Historically, within the family of Clusiaceae, two distinct monophyletic subfamilies could be distinguished: Clusioideae and Kielmeyeroideae (Stevens 2005). In more recent times, Kielmeyeroideae has been recognized as a distinct family nowadays known as Calophyllaceae, making the two-subfamily system of Clusiaceae obsolete. The Clusiaceae *sensu stricto* (formerly subfam. Clusioideae) can be divided in the three distinct tribes: Garcinieae (**Figure 2A**), Symphonieae (**Figure 2B**) and Clusieae (**Figure 2C**) (Ruhfel et al., 2011).

Figure 2: Flowers of: A) *Garcinia mangostana* (Garcinieae), B) *Symphonia globulifera* (Symphonieae), C) *Tovomita parviflora* (Clusieae). (Source: powo.science.kew.org, Gustavo Shimizu)

The Clusieae tribe is the one to which the genus *Clusia* (**Figure 3B**) belongs to, along with the genera *Chrysochlamys* (**Figure 3A**), *Dystovomita* (**Figure 3C**), *Tovomita* (**Figure 3E**), *Tovomitopsis* (**Figure 3F**) and the freshly new categorized genus *Arawakia* (**Figure 3D**) (Marinho et al., 2019).

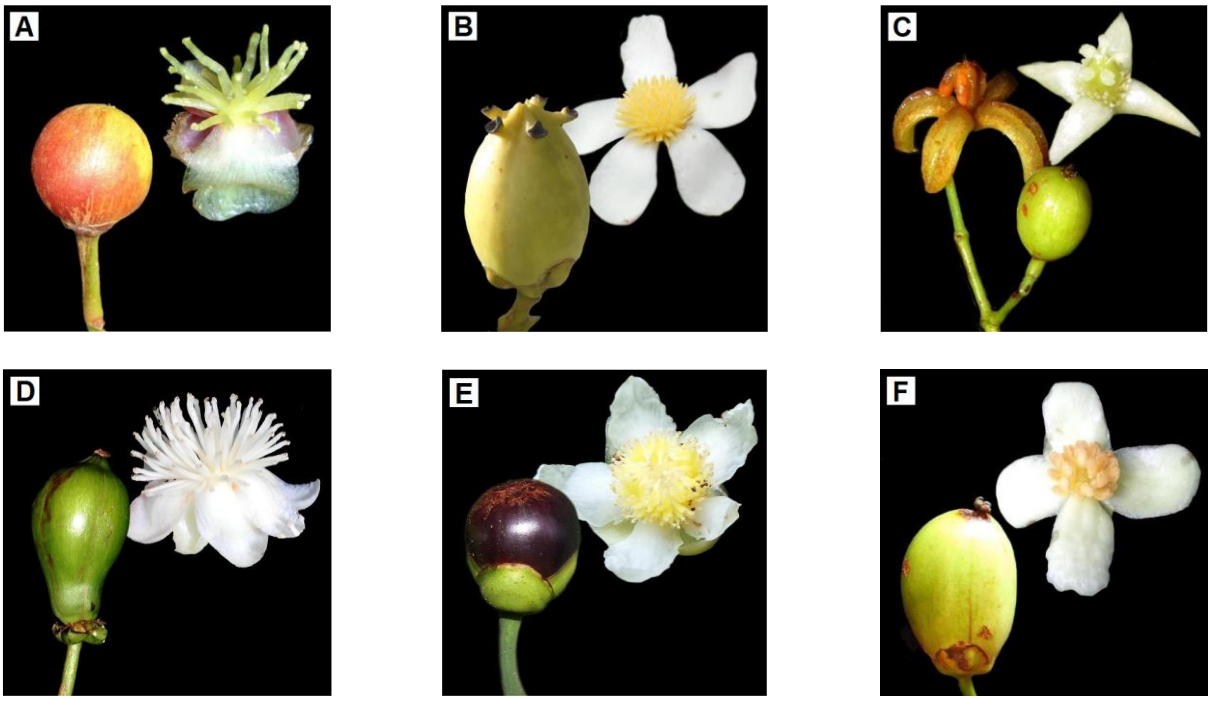

Figure 3: Fruits and flowers of: A) *Chrysoclamys* sp., B) *Clusia* sp., C) *Dystovomita* sp., D) *Arawakia* sp., E) *Tovomita* sp., F) *Tovomitopsis* sp. (Source: Marinho et al., 2019)

2.1.2 Diversity

The most complete list of *Clusia* spp., the website Plants of the World Online (viewed in 2022), recognizes 321 accepted species. In reality, the list is not updated and there are quite some missing species not to mention the conspicuous number of undescribed species. Sensible estimates set the total number of *Clusia* spp. to be up to 400 species (Gustaffson et al., 2007). Historically the genus *Clusia* was divided in various genera (*Arrudea, Androstylium, Clusia, Cochlanthera, Decaphalangium, Havetia, Havetiopsis, Oedematopus, Oxystemon, Pilosperma, Quapoya, Renggeria and Rengifa)* (Aublet, 1775; Planchon & Triana, 1860) that were incorporated in one genus (Pipoly et al., 1988) and later recognized as monophyletic when grouped together (Gustafsson & Bittrich, 2002; Gustafsson et al., 2007). The merging of two phylogenetic analysis papers (Gustafsson & Bittrich, 2002; Gehrig et al., 2003) gave rise to the most comprehensive knowledge of the internal phylogenetic tree of *Clusia* to date (**Table 1**) (Gustafsson et al., 2007). A more recent study was conducted in 2022 (Luján et al.) with a large subset of the one from Gustafsson et al., 2007. The genus divides in numerous subgroups referred as sections (sect.): Anandrogyne, Havetia, Cochlanthera, Retinostemon, Clusiastrum,

Cordylandra, flava-group, Omphalanthera complex, Phloianthera, Chlamydoclusia, Androstylium, Criuvopsis, Criuva and Oedematopus.

Table 1: Species of *Clusia* with known section (Gustafsson et al., 2007)

Section	Species	Section	Species
Anandrogyne	*coclensis*	Criuva	*burle-marxii*
	congestiflora		*criuva*
	cupulata	Criuvopsis	*amazonica*
	ducu		*hammeliana*
	elliptica	Flava-group	*flava*
	flaviflora		*quadrangula*
	melchiorii		*torresii*
	multiflora	Oedematopus	*cylindrica*
	palmana		*gundalchii*
	salvinii		*octandra*
	stenophylla	Unnamed distinct clade	*clusoides*
	tocuchensis	Unnamed distinct clade	*osseocarpa*
	triflora	Omphalanthera complex	*aripoensis*
Androstylium	*fockeana*		*columnaris*
Chlamydoclusia	*grandiflora*		*favum*
	insignis		*gardneri*
	nemorosa		*major*
	palmicida		*schomburgkiana*
	platystigma		*scrobiculata*
	rosea		*valerioi*
	viscida	Phloianthera	*hilariana*
Clusiastrum	*pusilla*		*lanceolata*
Cochlanthera	*celiae*		*mexiensis*
	orthoneura		*studartiana*
Cordylandra	*burchelli*	Retinostemon	*croatii*
	diamantina		*divaricata*
	fluminensis		*fructiangusta*
	leprantha		*liensneri*
	panapanarari		*lineata*
	paralicola		*longipetiolata*
	pernambucensis		*minor*
	renggerrioides		*odorata*
	spiritu-sanctensis		*pallida*
	weddeliana		*pratensis*
Havetia	*colombiana*		*uvitana*

2.1.3 Distribution

This genus is endemic solely to the neotropics with a geographical range that extends from Florida (USA) to the extreme north, up to Grande do Sul (Brazil) to the extreme south (**Figure 4**) (do Nascimento et al., 2019). The countries with the most abundance and diversity of species are Colombia, Ecuador, Brazil and Venezuela (do Nascimento et al., 2019).

Figure 4: Distribution of *Clusia* spp. (Source: powo.science.kew.org)

The genus covers the full range of altitudes available, from the very shoreline at ~ sea level up to more than 3500 m a.s.l. on the mountain ranges (Gustaffson et al., 2007). In terms of habitats the genus is widely spread and can be found: along the coast on sandy dunes beaches or rocky shores, on coastal mounts, in drier areas like the savana or the cerrados, in human-modified areas like in green corridors up to the cities, at high altitude whether rocky highlands or on mountain slopes and in all possible tropical forests whether dry, rainy-humid, cloudy-humid or in tropical planes (Weckwerth, 2022). Humans have introduced *Clusia* also in other parts of the world such as Hawaii, Sri Lanka, Guam and South Africa where it has become a serious threat to the local flora as an invasive species. (Weerawardane & Dissanayake, 2003; Pacific Islands Ecosystems at Risk, viewed 2022; Plants of the World Online, viewed 2022).

2.1.4 Morphology

Clusia is a genus of solely woody plants that can be found in all forms: tree, shrub and liana (Gustaffson et al., 2002; Lüttge, 2008). All species have a duct system that carries a milky sap in all parts of the plant (Stoffer & Lindeman, 1966). Many of these species produce aerial adventitious roots system reaching down to the ground from the branches (Gustaffson et al., 2007). The phyllotaxis of the genus is of opposed and decussated leaves (Stoffer & Lindeman, 1966). The leaves (**Figure 5A**) are thick, waxy, elliptical to lanceolate, with clear margins and one main vein from which secondary veins depart parallel to each other (Stoffer & Lindeman, 1966). The leaves have a multilayered and waxy epidermis that protect the leaf from water loss, a thick upper hypodermis that filtrates the UV radiation, a multilayered palysade mesophyll typical of CAM plants (Lüttge, 2008). Latex ducts are found also in the leaves, and their size, position and content is species-specific. In the leaves round calcium oxalate crystals called druses may be present, whose abundance and position depends on the species and the ontogeny of the leaf. The flower's petals (**Figure 5B**) are thick, waxy with smooth curves and clear margin (Stoffer & Lindeman, 1966). The number of structural parts of the flower is highly variable and dependent on the species. Many species are dioceous (Stoffer & Lindeman, 1966). The fruits appear as oval to spheroidal capsules when unripe (**Figure 5C**) that, once mature, split open into slices forming a star-like shape with a ribbed core at the center (**Figure 5D**) (Mourão & Marzinek, 2009). The opening of the capsule exposes the diaspores, which are sticky, bright-colored arills disposed on the star-shape arms or in the ribs of the central core (Mourão & Marzinek, 2009).

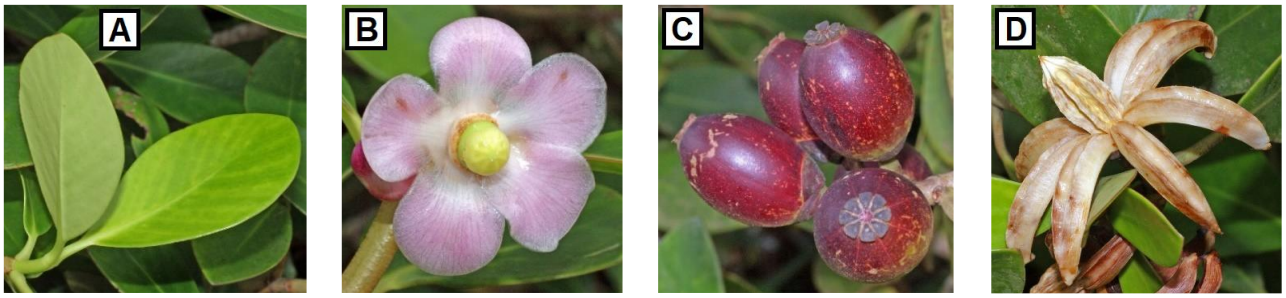

Figure 5: *C. minor*: A) leaves, B) female flower, C) unripe fruit capsule, D) mature, empty fruit capsule (Source: Andrés Hernández)

The genus does not possess unique synapomorphies, though it has some traits that are common to most of the members of this genus and are mostly absent in its sister genera:

seeds that are <5 mm long, the aril is not vascularized, there's more than one seed per carpel, a multilayered hypodermis of leaves and a tendency to a hemi-epiphytic lifestyle, with long adventitious aerial roots (Lüttge, 1991; Gustaffson et al., 2007).

2.1.5 Key innovations

The genus *Clusia* has a noticeable repertoire of special adaptations that caught the attention of many researchers. Among these adaptations, three have been pointed out as the key innovation, which are considered to be the main explanation on the great success of this genus in terms of diversity and distribution: hemi-epiphytism, floral resin reward and CAM photosynthesis (Gustafsson et al., 2007).

2.1.5.1 Hemi-epiphytism

Clusia spp. mainly grow as phanerophyte and primary hemi-epiphyte (Stoffer & Lindeman, 1966). The phanerophytic strategy is the one exploited by most trees in which the seed sprouts directly on the ground and, without any support, it elevates its canopy vertically (Raunkiaer, 1934). The hemi-epiphytic strategy involves a stage of life where the plant lives on the canopy of other trees with no connections to the ground and another stage of life where the plant has a root system connected to the understory soil (Zotz, 2013). If the hemi-epiphyte starts on the other tree's canopy and then develops a connection to the soil, it's called a primary hemi-epiphyte otherwise, if it starts from the soil and climbs its way up to the other tree's canopy it's called a secondary hemi-epiphyte (**Figure 6**) (Zotz, 2013). In the case of *Clusia* spp. only primary hemi-epiphytes can be found. Hemi-epiphytes are characterized by the production of aerial and adventitious roots though this trait has been observed also on phanerophytic *Clusia* spp. (Universidad Catolica de Oriente, viewed 2022).

Figure 6: *C. rosea* sprouting out from the wood of another tree species (Source: Forest & Kim Starr)

When a hemi-epiphytic *Clusia* establishes its connection to the ground it starts a rapid growth of both the canopy and the root system, posing an aggressive competition to the host tree in terms of both light exposure, soil nutrients and physical space constraint. The competition ultimately ends with the death of the host tree and that is the reason for why *Clusia* are also known as strangling trees (Ball et al., 1991).

2.1.5.2 Floral resin reward

Usually, a flower rewards its pollinators by supplying a service for the most basal need of the pollinators whether food, shelter or a deception of sex. It is therefore very atypical for a flower to propose as a reward an inedible waxy material (Armbruster, 1984). Rewarding with resin is a very rare trait and restricted to few specialized taxa, *Clusia* being one of them (Armbruster, 1984). The reason for why many species of this genus evolved to produce this rare reward stands in a long story of coevolution with the stingless bees, family Apidae, tribe Meliponini (**Figure 7**) (Armbruster, 1984).

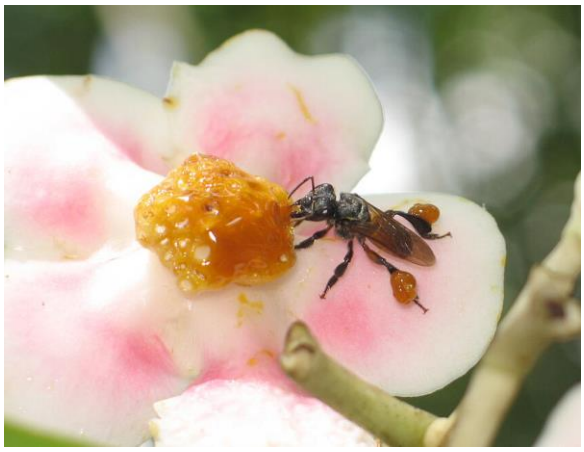

Figure 7: Stingless bee collecting resin from a flower of *Clusia* (Source: W. Hödl)

These bees build theirs hives using waxy materials, which, in the absence of *Clusia* would be quite difficult to find in the tropical forest. The floral resin is malleable and waterproof and is therefore perfect for being shaped as required, preventing water infiltrations in the hive. The floral resin is enriched with a combination of bioactive, lipophilic phenolic compounds, which confer antibacterial and antifungal properties to the wax (Lokvam & Braddock, 1999; Bicalho et al. 2003). The hygiene of the nest is a particularly important feature for the correct functioning of the communities in social insects. Moreover, predatory ants, which are the main biological drivers in the tropics, selectively avoid walks on *Clusia* waxy cuticle, and it is believed that this quality is also transposed in the wax produced by the flowers and transferred to the hives.

2.1.5.3 C3 to CAM photosynthesis

Photosynthesis is the mechanisms used by plants to fixate sunlight's energy inside of chemical bonds of energetical molecules that subsequently can be used to fuel the life of the plants and sustain the food web of all those ecosystems that rely on this form of primary production. Such a fundamental biochemical process is highly preserved but nonetheless it has evolved into few forms, which have some adaptation that give an advantage under specific environments. The main issue of photosynthesis stands in the requirement of two compounds necessary for this process, CO_2 and H_2O. Carbon dioxide is collected through diffusion by exposing the plant's internal tissues to the air, but this inevitably results also in a consistent loss of water from the plant's tissues in the air (Willmer & Fricker, 1996). The gateways that con-

trol this exchange of molecules are the stomata, found typically in the abaxial lamina of leaves (Willmer & Fricker, 1996).

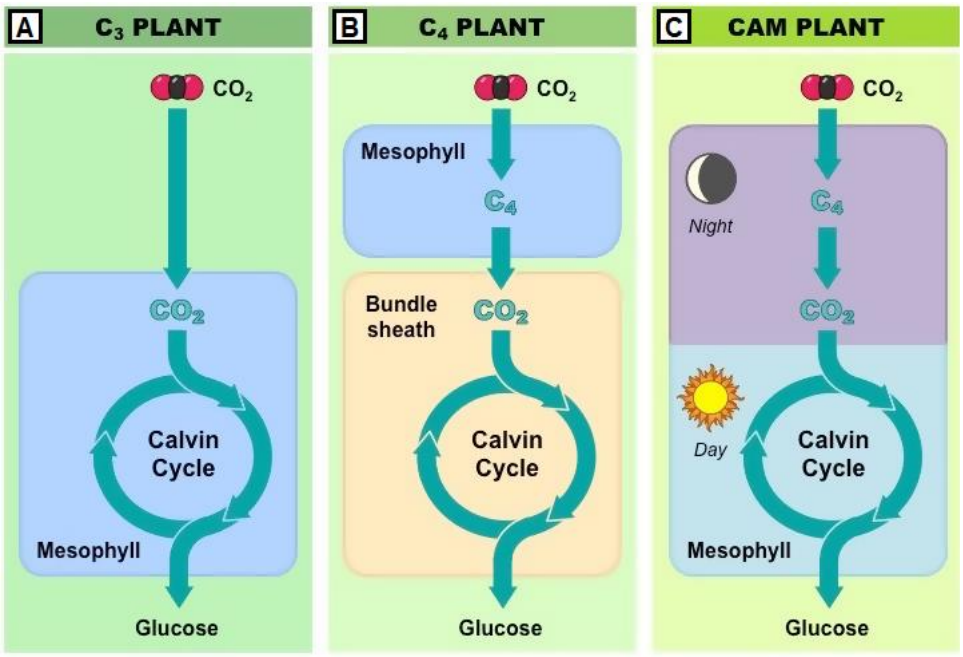

Figure 8: Photosynthetic strategies: A) C3, B) C4, C) CAM (Source: cid-inc.com, viewed September 2022)

The most simple, diffused and ancient version of photosynthesis is called C3 (**Figure 8A**) (Calvin, 1997). In this strategy, the stomata are the main controllers, opening when the plant is exposed to light and closing if it's dark or the water loss is too severe. The CO_2 in this case is directly directed to the Calvin cycle for immediate fixation in the 3-carbon molecule 3-phosphoglycerate and consequentially into sugars (Calvin, 1997).

A second photosynthetic strategy is called C4 in which the CO_2 is fixated in the 4-carbon molecule malate in the chloroplast of the mesophyll and furtherly gets directed to the chloroplasts of the bundle sheath cells where CO_2 is released again and follows the Calvin cycle (**Figure 8B**) (Christin & Osborne, 2014). The specialization and spatial separation of the chloroplasts along a working chain improves the CO_2 intake ability with a consequential lower water loss (Osborne & Sack, 2012). It's the photosynthetic strategy adapted to withstand heat stress and is found almost only in grassy plants. The separation of carbon fixation through tissues is known as Kranz anatomy (Lundgren et al., 2014). In recent years a single-cell form of C4 was discovered with specialized peripheral and central chloroplast inside of single cells too (Stutz et al., 2014).

The last photosynthetic strategy is called crassulacean acid metabolism or CAM (**Figure 8C**). Instead of creating a spatial separation like C4, it makes one timewise: the stomata open during the night when the air is cooler and moister to minimize the water loss and the CO_2 is fixated in malate and its derivatives and stored up to the daytime when the stomata close and the stored carbon dioxide can be used for the photosynthesis (Winter & Smith, 1996). This is the winning strategy of plants that grow in environments, subject to droughts (Winter & Smith, 1996). Between C3 (**Figure 9A**) plants and fully CAM (**Figure 9D**) there are two transitional subcategories: CAM-cycling plants (**Figure 9B**) resembles C3 in terms of open stomatal timings but additionally fixates the CO_2 produced during the nighttime respiration, CAM-idling (**Figure 9C**) plants keep the stomata closed most of the time and rely mainly on the CO_2 produced via respiration (Tay et al., 2021). Additionally, some species can do facultative CAM, which is the ability to switch in between C3 and the various CAM strategies to better reflect the environment conditions on the moment (Winter et al., 2008).

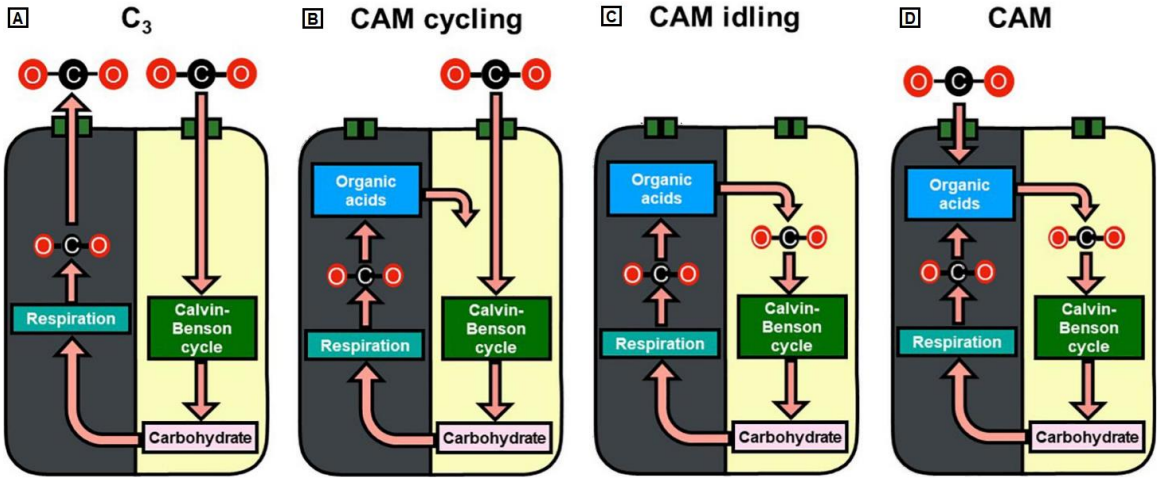

Figure 9: The C3-CAM spectrum of strategies: A) C3, B) CAM-cycling, C) CAM-idling, D) CAM (Source: Tay et al., 2021)

Clusia, depending on the species, can perform C3, facultative CAM or mainly CAM (Barrera Zambrano, 2012). This adaptation gives an advantage to *Clusia*, especially when considered that the tropics are alternated by rainy, wet seasons and sunny, dry ones. What makes this genus particularly remarkable stands in the fact that *Clusia* is the only known woody taxa able to perform forms of CAM photosynthesis (Lüttge, 2007). This unique characteristic is the main subject topic of studies conducted on *Clusia*.

2.1.6 Ecological interactions

Clusia are flowering plants that rely solely on animals for pollination (Hochwallner et al., 2012). The type of pollination syndrome is species-specific. The most abundant one is the bee pollination syndrome which relies on two neotropical tribes of the family Apidae, the Meliponini also known as stingless bees, and the Euglossini also known as orchid bees, respectively (Kaminski & Absy, 2006). Bee-pollinated flowers of *Clusia* are scented and produce a floral resin as reward. The second most abundant pollination syndrome is the one mediated by birds, more specifically by flower piercing birds of the genus *Diglossa* and by some member of the Trochilidae family, better known as hummingbirds (Rojas-Nossa, 2007, Rojas-Nossa et al., 2016). The flowers pollinated by birds produce a very sugary nectar instead of floral resin (Schondube & Del Rio, 2003). The pollination syndromes of *Clusia* spp. are a proxy of the altitude where these species are found. Due to the fact that the higher the elevation, the lower the insects' abundance becomes, and consequently, bird-pollination becomes more trending (Dellinger et al., 2021). Nectariferous flowers are suspected to be pollinated also by some bats, since *Clusia* pollen grains were collected on bats of the genus *Anoura* (Muchhala & Jarrin, 2002). Other minor pollinator syndromes related to just few *Clusia* spp. are mediated by pollen-eating beetles of the genus *Dinaltica,* or by cockroaches of the genus *Amazonina,* which get attracted to the flowers by compounds that emulate their pheromones (Vlasáková et al., 2008). Apart from these known pollinators, other flower visitors, who's roles have not been defined yet, have been reported. Flying insects such as wasps, flies and moths visit the flowers and may be involved in pollination. Non-flying flowers visitors reported on *Clusia* are ants, crickets, other genera from cockroaches, spiders, scorpions and springtails (Vlasáková et al., 2019), but their role is in the dark.

Like the pollination, also the seed dispersal relies solely on animal interactions, more specifically epizoochorally, where the seed gets transported by the animal without passing through the digestive tract. The main dispersal method is ornitochory (Passos & Oliveira, 2002). The birds are attracted by the bright red/yellow arils exposed on the mature fruits and by slopy feeding they get some of the sticky diaspores on their body. If a fruit happens to fall to the understory, the diaspores will be collected by ants, a dispersal method also known as myrmecochory (Passos & Oliveira, 2002).

Clusia are involved with few biotic interactions that are specific to this genus only. The leaves are parasitized by some gall-inducing flies of the family Cecidomyiidae (**Figure 10A**) (Maia, 2021). This is a wide and understudied family of flies and though few genera (*Parazalepidota* and *Clusiamyia*) have been reported on *Clusia*, there are probably more members of this family still to be discovered and described. The female flies deposit an egg inside the leaf tissue that develops a bump shaped gall from which the next generation fly will emerge. Another insect symbiosis is given by the selective choice of wasps to build their nests on *Clusia* spp. (**Figure 10B**). The reason is to be found most likely in the selective avoidance of *Clusia* spp. from ants and therefore a favorable nesting site for the wasps (Corbara et al., 2009). There is no study on beneficial support the wasps provide to the *Clusia* so it is to be considered just as a commensalism up to further proof. Lastly, *Clusia* litter, mostly leaves and sometimes fruits, are the only substrate where the fungi of the family Xylariaceae *Xylaria clusiae* was found producing fruiting bodies (**Figure 10C**) (Ju et al., 2018). The fungus serves a saprotrophic role in this stage, but no study has been carried to assess the potential as parasite, eusymbiont or possibly endophyte in the living tissues.

Figure 10: Biotic interactions specific to *Clusia*: A) Galls of *Parazalepidota clusiae* on *C. fluminensis*, B) Nest of *Polybia scrobalis*, C) Fruiting body and spores of *Xylaria clusiae* (Source: Guimarães et al., 2020, Maccann et al., 2014, Ju et al., 2018)

Other than the sustainment of the local bees' communities and the enhancement and diversification of niches like in the ant-wasp's nest reported cases, *Clusia* has been demonstrated to play additional roles in the ecological interactions. In arid, nutrient poor soils, the presence of *Clusia* in the canopy is correlated with an additional production of leaf litter, which enriches the soil and promotes the nutrients' cycling (Villela et al., 2020). In areas of scarce freshwater availability, like on beaches or arid areas, resistant species of *Clusia* can become the first colonizers of these locations (Lüttge, 2007). With the consolidation of the

sand or soil through the root system and thanks to the partial shading with the wide leaves, *Clusia* produce a local microclimate with slightly more favorable conditions for the sprouts of other species to survive their critical early stages. This process is also known as Nurse-plant syndrome (Lüttge, 2007).

2.1.7 Uses

Humanity has found various ways to put *Clusia* spp. to good use. Due to its resistance to many factors, *C. rosea* has been largely exported as an ornamental plant for inhouse, garden and fencing (**Figure 11B**). The aerial roots are very resistant and are a valuable source of fibers or can be knotted directly to produce baskets (Plants of the World Online, viewed 2022). The wood has been used for construction and cabinetry (Plants of the World Online, viewed 2022). The thick leaves that scar when cut (**Figure 11A**) can be used as substitutes for playing cards, as ornaments or sewed together as a cheap version of leather to make playing balls and hats (Plants of the World Online, viewed 2022). The leaves can also be used to feed livestock (Plants of the World Online, viewed 2022). The milky sap has been used as incense and in ethnomedicine to treat fever, flu, cold, rheumatic pain, hernias, promote wound healing, as laxative and astringent (Plants of the World Online, viewed 2022).

Figure 11: Examples of human uses of *C. rosea*: A) leave's art project; B) fencing (Source: zetangle.blogspot.com, kens-nursery.com)

Thanks to its plethora of special adaptations and ecological services, *Clusia* has been proposed for several ecological uses. The complex root system could serve a role in consolidating unstable masses of soil such as riverbanks, deforestated areas, or beach dunes (Lüttge, 2007). The resistance to heat, drought, salinity and shading of certain species like *C. rosea*, combined with a relatively fast growth, make this species a candidate for ecological restora-

tion in green corridors and areas prone to desertification. The combination of salinity and drought resistance combined with the nurse-plant syndrome led to the proposal that *Clusia* could play a crucial role in its own colonization and that of other species on new islands (Lüttge, 2007).

2.1.8 Species used in the study

This study focuses on the species *Clusia minor* (L.) (**Figure 5, Figure 12**) which belongs to the sect. Retinostemon (Gustafsson et al., 2007). It is a woody tree that can reach up to 10 meters in height (Croat, 1978). It can be found in humid forests or close to the shoreline, typically as a hemi-epiphyte over rocks or trees (Croat, 1978). Its photosynthesis is CAM facultative and can switch in response to the environmental conditions (Borldan et al., 1992). The species is dioceous, with flowers producing resin as a reward (Salatino et al., 2005).

Figure 12: *Clusia minor* (Source: powo.science.kew.org)

2.2 Specialized metabolites

The taxa used in this study are of particular interest concerning chemodiversity, as they represent the main natural sources of certain compound classes. In particular, the vast majority of xanthones are found within the family Clusiaceae (Vieira & Kijjoa, 2005) and the vast majority of benzophenones are typically found in the genus *Clusia* (Baggett et al., 2005). Many of these compounds have shown interesting bioactive properties that could find useful human applications. Way before the understanding of chemistry, many of these plants were already used in ethnobotanical medicine because of their properties (Plants of the World Online, viewed 2022).

The best exploited biosynthetic pathways in Clusiaceae are the terpenoid and the phenolic ones. These just mentioned classes of compound are characterized mainly by carbon and oxygen. On the other hand, classes of compounds that require other elements, such as nitrogen for alkaloids, are virtually absent from the Clusiaceae's literature and seem to be restricted to the primary metabolism only. This is most likely reflective of the fact that plants in the tropics, and Cusiaceae in particular, grow on soils that are notoriously poor in nutrients. Therefore, it is reasonable to believe that important and rare nutrients are not to be exploited for purposes other than survival. This last fact on the other hand, may, at least partially, explain itself why this family did receive a major evolutionary pressure to radiate its arsenal of carbon-oxygen-based compounds. A comprehensive list of SPM isolated from the genus *Clusia* can be found in the Appendix section.

2.2.1 Terpenoids

The terpenoids, with over 60,000 compounds are by far the largest class of secondary metabolites (Zeng et al., 2019) and are present in almost every life form (Oldfield & Lin, 2012). The building blocks of all terpenoids are the pyrophosphate forms of the branched 5-carbon molecules; isopentenyl pyrophosphate (IPP) and its allyl isomer, dimethylallyl pyrophosphate (DMPP). The condensation in various forms, the numbers of elements, cyclizations and additional modifications explains the vastness of this compound class. There are two possible biosynthetic pathways two obtain of IPP and DMPP units: the mevalonic acid (MVA) in the cytosol and the methylerythritol phosphate (MEP) in chloroplast (**Figure 13**) (Dudareva et al., 2013).

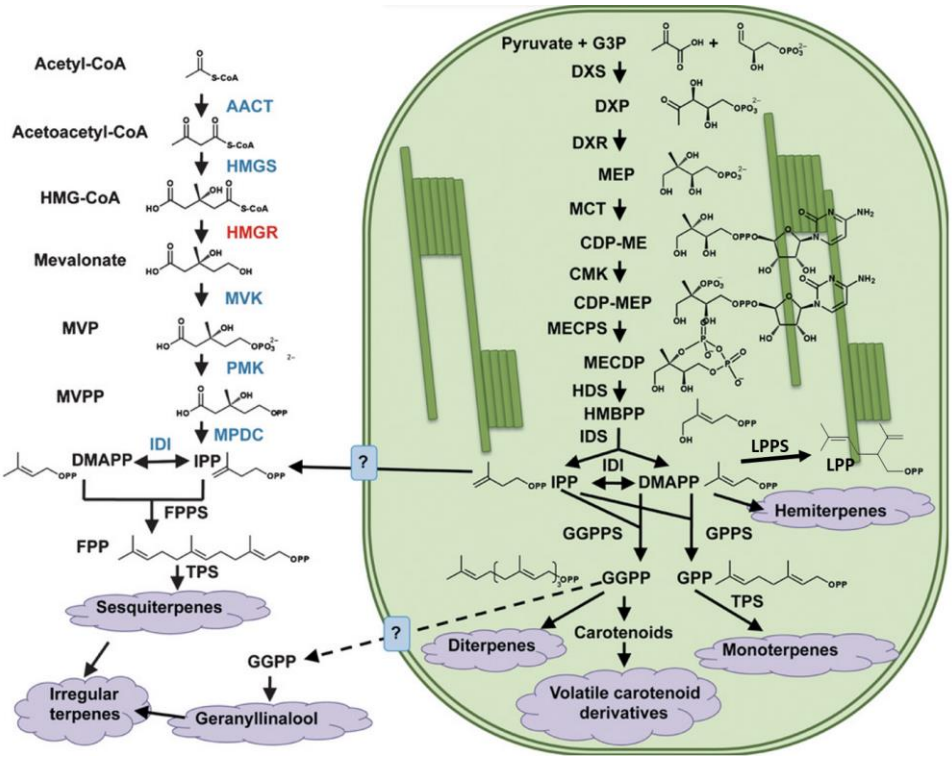

Figure 13: MVA and MEP biosynthetic pathways leading to the isoprene and polyisoprene units (Dudareva et al., 2013 [modified])

The function of terpenoids is as vast as its class, ranging from direct and indirect defense molecules to interspecific positive interactions and more (Cheng et al., 2007). Purely terpenoid-based compounds and moieties can be subclassified depending on the number of isoprene units involved in its synthesis. What often happens is that the terpenoid pathway combines with another biosynthetic pathway, producing hybrid molecules. This can happen with a single condensation of the molecules from each pathway or with multiple side additions of terpenoid moieties to the other molecule. These modifications change the molecule, giving it new qualities, enhancing the number of exposed carbons, with the possibility of further modifications. *Clusia* spp. are reported to be a valuable source for pure terpenoid-based classes, especially sesquiterpenoids and triterpenoids. There are some publications on common hybrid molecule classes, like pheophytins and tocotrienols (Teixeira et al., 2006; Ferreira et al., 2016). The addition of multiple terpenoid moieties, especially to phenolic cores, is the main reason for the extreme radiation of apolar compounds in *Clusia* spp. These compounds are frequently addressed as polyisoprenylated phenols (or analogous phenol's subclasses name) and exploit the use of various terpene moieties such as isopentenyl, dimethylallyl,

geranyl, geranylgeranyl and lavandulyl (Cahoon et al., 2003; Baggett et al., 2005; Teixeira et al., 2005)

2.2.2 Phenolic compounds (Phenylpropanoids)

There are estimated to be several thousands phenolic compounds in the plant kingdom of which the vast majority of these can be found in land plants. The major biosynthetic pathway for the biosynthesis of phenolics is called the shikimate pathway (**Figure 14**). This pathway roots in the combination of the primary metabolites erythrose-4-phosphate (Ery4P) and phosphoenolpyruvate (PEP) that combine to produce first dihydroshikimate and consequentially shikimate. The pathway leads then to the production of the fundamental aromatic amino acids, phenylalanine and tyrosin and further on to cinnamate as the basic phyenylpropanoid structure. Cinnamic acid is the precursor *inter alia* to benzoic acid and *p*-coumaryl-CoA. These last two mentioned compounds represent the main crossway of the shikimate pathway, with numerous pathways departing from these starting molecules leading to various phenolics and polyphenolics (Vogt, 2010).

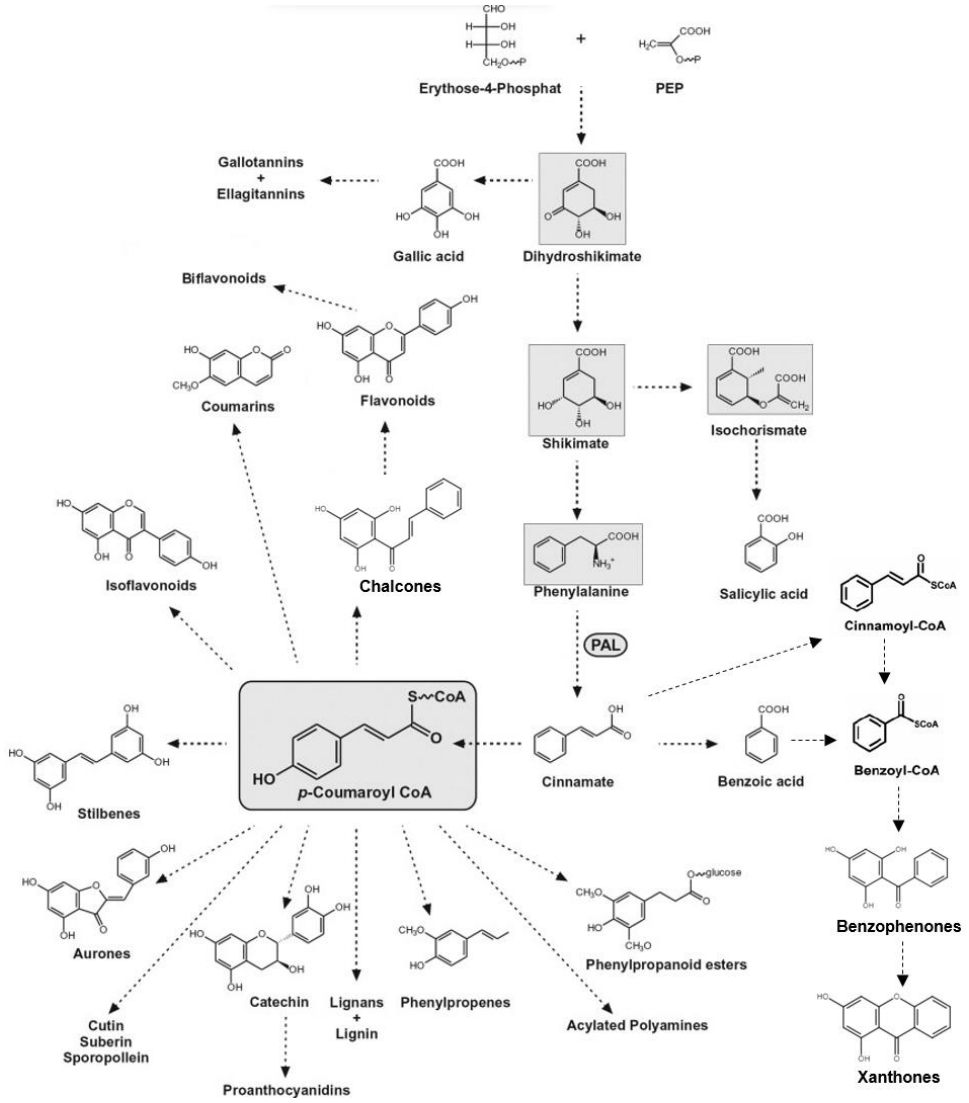

Figure 14: Shikimate biosynthetic pathway leading to some of the major classes of phenylproanoid derivatives (Vogt, 2010 [modified])

The radiation of the phenolics is mediated more than often by the combination with the polyketide pathway, where the condensation of three malonate molecules on an organic acid (e.g.: benzoic and *p*-coumaric acids) sets the conditions for the condensation of an additional aromatic ring, obtaining this way polyphenolic compounds (Johnson et al., 2017). Another important addition that enhances the phenolics comes from the addition of terpene moieties. The simplest of these moieties, the isoprene units, can then combine with adjacent hydroxyl groups already present on the phenol to produce furan- or pyran- like rings or directly on nearby carbons to produce purely carbon-based cycles.

Phenolics abundance in the upper plants is believed to be in response to the plant colonization of the land, as most of the phenolics possess UV protectants and antioxidative properties (Rensing, 2018). Phenolics are exploited in a wide variety of stress responses, whether abiotic and biotic (Chalker-Scott & Fuchigami, 2018). Clusiaceae have mastered the production of exotic phenolic compounds and the members of this family are the main producers of xanthones and benzophenones in nature (Vieira & Kijjoa, 2005; Baggett et al., 2005). Benzophenones in particular are widely produced specifically in the genus *Clusia* (Baggett et al., 2005). Other phenol subclasses found in Clusiaceae are benzoquinones, chromenes, chromanes, chromones, phloroglucinols, biphenyls, dihydrophenantrenes, flavonoids, biflavonoids and derivatives of all these mentioned (more detailed list in the Appendix).

2.2.2.1 Tocotrienol derivatives

Tocotrienols are a class of lipophilic compounds that can be found in a widespread set of plant taxa (Tan et al., 2019). The biosynthesis of these compounds involves two different molecules from two different pathways: homogentistic acid, which derives from tyrosine in the shikimate pathway and a geranylgeranyl moiety produced from the terpenoid pathway (**Figure 16**) (Cahoon et al., 2003).

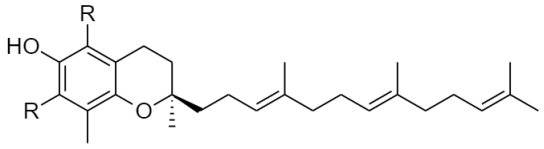

Figure 15: Tocotrienol general structure

Tocotrienols have a single chiral carbon that in nature always occur as dextrorotatory. Four tocotrienols exist (α-/β-/γ-/δ-) which differ from each other from the moieties at R^1 and R^2 (**Figure 15**) that can be either H or CH_3 in all four possible permutations. Four corresponding tocopherols exist, which are part of a class corresponding molecules that differ from tocotrienols by having a completely saturated prenyl chain, and all together they form what are commonly known as vitamins E.

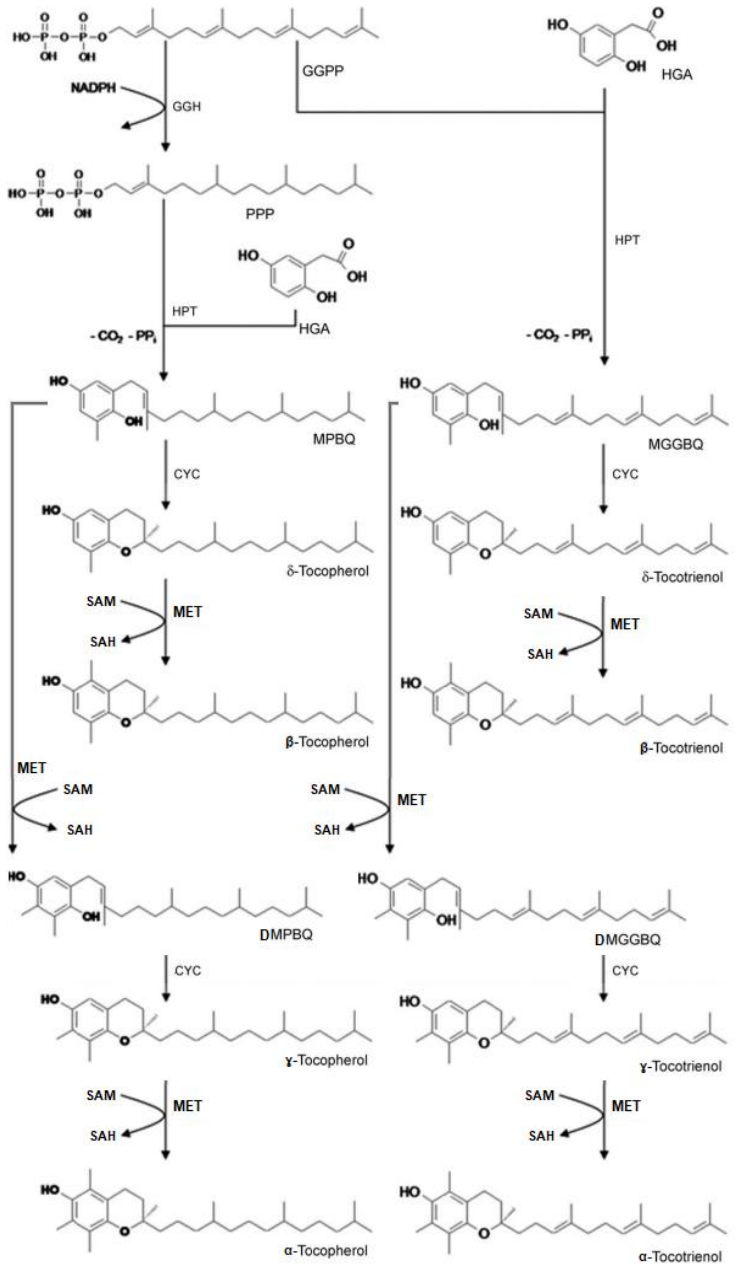

Figure 16: Biosynthetic pathway of tocotrienols and tocopherols (Source: Albermann et al., 2008 [modified])

All vitamins E share antioxidative qualities (Cercetto & Lopez, 2007) and are studied for their beneficial potential implication in aging, diabetes, heart diseases and cancer treatment (Meganathan & Fu, 2016; Georgousopoulou et al., 2017). Though simple tocotrienols are very common in plants, they usually represent the final product of their biosynthetic pathway and, therefore, the derivatives of tocotrienols are instead the product of characteristic biosyn-

thetic pathways that are restricted to just few specialized taxa, one of which is the family Clusiaceae.

2.2.2.2 Benzophenone derivatives

Benzophenones are a class of lipophilic compounds. In plants, their biosynthetic pathway branches off from the shikimate pathway, starting from cinnamic acid which is cleaved to benzaldehyde and later oxidized to benzoic acid. The benzoic acid is loaded as a thioester in a polyketide synthase complex in which three malonate moieties are condensed in sequence and later cyclized to form the second aromatic ring (**Figure 17**) (Anholeti et al., 2015). The secondarily biosynthesized aromatic ring carries three OH groups in alternated fashion which can be referred to as a phloroglucinol ring.

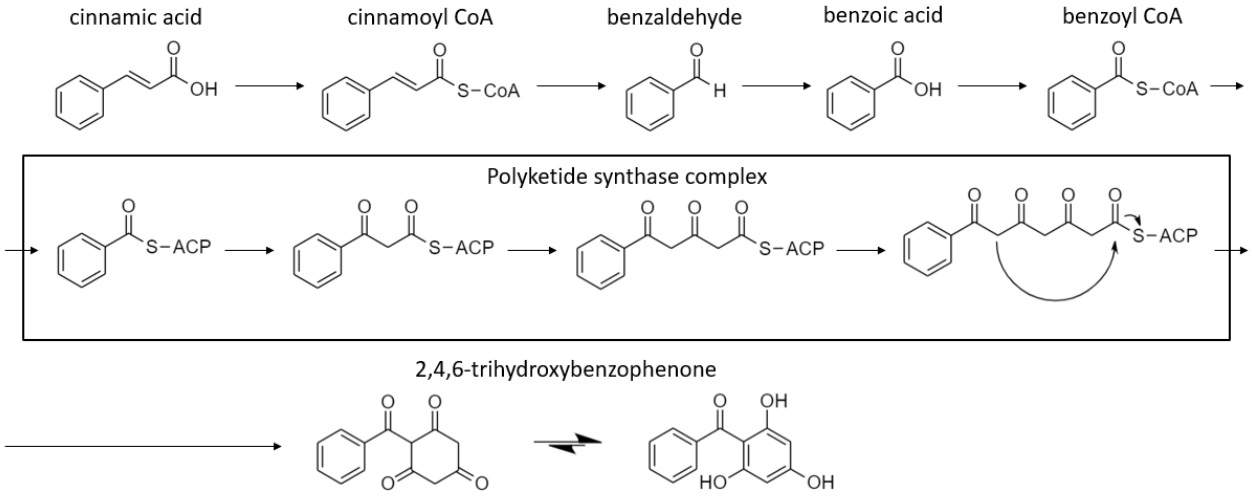

Figure 17: Biosynthetic pathway of benzophenone

There are more than 300 natural benzophenones known, with many new ones discovered each year (Wu et al., 2014). The genus *Clusia* accounts alone for 2/3 of all the natural benzophenone derivatives known, 90% of which are exclusively found only within this genus (Baggett et al., 2005). Possibly the most common modifications to the molecule, which in turn explains the diversity within this class, is given by the addition of prenyl moieties derived from the terpenoid pathway (Baggett et al., 2005). These last-mentioned carbon chains can then rearrange themselves forming an additional ring with other carbons or OH groups found in the molecule or by reacting with each other (Baggett et al., 2005). The large diversity of benzophenone derivatives can be subdivided like described in Wu et al., (2014) in basic ben-

zophenones (BBS) and polyprenylated benzophenones (PPBS) with this last moreover subdivided in four types (A/B/C/D). Benzophenones and their derivatives are considerably bioactive molecules and have a wide range of potential applications such as antiviral, antimicrobial, antifungal, antitumoral and antioxidative (Baggett et al., 2005). Moreover, benzophenones are an intermediate biosynthetic step for the synthesis of xanthones, another subclass of phenols very abundant and diverse within the Clusiaceae (Vieira & Kijjoa, 2005; Negi et al., 2013).

3 Material & Methods

3.1 Plant Material

The roots of *C. minor* (344.8 g) used in this work came from two specimens cultivated in a University of Vienna greenhouse, propagated from the individual 5919-1-1993. The two specimens were disassembled in their various organs and frozen for future use.

3.2 Laboratory techniques

3.2.1 Extractions

3.2.1.1 Crude extract

The frozen, fresh, roots of *C. minor* were ground into fine chips using a kitchen blender. A small amount of chips was put in the oven to dry for several hours to determine the water content. The rest of the chips were stored in glass jars and covered with methanol (MeOH) and left for extraction overnight. The upcoming day, the jars were sonicated for 25 minutes and the methanol was then filtered and set to dry using a rotary evaporator (40°C). The same chips were subjected again to the up mentioned process for a total of four rounds.

3.2.1.2 Liquid-liquid phase separation

The crude extract (CE) was resuspended in 200 mL of a solution 97: 3 of *n*-heptane and ethyl acetate (EtOAc) followed by 200 mL of water (H_2O). All CE was resuspended in the two liquids and both were collected in a separation funnel. The funnel was shaken to facilitate the separation of compounds according to their phase affinity. The *n*-heptane was extracted and set to dry in the rotary evaporator, while 200 mL of fresh *n*-heptane were added to the separation funnel with the water to repeat the process. The process was repeated four times after which the whole process was repeated using instead of *n*-heptane respectively chloroform ($CHCl_3$) and EtOAc in this order. Each phase had its process repeated for four rounds. The final result is of four extracts, one for each phase.

3.2.2 Analytical methods for control

The analytical methods were conducted consistently throughout the fractioning and isolation procedures and at the end of each chromatography to track down the separation of the compounds inside the fractions and to guide the decisions on which fractions were of interesting for further analysis and the isolates purity.

3.2.2.1 Analytical thin layer chromatography (ATLC)

ATLCs were conducted on 0.2 mm layers of silica gel 60 (grain size 10–20 μm) on aluminum plates. Different eluents were used to run the fractions on the plate depending on the range of polarity of the comprising compounds. The ATLCs plates were observed under visible and UV lights (254 and 366 nm) and later treated with anisaldehyde and heat to render the compounds visible under ambient light.

3.2.2.2 High performance liquid chromatography (HPLC)

The HPLC is a machine-performed high pressure column chromatography. The instrument involved in the study is an Agilent 1100 series. The machine was equipped with a Hypersil BDS-C18 column (250 × 4.6 mm) with 5 μm grains, which use reversed phase elution, with modified silica gel as apolar, static phase and liquid, mobile phases solutions from the most polar to more apolar. The liquid phase comprised of a mixture of two eluents: (A) An aqueous solution of ammonium acetate (CH_3COONH_4, 10 mM) and (B) MeOH. The eluent mixtures used were: t= 0 min B=55%, t=17 min B 90%, t=20 min B 100% and t=28 min B 100%. The flow rate was set to 0.8 L/min with an injection volume of 10 μL. The HPLC is equipped with a UV-VIS DAD at 230 nm to detect the compounds' spectra and build the sample's chromatograms for following the separations of compounds and comparing them with the software library.

3.2.3 Isolation preparative methods

Several chromatographic techniques were used in sequence to achieve more and more pure fractions up to the isolation of the almost pure compounds. Depending on the necessity, different heights and widths of columns/plates were used in combination with different liquid

eluents. The technical specifications of the single steps taken are mentioned in the in the processing scheme paragraph.

3.2.3.1 Column chromatography (CC) over Silica gel

Silica gel is a type of column chromatography (and will be referred with the CC abbreviation). The silica gel grains provide a polar stationary phase. The mobile phase is added gradually moving from a very apolar phase to a gradually more polar one. The compounds therefore elute with a speed proportional to their polarity. The silica gel used in the CC of this study was size 40–63 μm. The column used was ~60 cm in length and ~2 cm in diameter.

3.2.3.2 Medium pressure chromatography (MPC)

Medium pressure chromatography (MPC) has an analogous functioning of the CC with the only addition that the entire process takes place in an artificially pressurized system. The advantage of adding pressure stand in the higher accuracy of the separation process due to the small particle sizes of the stationary phase. The column used silica gel 40–63 μm.

3.2.3.3 Size exclusion chromatography over Sephadex LH-20 (SC)

Size exclusion chromatography over Sephadex LH20 (SC) is another type of column chromatography. This time the grains used in the stationary phase are consisting of a polysaccharide with isopropyl side chains and the mobile phase used is the same through all the process (isocratic elution). The compounds get separated according to relative size, with bigger compounds moving more straight onwards while the smaller ones have a higher tendency to entangle in the polysaccharide mesh. The column used was a ~70 cm in length and ~1.2 cm in diameter

3.2.3.4 Preparative thin layer chromatography (PTLC)

PTLCs were conducted on 0.5 mm layers of silica gel 60 (grain size 10–20 μm) on 20 cm glass plates. Different eluents were used to run the fractions on the plate depending on the range of polarity of the comprising compounds. To identify the separated lines, the PTLCs plates were observed under visible and UV lights (254 and 366 nm) and marked with a pencil. After scratching off the silica gel with the marked bands, the silica gel was extracted with

methanol and filtered through a 5G glass frit. The filtered solution was evaporated and the amounts of the purified compounds determined.

3.2.4 Molecular structure elucidation

Once a compound resulted isolated over a minimum threshold of purity (>95%) and over a minimum amount threshold (>1 mg), then a small aliquot was transferred in a glass vial for recording the mass spectrum while the remaining amount of compound was dissolved in deuterated methanol (CD_3OD) and the solution transferred into a NMR glass tube. Both, the NMR and the mass spectra were recorded at the Department of Organic Chemistry of the University of Vienna.

3.2.4.1 Electrospray ionization mass spectrometry (ESI-MS)

ESI-MS is an analytical technique used on unknown compounds to infer the molecular formula of the isolated compound. The compound is dissolved in a highly volatile solution which gets subjected to a voltage of ~3 kV and sprayed into a vacuum chamber, sometimes with additional heating involved too. The high volatility, the vacuum and the heat all promote the fast shrinking of the droplets in the sprayed aerosol. Since the droplets carry an electric potential given by the induced voltage, at a critical shrinking threshold, the electrostatic repulsion overpowers the surface tension and the droplets undergoe Coulomb fission producing smaller droplets. The sequential fragmentation through Coulomb fission results in a phase of ionized gas which is passed through a magnetic field and on to a detector, which records the fragments, also known as mass spectra, as a ratio of their mass over their charge (m/z) and outputs a mass chromatogram.

3.2.4.2 Nuclear magnetic resonance (NMR)

NMR is a technique that provides information on the magnetic interactions the atoms in a molecule exert on their neighboring atoms and is the main technique for structure elucidation. The technique measures the precession of the spin of atoms that possesses a magnetic momentum when subjected to a strong constant magnetic field. The two most important isotopes with non-zero nuclear spin are ^{1}H and ^{13}C. Various types of 1D and 2D NMR measurements can be run each providing different spectra of interactions between the atoms and, by

correctly interpreting the data, it is possible to infer the molecule's structure. For each compound, the following NMR-spectra were provided: proton NMR spectroscopy (^{1}H), correlation spectroscopy (COSY), heteronuclear single quantum correlation spectroscopy (HSQC), heteronuclear multiple-bond correlation spectroscopy (HMBC), nuclear Overhauser effect spectroscopy (NOESY), Malcolm Levitt's CPD sequence (MLEV) and distorsionless enhanchement by polarization transfer (^{13}C DEPTq).

3.3 Processing (**Figure 18**)

An amount of 344.8 g of fresh, frozen roots of *C. minor* were used. Of these, 12.3 g were put in the over for the water mass content estimation that resulted in a dry/wet ratio of ~22.0%. This means that the 344.8 g of fresh corresponded to 75.7 g of dry roots. The crude extract resulted in 22.5 g. The liquid-liquid phase separation yielded 0.4238 g of material in the *n*-heptane phase, 2.1 g in $CHCl_3$ phase, 3.8 g in EtOAc phase and 13.7 g in H_2O phase. The *n*-heptane and $CHCl_3$ phases were chosen for further analyses while the residue was stored.
A *n*-heptane phase's aliquot (~265.9 mg) was subjected to SC with MeOH resulting in 33 fractions (S1-01:33). Fractions S1-07:11 were run through an ATLC with a mixture 70:30 of *n*-heptane/EtOAc. All remaining S1 fractions were run through a TLC in 90:10 $CHCl_3$/MeOH, merged according to similarity, weighted and stored: S1-03:07 (262.5 mg), S1-08:10 (111.5 mg), S1-11:12 (8.7 mg), S1-18:21 (10.9 mg), S1-24:27 (4.8 mg), and all the remaining ones (86.1 mg). Fractions S1-08:10 was subjected to a double run on a PTLC in 95:5 $CHCl_3$/MeOH resulting in six interesting strips (Ø–V) of which only strip III had an appreciable amount of purity and weight (5.6 mg) and was renamed PK-CM01. All $CHCl_3$ phase was subjected to MPLC with a pressure of ~1.8 bar. The eluting solutions were in aliquots of 100 mL and are here resumed in **Table 2**:

Table 2: Eluents used in the MPLC1. Values given in % (v/v)

Solvent	*n*-heptane	$CHCl_3$	MeOH
I	90	10	
II	90	10	
III	80	20	
IV	70	30	
V	60	40	
VI	50	50	
VII	60	40	
VIII	80	20	
IX		100	
X		80	20
XI		60	40
XII			100
XIII			100

The MPLC resulted in 35 fractions named M1-01:35. All fractions were subjected to a TLC in 70:30 *n*-heptane/EtOAc. The following fractions were merged and weighted: M1-05:06 (132.5 mg), M1-11:14 (511.9 mg), M1-20:23 (124.9 mg), M1-24:27 (118.2 mg). M1-05:06 were then run for a SC in MeOH resulting in fractions S2-01:20 that were all run through ATLC in 70:30 *n*-heptane/EtOAc. All S2 fractions were also run through ATLC in 80:20 *n*-heptane/EtOAc. Fractions M1-11:14 were divided in two batches run for a SC in MeOH resulting in fractions S3-01:33 and S4-01:17. Both S3 and S4 had all their fractions run through ATLC in 70:30 *n*-heptane/EtOAc. Fractions S2-16:18 were merged and similarly happened for fractions S3:18+S4-15. Both last mentioned merges were run in PTLC in 80:20 *n*-heptane/EtOAc. S2-16:18 resulted in three interesting strips (I–III) and S3-18+S4-15 in two strips (IV–V). Strip IV was a replicate of PK-CM01 (3.1 mg) and was stored. Strips II, III and V had enough material to be processed for NMR analysis and were renamed respectively PK-CM02/03/04. The following fractions were merged, weighted and stored: S3-12:14+S4-09:11 (183.1 mg), S3-26:30 (1.4 mg), S3-16:17+S4-12:14 (170.2 mg). A SC was made for fractions S3-12:14+S4-09:11 resulting in fractions S5-01:19 of which were subjected S5-10:16 to a TLC in 70:30 *n*-heptane/EtOAc. Fractions S5-10:12 were run on PTLC with two runs, the first one in 80:20 *n*-heptane/EtOAc and the second one in 75:20:05 *n*-heptane/EtOAc/MeOH. Ten stripes (I–X) were scratched from the plate that didn't have enough material to be used. Fractions M1-20:23 were run for SC resulting in fractions S6:01:24 that were run in 60:20:20 *n*-

heptane/EtOAc/MeOH and after in 70:30 *n*-heptane/EtOAc. Fractions S6-13:15 were merged and weighted (22.5 mg) and subjected to MPC resulting in fractions M2-01:15. The 50 mL aliquots of eluents are resumed in **Table 3**:

Table 3: Liquid phase eluents used in the MPLC2

Solvent	$CHCl_3$ [%]	MeOH [%]
I	100	
II	100	
III	97.5	2.5
IV	95	5
V	92.5	7.5
VI	90	10
VII	87.5	12.5
VIII	85	15
IX	82.5	17.5
X	80	20

The rinsing of S6 column resulted in a very pure fraction of a biflavonoid isolated in a previous study (coded SI-CM03) that was stored. M2 fractions were subjected to ATLC in 95:05 $CHCl_3$/MeOH followed by 80:20 $CHCl_3$/MeOH. Fractions M2-8:15 were merged. A new SC was conducted on fractions S1-08:10 in 50:50 $CHCl_3$/MeOH resulting in fractions S7-01:15. Fractions S7-7:11 were singularly compared with the already stored PK-CM01 samples on ATLC in 90:10 $CHCl_3$/MeOH. Fractions S7-07:08 and all the remaining were respectively merged and stored. A new SC was conducted with the merged fractions S1-11:12+M2-04:07 in 50:50 $CHCl_3$/MeOH resulting in fractions S8-01:15 that were then subjected to ATLC in 90:10 $CHCl_3$/MeOH. Fractions S8-14:15 were merged, weighted, renamed PK-CM05 and sent to the NMR for isolated compound structure elucidation.

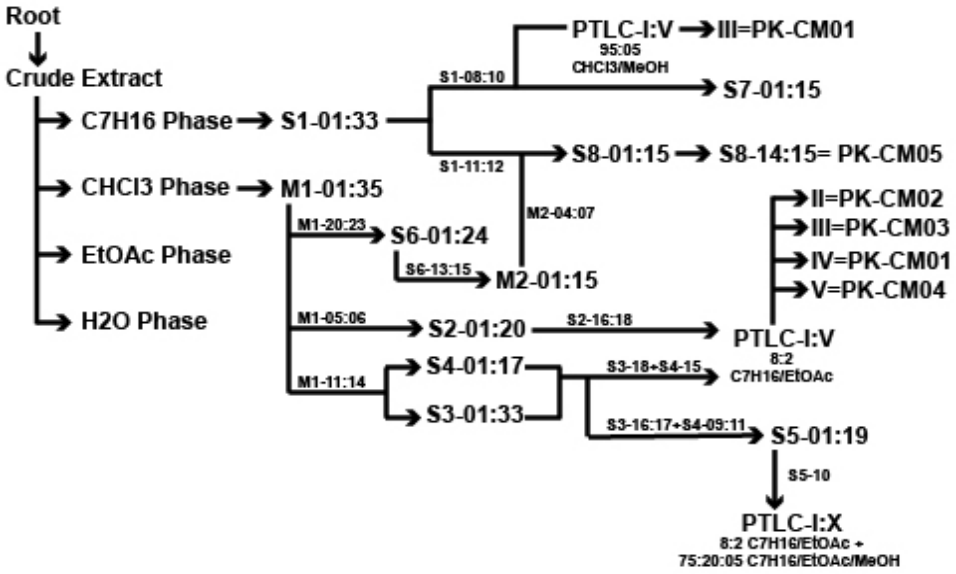

Figure 18: Scheme summarizing the processing paragraph. Subscription numeration is kept in standard font for visual purpose.

4 Results

The conducted study resulted in the isolation of five compounds (PK-CM01–05). Unfortunately, sample PK-CM04 didn't produce signals clear enough to make it possible to work with for structure elucidation due to either a too low level of purity or due to a too little amount. PK-CM03 initially showed a promising level of purity at the HPLC but at the NMR resulted in a confuse mix of compounds. Later readings at the HPLC confirmed the NMR results. The reason for this happening is suspected to be a spontaneous degradation of the sample during time. For these reasons PK-CM03 and PK-CM04 do not appear in the paragraphs of the results. **Figure 19** shows the chromatogram of the root crude extract with the isolated compounds tagged along with some biflavonoids' peaks previously isolated from our research group (Schinnerl, personal communication). The structure of each compound was elucidated thanks to the MS and the various NMR analyses mentioned in the Material and Methods section. All the raw results of the NMR analyses can be found in the Appendix.

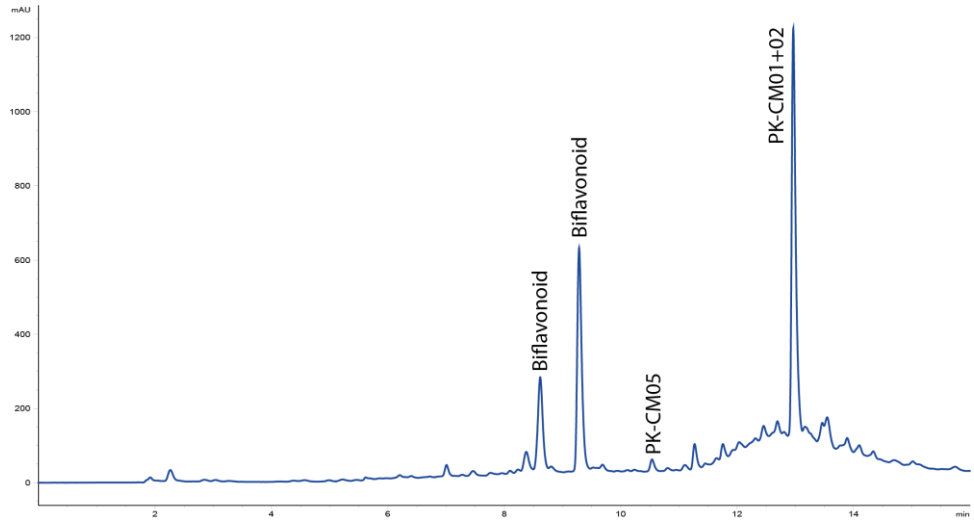

Figure 19: Chromatogram of the crude extract

4.1 PK-CM01

The UV absorption spectrum of PK-CM01 shows characteristic absorption maxima at λ_{max} = 296nm and λ_{max} = 214nm (**Figure 20**). A search in literature on the UV absorption spectra of compounds already identified in *Clusia* helped to determine that PK-CM01 was most likely related to the Vitamins E even before the elucidation of the molecule.

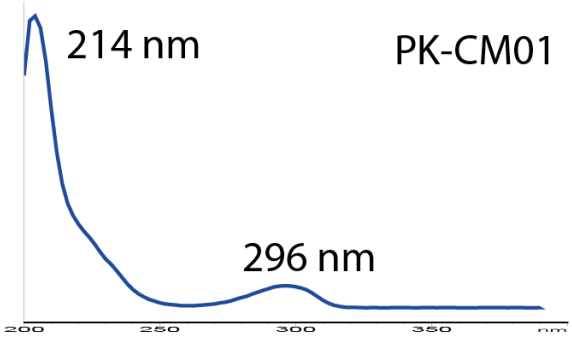

Figure 20: PK-CM01 UV absorption spectrum

The MS of PK-CM01 shows a single main molecular ion peak for each MS charge: a sodium adduction $[M+Na]^+$ in the +MS at 449.2666 and a parent peak $[M-H]^-$ in the -MS at 425.2698 (**Figure 21A–B**). With an inferred mass of 426.27, the corresponding molecular formula resulted to be $C_{27}H_{38}O_4$ and therefore a double-bond-equivalent (DBE) of 9.

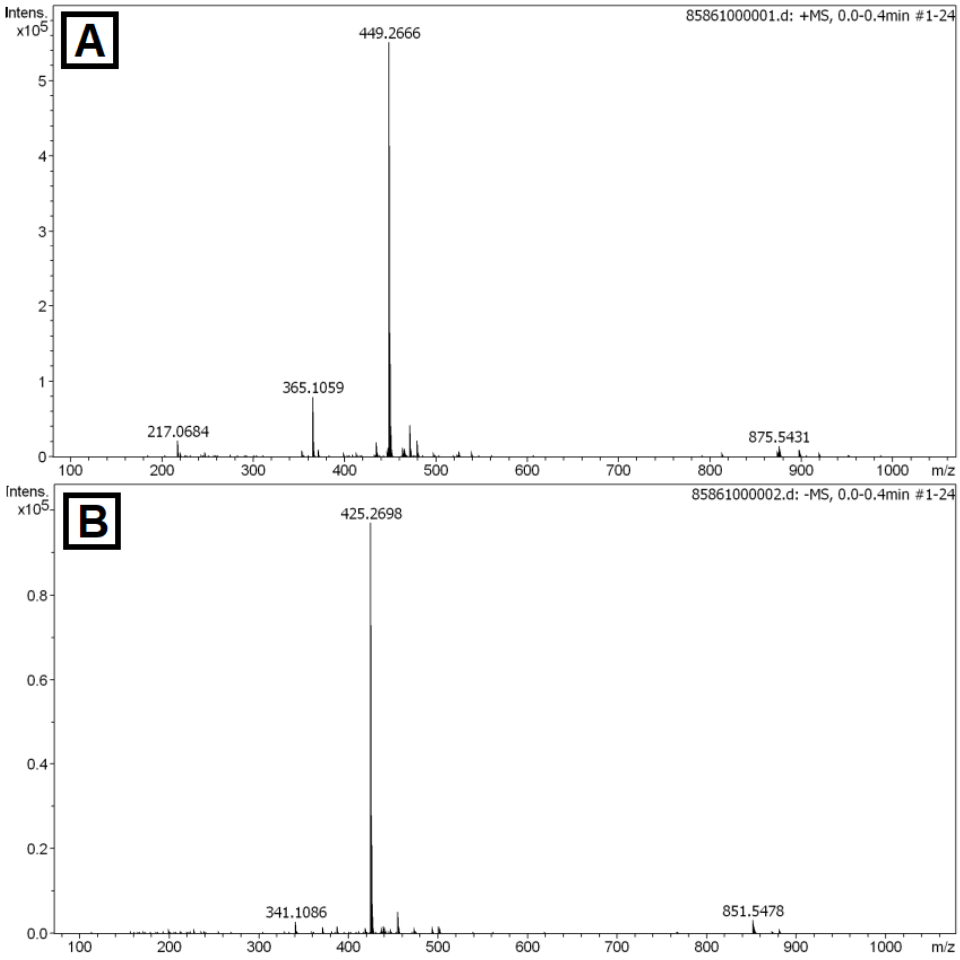

Figure 21: Mass spectra of PK-CM01: A) positive mode; B) negative mode

The NMR data was very clear, with no signals missing and only few long-range couplings appearing (**Figure 23A–E**). HMBC alone was sufficient to clarify most of the molecule. The *E*-/*Z*- isomerism was solved by simulating both isomers (on the website cheminfo.org) and taking the measurements of shift and coupling constant of the hydrogen bonded to the close by double-bond as it is predicted to be the mostly affected by the difference in isomers. The data of the simulations and their comparison with the empirical data is recapped in **Table 4**. The empirical data has a much higher match with the *Z*- isomer simulation demonstrating this way it is the right configuration.

Table 4: Empirical data of PK-CM01 compared with the simulated *E*-/*Z*- isomers. In brackets the absolute difference with the empirical data.

Case	$H_{11''}$ shift [ppm]	$H_{11''}$ coupling constant [Hz]
Empirical data	5.91	7.3
Z- isomer	6.26 (0.35)	7.3 (0)
E- isomer	6.90 (0.99)	6.8 (0.5)

PK-CM01 resulted to be *Z*-δ-garcinoic acid (**Figure 22**). The data of the molecule's carbons and non-heteronuclear hydrogens, which comprises the ^{1}H and DEPTq data, is recapped in **Table 5**.

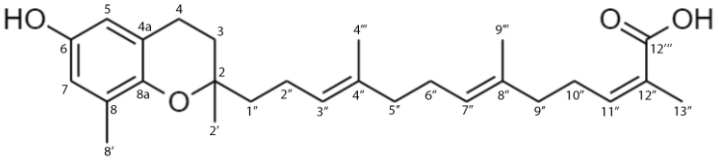

Figure 22: Structural formula of *Z*-δ-garcinoic acid

Table 5: ^{13}C and ^{1}H shift values of PK-CM01 according to 1-dimensional NMRs

C Number	C Shift [ppm]	H count	H shift [ppm]	H Peak Multiplicity	H Coupling constant [Hz]
2	76.23				
3	32.76	2	1.73 / 1.78	ddd	6.8
4	23.45	2	2.67	ddd	6.8 / 2.2
4a	122.3				
5	113.58	1	6.32	dd	2.9 / 0.5
6	150.38				
7	116.6	1	6.4	dd	2.9 / 0.5
8	127.79				
8a	146.37				
2'	24.46	3	1.24	s	
8'	16.31	3	2.07	s	
1''	40.49	2	1.52 / 1.60	m	
2''	23.22	2	2.12	q	8.2
3''	125.86	1	5.14	tm	7.3
4''	135.82				
5''	40.72	2	1.98	t	7.6
6''	27.5	2	2.07	m	
7''	125.93	1	5.11	tm	7.1
8''	135.4				
9''	40.24	2	2.03	t	7.4
10''	29.11	2	2.53	m	7.7
11''	143.34	1	5.91	tm	7.3
12''	128.77				
13''	21.03	3	1.85	s	
4'''	15.97	3	1.57	s	
8'''	15.89	3	1.57	s	
12'''	171.68				

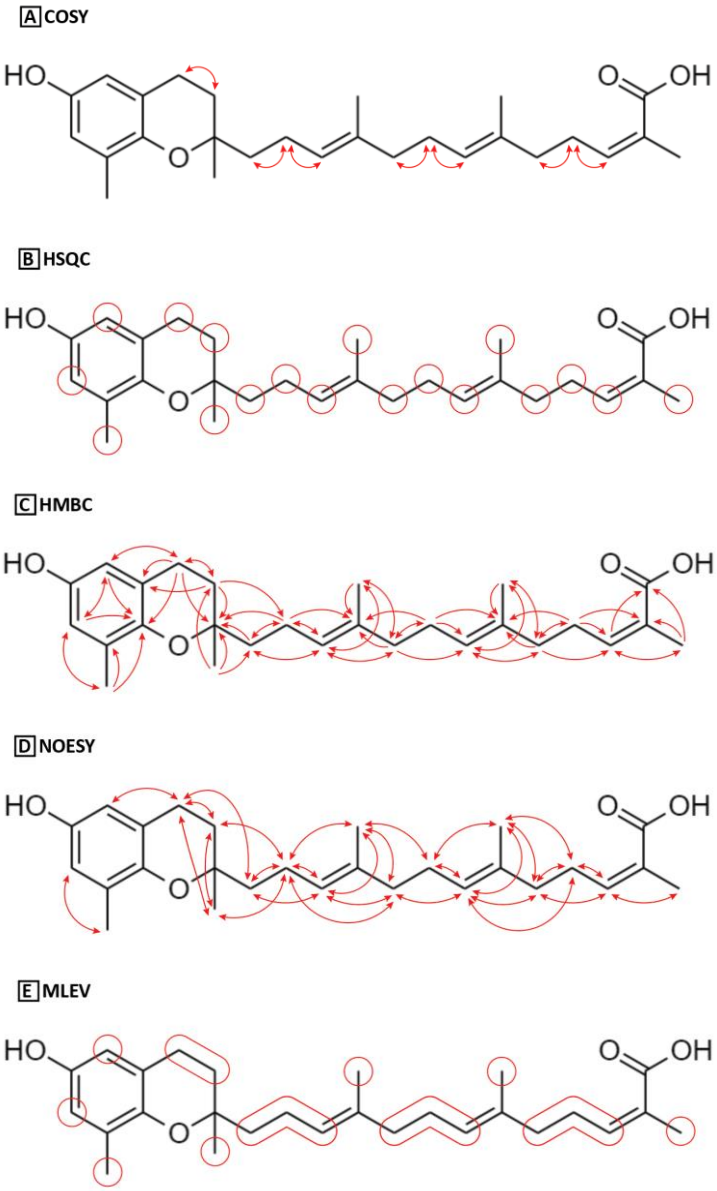

Figure 23: Relationships between the molecule's atoms inferred with the NMR analyses: A) COSY; B) HSQC; C) HMBC; D) NOESY; E) MLEV

4.2 PK-CM02

PK-CM02 exhibited a UV absorption spectrum (**Figure 24**) with λ_{max} = 214nm, 296nm. The spectrum is virtually identical to the one of PK-CM01, suggesting PK-CM02 is also related to vitamins E.

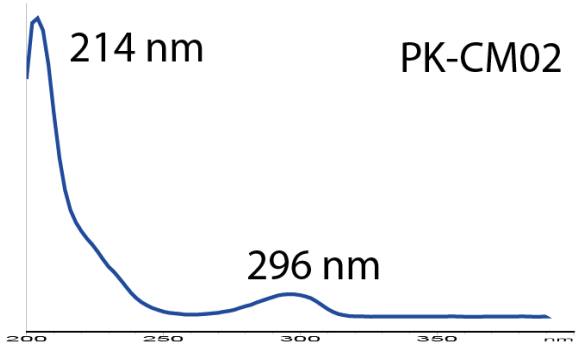

Figure 24: PK-CM02 UV absorption spectrum

The +MS of PK-CM02 (**Figure 25A–B**) shows a sodium adduction at peak at 419.2922 $[M+Na]^+$ and a major peak at 593.3602 $[M+197]^+$. The -MS shows a major peak at 569.3639 $[M+173]^-$. The proposed mass is 396.29 with a corresponding formula of $C_{27}H_{40}O_2$ and a DBE=8.

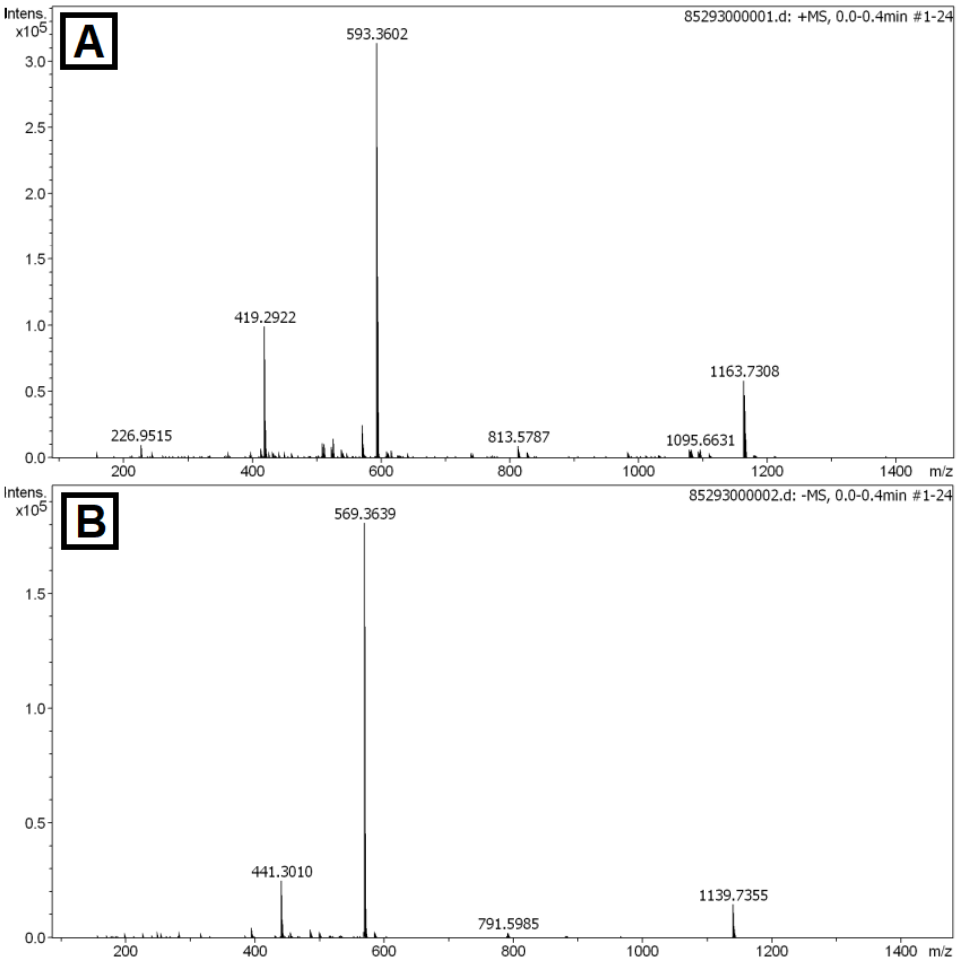

Figure 25: Mass spectra of PK-CM02: A) positive mode; B) negative mode

PK-CM02 had very clear signals in its NMR analyses (**Figure 27A–E**) and thanks to the relatedness with the previous compound, hinted by the UV absorption spectra, it was even easier to assign the correct atoms' positions. PK-CM02 resulted to be δ-tocotrienol (**Figure 26**). The data of the molecule's carbons and non-heteronuclear hydrogens is recapped in **Table 6**.

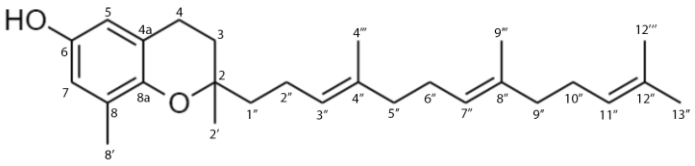

Figure 26: Structural formula of δ-tocotrienol

Table 6: ^{13}C and ^{1}H shift values of PK-CM02 according to 1-dimensional NMRs

C Number	C Shift [ppm]	H count	H shift [ppm]	H Peak Multiplicity	H Coupling constant [Hz]
2	76.22				
3	32.75	2	1.74 / 1.78	ddd	6.7
4	23.45	2	2.68	ddd	6.8 / 2.3
4a	122.28				
5	113.58	1	6.32	d	2.7
6	150.39				
7	116.6	1	6.4	d	2.7
8	127.78				
8a	146.36				
2'	24.45	3	1.25	s	
8'	16.29	3	2.07	s	
1''	40.54	2	1.54 / 1.61	t	4
2''	23.23	2	2.13	q	8.3
3''	125.85	1	5.14	tm	7.3
4''	135.9				
5''	40.76	2	1.98	t	7.5
6''	27.47	2	2.08	m	7.4
7''	125.47	1	5.08	tm	7.2
8''	135.83				
9''	40.84	2	1.94	t	7.3
10''	27.8	2	2.05	m	
11''	125.4	1	5.08	tm	7.2
12''	132.02				
13''	25.89	3	1.66	s	
4'''	15.88	3	1.58	s	
8'''	16.06	3	1.57	s	
12'''	17.75	3	1.59	s	

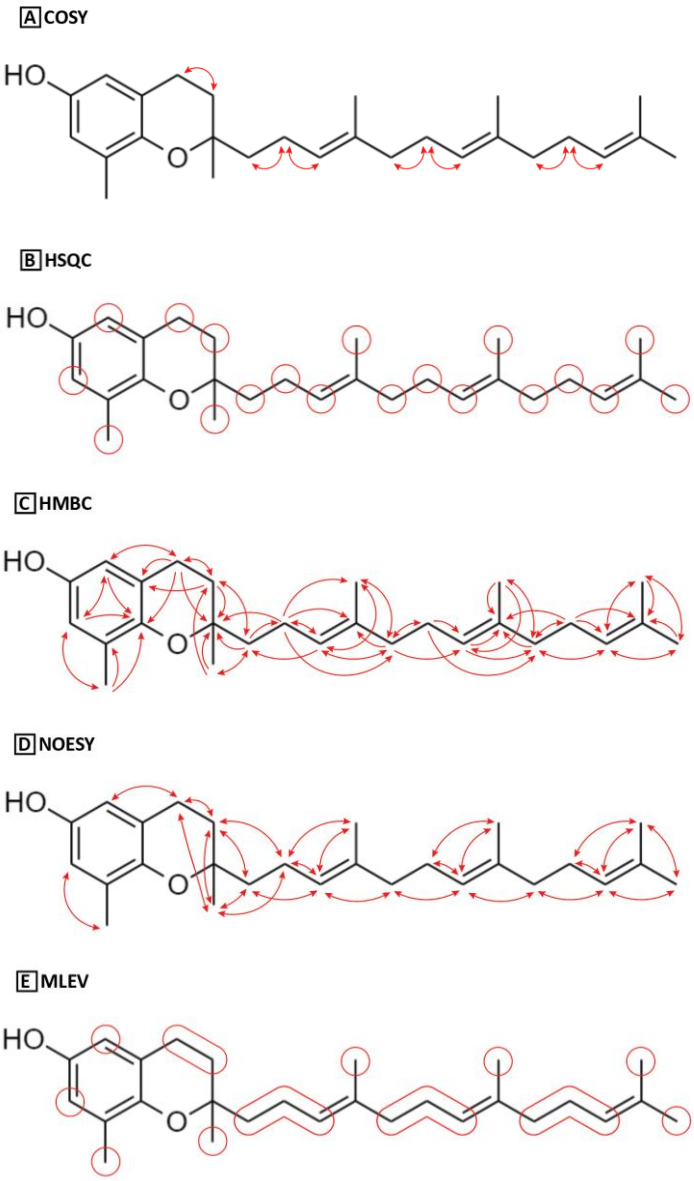

Figure 27: Relationships between the molecule's atoms inferred with the NMR analyses: A) COSY; B) HSQC; C) HMBC; D) NOESY; E) MLEV

4.3 PK-CM05

PK-CM05 exhibits UV absorption maxima at λ_{max} = 202nm, 272 nm, 338nm. The absorption spectrum shows this compound belongs to a different class compared to the previously isolated compounds, though which class was not inferred prior to the structure elucidation. The possible explanation for the observed absorption maxima is discussed as a matter of sensible hypotheses in the discussion section.

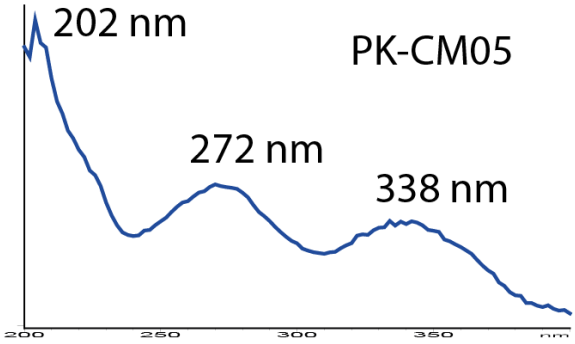

Figure 28: PK-CM05 UV absorption spectrum

The +MS of PK-CM05 (**Figure 29A–B**) shows a major peak at 465.2012 possibly due to a double adduction of sodium and the loss of a hydrogen $[M-H+2Na]^+$. Peak 443.2192 corresponds to a single sodium adduction $[M-Na]^+$. The -MS shows a main peak at 419.2209 $[M-H]^-$. The proposed mass for PK-CM05 is 420.22 corresponding to a formula of $C_{27}H_{32}O_4$ with a DBE=12.

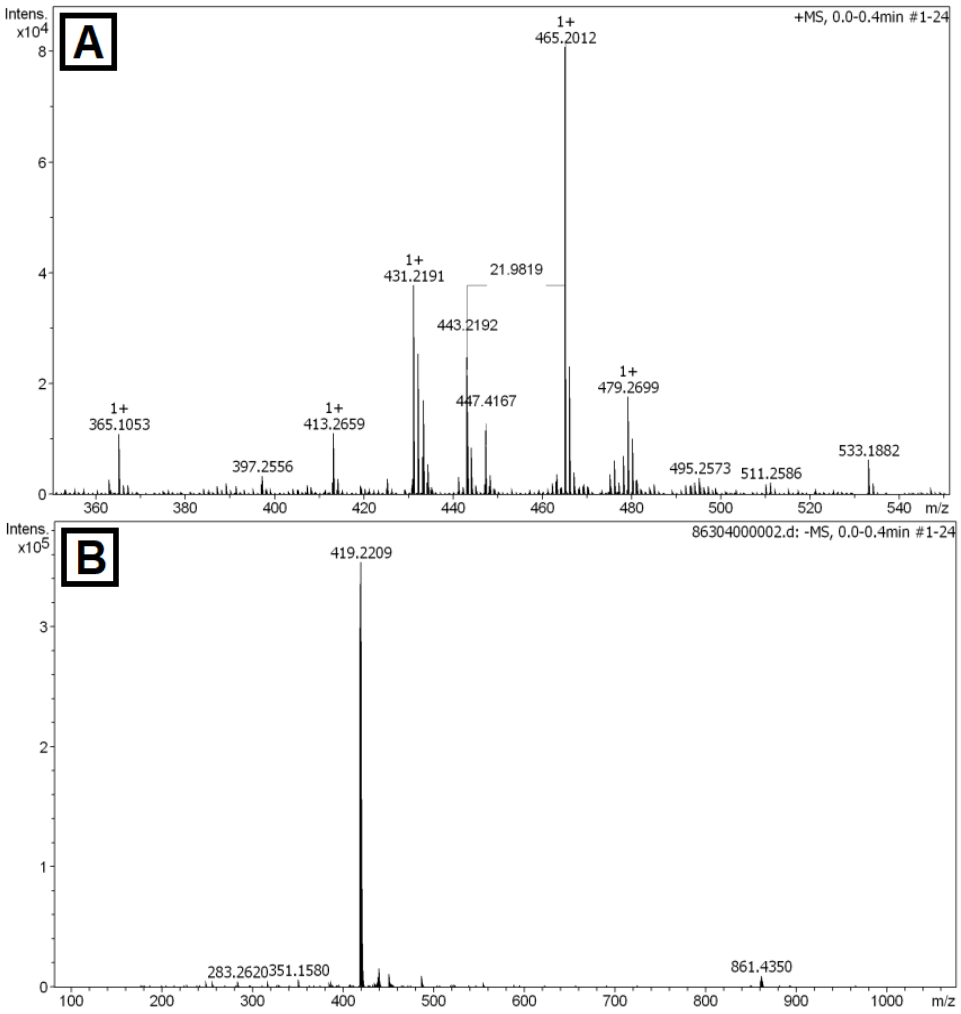

Figure 29: Mass spectra of PK-CM05: A) positive mode; B) negative mode

Contrary to the previous compounds, the NMR analyses showed a fairly good quality just for the apical chains of the molecule. The core part of the molecule resulted to be very rich in oxygen and almost free from hydrogens hence the absence of useful signals and eventually also with missing signals given by acidic protons due to the oxygen presence. The part of the molecules that were elucidated with a high confidence along with missing molecule's atoms and DBE are reported in **Figure 30**. For the sake of argumentation fluency in further results analysis, the three chains shown in **Figure 29** will be referred respectively from left to right, top to bottom, as benzoyl, isoprenyl and monoterpenyl chains.

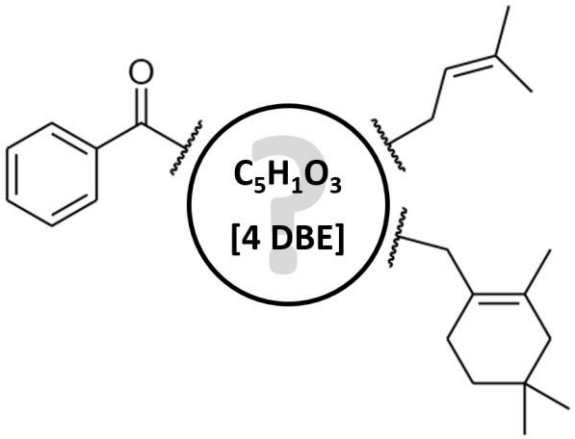

Figure 30: PK-CM05 fragments from NMR elucidation

The shifts of the not yet elucidated part of the molecule suggests the presence of two ketones, a carbon-carbon double bond and a sp^3 carbon. Both, the isoprenyl and the monoterpenyl chains have shifts in the carbon positioned at the attachment point with the rest of the molecule that suggests the bonding to a sp^3 and show a signal of a ketone closed by, suggesting at least one ketone is directly bonded to the sp^3 carbon too. The ketones and the double bond account for three DBE, leaving just one which must be accounted for the presence of a cycle. Research in literature didn't produce any result of natural occurring cycles with ≤4 carbons and ketones all in one, nor the occurrence of cyclopropene so it is therefore safe to consider a 5-membered ring as the only plausible option. By combining all the up mentioned restrictions for the construction of all possible conformations leads to just three possible candidate structures (**Figure 31A–C**).

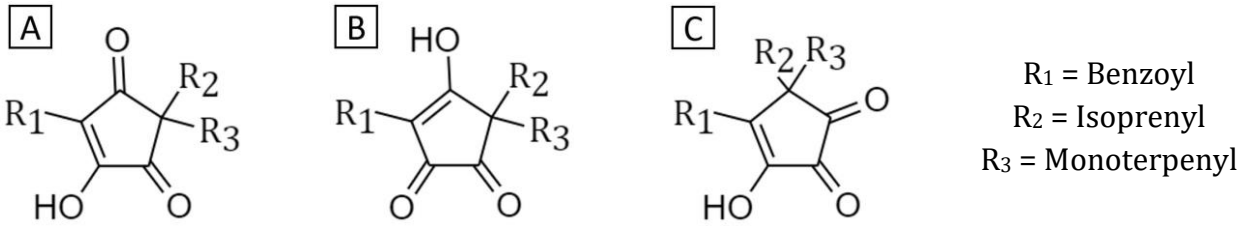

Figure 31: The three candidate structures for the core ring of PK-CM05

It must be noticed that candidate structures A and B are in a β-diketone tautomeric equilibrium and, since the methods used in the study allow for the free reconfiguration between tautomers, candidate A and B represent an equilibrium of a single entity (**Figure 32**).

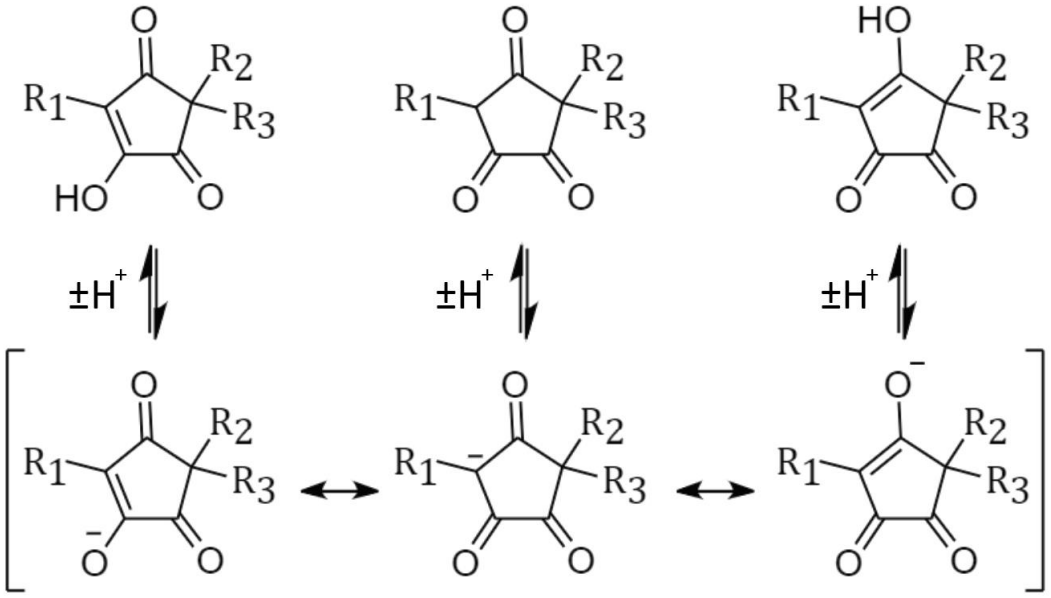

Figure 32: β-diketone tautomeric equilibrium between candidate structures A, B and an intermediate diketonic configuration

Nevertheless, the NMR spectra for ^{13}C for all three candidate structures were simulated and compared with empirical measurements. The results of the simulations are recapped in **Table 7**. The candidate structures are referred with the letter used in **Figure 31**.

Table 7: NMR carbon shifts [ppm] of the 5-membered ring candidates for PK-CM05 in decreasing order of shift. In brackets the absolute difference from the empirical data

Case	C_I	C_{II}	C_{III}	C_{IV}	C_V	Total difference
Empirical data	212.9	201.8	181.8	115.3	56.6	
Candidate A	209.7	196.6	151.3	114.5	40.6	
	(3.2)	(5.2)	(30.5)	(0.8)	(16)	55.7
Candidate B	203.2	188.7	175.0	114.5	40.6	
	(9.7)	(13.1)	(6.8)	(0.8)	(16)	46.4
Candidate C	203.2	180.9	151.3	127.3	40.6	
	(9.7)	(20.9)	(30.5)	(12)	(16)	89.1

Candidate B exhibits the lowest difference with empirical measurements and is therefore, most reasonably, the favorable state of the tautomeric equilibrium (**Figure 33**). The NMR data of the molecule is resumed in **Table 8** and **Figure 34**. The proposed structure has

never been reported before and, therefore, the name minorone is proposed and used to refer to PK-CM05 further on in this study.

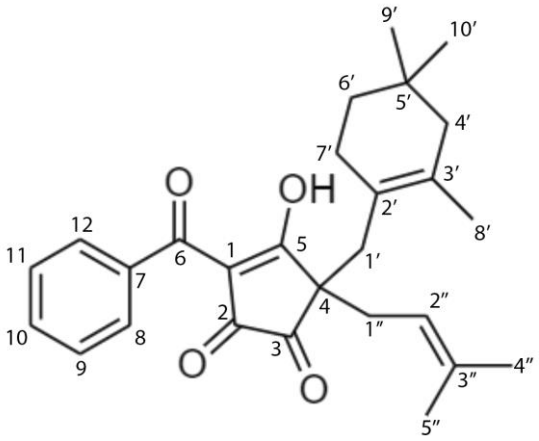

Figure 33: PK-CM05 proposed structure

Table 8: ^{13}C and ^{1}H shift values of PK-CM05 according to 1-dimensional NMRs

C Number	C Shift [ppm]	H count	H shift [ppm]	H Peak Multiplicity	H Coupling constant [Hz]
1	115.3				
2	201.8				
3	212.9				
4	56.6				
5	181.8				
6	194.9				
7	140.5				
8	130.5	1	7.74	d	7.5
9	128.8	1	7.37	t	7.6
10	133.2	1	7.48	t	7.3
11	128.8	1	7.37	t	7.6
12	130.5	1	7.74	d	7.5
1'	40.1	2	2.43 / 2.54	d	13.3
2'	130.5				
3'	125.9				
4'	47.5	2	1.69	s	
5'	29.8				
6'	37.3	2	1.22	tm	5.52
7'	30.5	2	1.76 / 1.91	m	
8'	20.5	3	1.59	s	
9'	28.7	3	0.79	s	
10'	28.9	3	0.8	s	
1''	35.2	2	2.41	d	8.13
2''	120.1	1	4.98	t	7.6
3''	135.7				
4''	26.2	3	1.63	s	
5''	17.9	3	1.59	s	

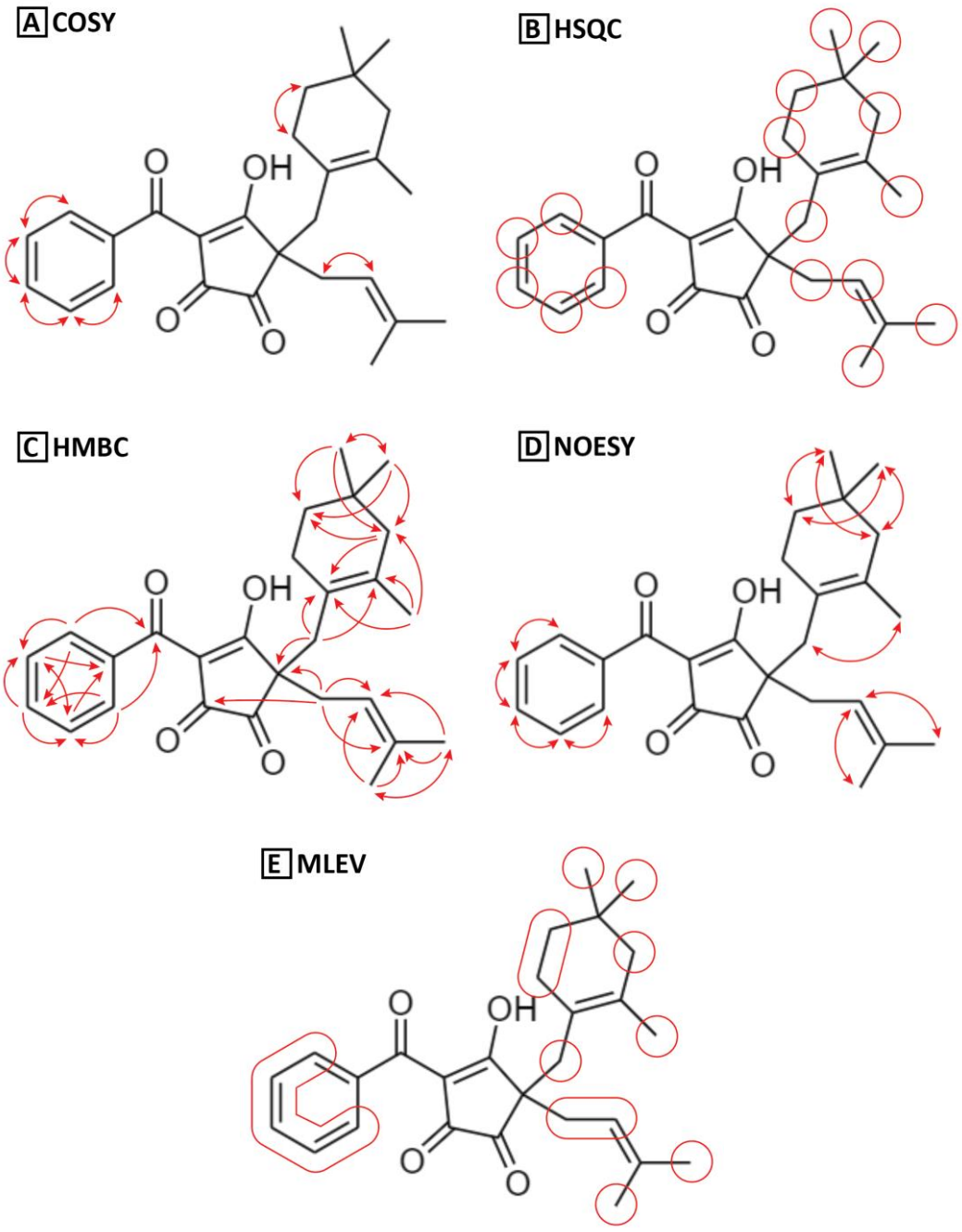

Figure 34: Relationships between the molecule's atoms inferred with the NMR analyses: A) COSY; B) HSQC; C) HMBC; D) NOESY; E) MLEV.

5 Discussion

The study has contributed to the knowledge of compounds found in *Clusia minor*. It re-confirms, once more, that the genus *Clusia* and the family Clusiaceae are a valuable source of characteristic compounds, many of which found exclusively in these taxa. A new molecule, for which the name minorone is proposed, has been elucidated, expanding this way the list of known natural benzophenone derivatives and being the first compound exclusively found in *C. minor*. In the following, some ideas on the biosynthetic background and ecological aspects are discussed in detail.

5.1 Biosynthesis

In *Clusia,* the exact biosynthesic sequences of both benzophenone and tocotrienol derivatives is not fully understood. Only sensible hypotheses can be made about the processes that guide the basic compound of each class to the final product. No biochemical study has been conducted on these derivatives syntheses up to now.

δ-Tocotrienol (δ-T3), (PK-CM02 in this study), is one of the four tocotrienols (T3), part of the Vitamins E (Vit.E). T3s have been reported in numerous, unrelated plants such as rice, oats, wheat, maize, rye, barley, hazelnut, walnut, pumpkins, grapefruit, poppy, coconut, palm, grape, buckthorn, olive, flax, safflower and many more (Aggarwal et al., 2010). One of the most abundant sources for specifically δ-T3 is the seed oil of annatto (*Bixa orellana* (L.)) (Frega et al., 1998). δ-T3 and γ-T3 are known to be constituents of the Clusiaceae, with several reports from genera *Clusia* and *Garcinia* while no presence of α- and β- forms is reported within this family.

The biosynthetic pathway of T3s is well understood, as described in the introduction of this study, so much it has also been used on recombinant *Saccharomyces cerevisiae* for the industrial fermentation of δ-T3 (Sun et al., 2020). The chromane ring acts as a chromophore enabling the absorption with a peak at 296 nm. The aliphatic chain provides the majority of lipophilic qualities to the molecule. Vitamin E is known to infiltrate the lipidic membranes and scavenge peroxyl radicals present, that would be damaging to the cell. This antioxidative quality is the main way these vitamins provide beneficials effects on health and against debilitating conditions like light damages, various cancers or aging.

Z-δ-Garcinoic acid (*Z*-δ-GA), (PK-CM01 in this study), is a derivative of δ-T3. The prefix *Z*- specifies the isomerism and δ- is referred to the tocotrienol from which it originates. Contrary to Vit.E, GAs production is solely circumscribed within the Clusiaceae family. GAs are reported from nature in both *E*-/*Z*- isomers, with δ-GA discovered in genera *Clusia*, *Garcinia* and *Tovomitopsis* (Terashima et al., 1997; Setzer et al., 1995; Teixeira et al., 2006; Silva et al., 2013; Lavaud et al., 2015; Marques et al., 2021) while γ-GA is only reported in *Garcinia amplexicaulis* (Vieill., 1883) (Lavaud et al., 2013). No α-GA and β-GA are known in nature.

There is no literature on the biosynthesis of GAs. A sensible hypothesis on how GA are biosynthetized would be that the corresponding tocotrienol undergoes oxidation on the apical part of the aliphatic chain, becoming first an alcohol, then an aldehyde and finally a carboxylic acid (**Figure 35**). This hypothesis is backed up by the report of both isomers of δ-tocotrienolic alcohol in *Clusia* and δ-garcinale in *Garcinia* (Terashima et al., 1997; Teixeira et al., 2006; Silva et al., 2013). The process most likely takes the form of a set of tandemed reactions produced by an enzymatic complex since aldehydes are typically reactive and potentially detrimental for the organism. Therefore, aldehydes are typically treated for further reactions as soon as they are formed. GA seems to be the final or close-to-final product of its pathway, with a single report of a GA derivative, GA methyl ester from *C. pernambucensis* G. Mariz (Silva et al., 2013). Clusiaceae produce a noticeable arrange of other exclusive tocotrienol derivatives through alternative modifications respect to the GA synthesis. These include a subclass, known as amplexichromanols, and their dimers, biamplexichromanols found in *Garcinia amplexicaulis* (Vieill. Ex Pierre) (Lavaud et al., 2013; Lavaud et al., 2015)

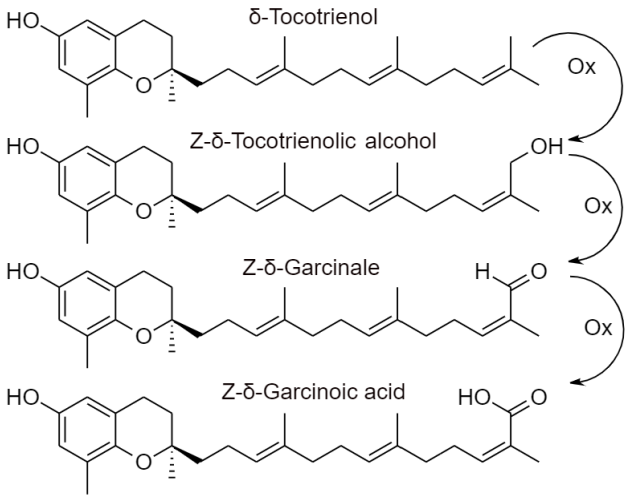

Figure 35: Putative biosynthesis of *Z*-δ-garcinoic acid

Garcinoic acids are quite similar to T3s and share therefore many common features. *Z*-δ-GA absorbs in the UV at 296 nm. It possesses strong antioxidant qualities (Terashima et al., 2002) and has been studied for its application as anti-inflamatory, antibacterial and against chronical degenerative diseases (Michel et al., 2016; Tchimene et al., 2016; Wallert et al., 2019; Hioki et al., 2020; Martinelli et al., 2020) similarly to the investigations on Vit.E.

Compound coded PK-CM05 is a newly discovered compound for which the name minorone is proposed. The compound resembles a polyprenylated benzophenone derivative with the peculiarity of having a 5-membered ring positioned where typically a phloroglucinol ring is expected. This type of ring system is not taken in account from the paper Wu et al. (2014) on subgrouping of benzophenones and, consequentially, no benzophenones' subclassification can be assigned to minorone. Such a change in structure is worth to be potentially considered a new class of compounds, similarly to how xanthones are derived from benzophenones but treated as their own category. The pentacycle bears a β-diketone with one of the ketones in an enol form. The system supposedly is in an equilibrium with the ketone and the enol alternating in their conformation. Only another benzophenone derivative with a similar pentacyclic structure appears in literature: paucinone I (**Figure 36**) extracted from *Garcinia paucinervis* (Chun & F.C. How) (Li et al., 2016) which differs from minorone just by the presence of two additional hydroxyl groups on the benzene ring and a different, uncycled long prenyl chain.

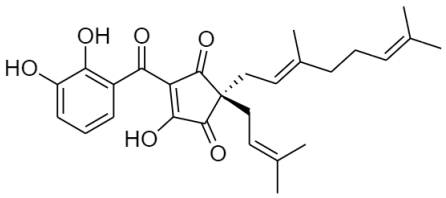

Figure 36: Structural formula of Paucinone I

Paucinone I and minorone most likely share very similar biosynthetic pathways, with eventually homologous genes that are responsible for the formation of the pentacycle. This type of reaction is quite atypical, where cycles usually are modified either by the level of insaturation, by the addition of lateral moieties or the opening of the entire cycle. A similar case of cycle isomerization that is well documented, concerns humulone, an α-acid that converts to isohumulone, a iso-α-acid, during processing of hops (*Humulus lupulus* (L.)) for beer production (De Keukeleire et al., 2008). The synthesis of isohumulone is heat induced when the hops are boiled (**Figure 37**).

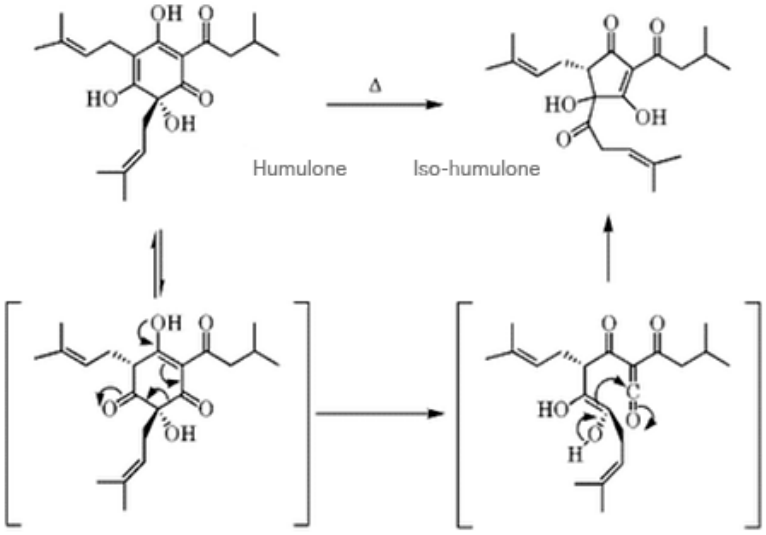

Figure 37: Humulone to isohumulone isomerization (Source: sciencebrewer.wordpress.com).

In the case of our study, the solutions were never raised above 40 °C during the isolation procedures, to avoid the formation of such artifacts. Supposedly Li et al., (2016) took similar precautions for the isolation of paucinone I. It may thus be assumed that the formation of these pentacycles in Clusiaceae results from reactions taking place within the living cells of the respective plants. Nonetheless, the isohumulone example is still worth noticing, as some enzymes in the Clusiaceae might mimic such chemcial reaction at environmental temperature.

Isohumulone is also involved in another noticeable reaction: isohumulone undergoes a spontaneous α-cleavage when exposed to UV-B radiation (280–320 nm) (**Figure 38**) (De Keukeleire et al., 2008). The resulting molecule, dehydrohumulinic acid, resembles even more the compounds found in the Clusiaceae regarding the number and disposition of degrees of unsaturation among the ring and the oxygen moieties attached. Given how much effort Clusiaceae are already known to invest in the synthesis of UV absorbing molecules, it is reasonable to suspect that UV radiation might somehow be linked with the formation or the role of these clusiacean pentacycles though the relationship between a UV absorbing molecule and its abundance in the roots remains unclear and seemingly counterintuitive.

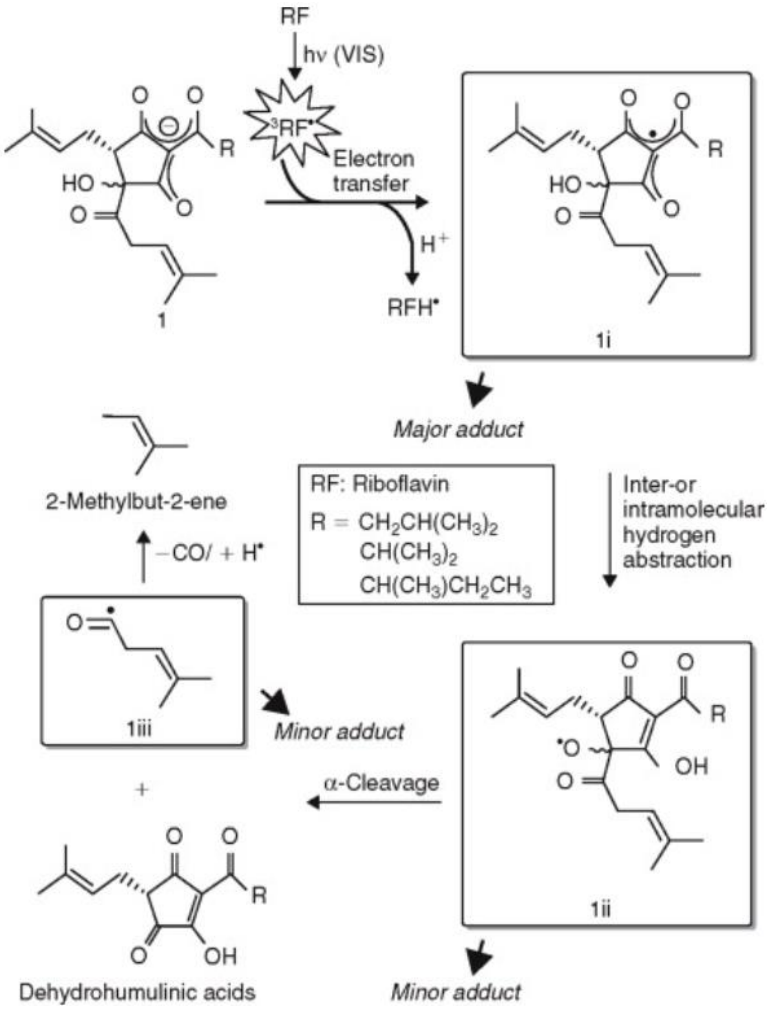

Figure 38: Light induced synthesis of dehydrohumulinic acid from isohumulone (Source: De Keukeleire et al., 2008)

The biosynthesis of minorone is unknown so far, and it can be only speculated that the already elucidated molecules act as intermediates for the biosynthesis of this compound in

Clusia. The most confident precursor may be 2,4,6-trihydroxybenzophenone, as it is the theorized precursor of most benzophenones in *Clusia*. Various, sequential prenylation reactions would be required resulting in various type-D polyprenylated benzophenones. At the end of these steps, the cyclization of the long prenyl chain to form the cyclohexene moiety would follow. This type of ring moiety is found only in another known benzophenone, hilarione (Porto et al., 2000), which would have potential to serve as a precursor to minorone. The hydroxylation of the phloroglucinol's carbon, which carries a single prenyl chain, would transform hilarione into a molecule that, analogous to the isohumulone and isohumulinic acid syntheses, would be subject to isomerization of the phloroglucinol ring, and the α-cleavage of a prenyl chain resulting in minorone. The oxidation of the phloroglucinol ring seems to destabilize the structure, and this is a key step for the ring isomerization. This might explain why benzophenones found in nature which carry a phloroglucinol ring never exhibit additional hydroxyl groups on that ring. Benzophenones having multiple hydroxyl groups in their secondary ring, also in *otho-* or *para-* position, do exist, but they never exhibit the phloroglucinol ring disposition and are restricted to the tribe Dalbergieae (Fabaceae) (Baggett et al., 2005).

In **Figure 39** a biosynthetic pathway for minorone is proposed. The putative pathway accounts for the chemistry that guides the isohumulinic acid synthesis and accounts also for the already known potential benzophenone intermediates which are tagged with their given names. Since the biosynthethic pathways of benzophenone derivatives are not explored, some ambiguity remains on the directionality of the reactions, like in the case of the steps that pass via weddelianone A and lanceolatone synthesis or, as another example, multiple steps of condensation of an isoprene unit (clusiaphenone B → grandone → lanceolatone/weddelianone A) might instead be performed by a single step of lavandulyl condensation (clusiaphenone B → lanceolatone/weddelianone A). It must also be noticed how the α-cleavage reaction requires the formation of a radical. Radicals are reactive species, and thus generally unfavored reactions in nature. However, it must be noted that Clusiaceae possess a wide arsenal of antioxidative compounds to deal with this type of chemical species.

The proposed pathway is only a sensible approximation of which reactions might take place. It does not take into account dangerous byproducts, enzymes, complexes, coenzymes and other eventual features that would be required for a proper biosynthetic pathway eluci-

dation. Possibly the gradual discovery of new benzophenones derivatives will fill the gaps of the tree of relationship for a better understanding of putative biosynthetic pathways.

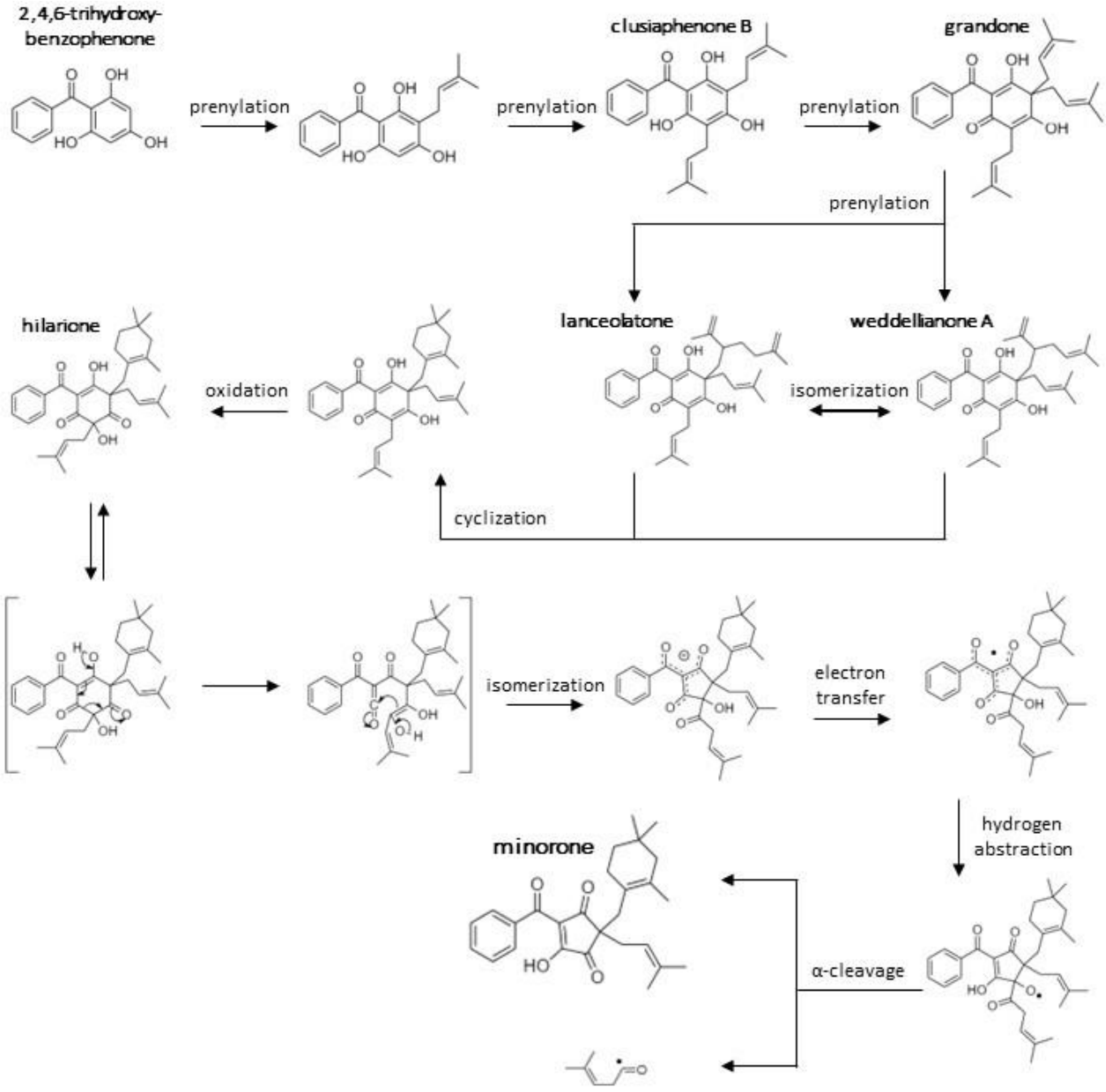

Figure 39: Putative biosynthetic pathway of minorone

5.2 *In planta* compartmentation and transport

What also remains obscure in the general biochemistry of *Clusia* spp. is compartmentation of the biosynthethic pathways concerning described compounds, and equally, on their

transport *in planta*. The synthesis of metabolites in specific organs followed by their transport to the final accumulation site is no news in the plant kingdom, like the well-studied example of nicotine being synthetized in the root of the *Nicotiana* spp. and transported to the aerial parts of the plant (Zenkner et al., 2019). In addition to the vascular system, *Clusia* spp. also possess a latex duct system that runs throughout the plant. The latex, which runs throught the plant organs, has already been highlighted as a source of benzophenone and terpenes (Lokvam et al., 2000; Camara et al., 2018) and has been used in ethnomedicine due to its bioactive chemicals (da Camara et al., 2021). However, nothing is known about the tissue or cells in which they were originally produced.

The roots, subject organ of this study, have widely been ignored in phytochemical studies on *Clusia* spp. Like all other parts of the plant, they have shown to be a valuable source of UV protecting molecules, which is a bit odd given that the roots typically are not exposed to a lot of sunlight. This apparent incongruence opens a question whether those compounds migrated from the root or to the root, though it must be taken into account that UV protection might not be the prime role for which the compound was synthetized. The positioning and the type of chemicals inside the plants all boils down to their functional role and when looking at secondary metabolites this translates to their ecological role.

5.3 Ecological Aspects

The literature on the ecology of *Clusia* spp. is scarce, except for the function of floral resin in the pollination syndrome. The studies are mostly generic, mentioning *Clusia* as part of the plants that inhabit tropical forests or serve ecological restoration roles. Those study that focused on specific biotic interaction only refer to macroorganisms, and no literature reports exist on microbial interactions even though it is known that *Clusia* spp. host quite a remarkable variety of epiphyllic organisms. Furthermore, it is suggested that there should be endophytes present with their own set of interesting compounds (Kavtaradze et al., 2014).

All compounds discovered in the study show high lipophilicity, UV absorption and antioxidant qualities, reinforcing this way these as the main feature of the SPM produced by this genus and family. With these recurring trends so do the questions revolving around them recur: what purpose have the members this genus and family to produce such large quantities and in such a diverse variety of compounds that shield from UV light and prevent from oxida-

tive damages? Surely in the tropics, where these plants are found, the UV radiation, and consequential production of free radicals, plays a much more significant role than the regions at higher latitudes. Nevertheless, the amounts of antioxidants and UV protectant molecules is exceptionally high also when compared to other tropically adapted species to the point that abiotic stress seems unlikely to be the only explanation for the syntheses of these compounds.

Many of these molecules, benzophenone derivatives in particular, have remarkable bioactivities, with many studies pointing them out for antiviral, antibacterial, antiprotozoan, antifungal and anticarcinogenic activities citation. One could be tempted to explain such a variety of chemicals as being a massive weaponry of defense molecules, in analogoy to the fact that there has been growing evidence for such an effect of floral resin benzophenones that bees collect for making their hives. However, this would only shift the paradigm of the original question to why a plant would need such an arsenal. Also, antioxidants are often preceived as matter of healthiness, but their role as being detrimental to other organisms is less understood. Therefore, another interesting question, would be if benzophenones of *Clusia* spp. might have some detrimental effect on other organisms. These molecules might possess specifical structures tailored to tackle some essential pathway of the targeted organisms. There are no studies on the molecular dynamics of benzophenone derivatives, and only one is available about garcinoic acid being identified as a selective antagonist of the pregnane X receptor (Bartolini et al., 2020). Moreover, some of these compounds might have positive effects or signaling purposes in specific biotic interactions in nature.

The newly described compound Minorone possesses a chromophore, with an aromatic ring joined to a set of three ketonic carbons resonance. The main absorption maxima at 272 nm is most likely the result of the aromatic ring in this complicated system, similarly to how basic benzophenones also absorb in the higher half of the UV-C range (Basu et al., 2009). The secondary absorption maxima in the UV-A range at 338 nm most likely follows similar dynamics of those that explain a similar absorption for 2-hydroxybenzophenones (Al-Malaika et al., 2017) and is given by the interaction of the benzoyl ketone with the close-by enol group. The complex chromophore of this compound suggests this molecule might have considerable antioxidant and UV-protectant qualities.

This atypical vast production of antioxidative and UV absorbing compounds is presumably evolutionarily advantageous, adding up to the other quirky key innovations mentioned in

the introduction. It is reasonable to consider that the key innovations have evolved synergistically during the process of occupying potential niches, and for this reason, part of the explanation on the chemistry of *Clusia* might lay in its hemi-epiphytism, the floral resin production and the CAM-like photosynthesis. Primary hemi-epiphytism is a strategy that inevitably results in low nutrient availability, at least for some periods and could have put evolutionary pressure to limit the use of nitrogen and fuel the evolution of organic acids instead though shikimate and polyketide pathways. Intense light exposure and biochemistry for the handling of organic acids are two common features shared both by the CAM photosynthesis and the benzophenones. Floral resin has also been linked to specific functions of benzophenones found in it.

What most likely is sure is that a whole class of compounds, especially big ones, cannot be affiliated to a single function and, therefore, depending on each specific compound. Benzophenones are synthetized by plants most likely for UV protection, control of free radicals, defense against pests, mutualistic interactions (e.g. pollination) and many more yet unidentified reasons. Tocotrienols and their derivatives occur lower in number and, consequentially, probably more limited to few specific functions, the most noticeable of which being the sequestration of free radicals. The derivatives of tocotrienols, by being modified, might gain additional functions which are still to be discovered.

6 Conclusion and Outlook

The study on the chemodiversity of *Clusia minor* led to the isolation of five compounds, of which three could be structurally characterized and identified as a tocotrienol, a tocotrienol derivative and a novel benzophenone derivative. These new reports will be an implementation of the ongoing knowledge revolving the compounds found in *C. minor* (and by proxy in *Clusia* and Clusiaceae more generally) and more specifically regarding the roots of this genus, which are very rarely studied. The newly discovered benzophenone derivative, minorone, adds a tile in the ongoing structuring of the unclear benzophenone class. Minorone, with its triketo- (one in enol- conformation) cyclopentene ring, calls for the naming of, either a new subclass of benzophenones, or a completely new class of phenolic derivatives, with more compounds to be assigned to it (e.g. paucinone I). The study highlights the necessity for more research to complement the gaps that are now present in our knowledge. As mentioned in the discussion, aspects linking these compounds to the tissue of origin, the biosynthetic pathway involved, the potential transport the role served to the plant is all virtually impossible with the current literature. There is plenty that can be uncovered on the topic and the technology is already advance enough to achieve this, with possibly even faster and more precise options to be available soon. The work of this study will become part of larger study (Schinnerl, personal communication) along with other three works that each focus on secondary metabolites isolated in different organs of *C. minor* to highlight the diversity of compounds within this species. Some of the isolated compounds in the other works have been tested for non-choice feeding assays and measurement of molar extinction coefficient. All collected samples will be sent to a collaborating research group to be tested for potential antimalarian properties.

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8 Appendix

8.1 Zusammenfassung

Die Arten der Gattung *Clusia* (Clusiaceae) sind verholzende Pflanzen der Neotropen und werden hauptsächlich bezüglich ihrer Fotosynthesestrategien analysiert. *Clusia* Arten akkumulieren jedoch auch spezielle Pflanzenmetabolite (SPM) von auffälligen Strukturen und bioaktiven Eigenschaften. Die Arten dieser Gattung zeigen eine Tendenz zur Biosynthese von Stoffen aus dem Phenylpropanoidstoffwechsel, oftmals in Kombination mit Bausteinen aus dem Terpenoid- und Polyketidstoffwechsel. Innerhalb des Pflanzenreichs ist die Gattung besonders bekannt für die Akkumulation von Benzophenonderivaten. Ziel der vorliegenden Arbeit war es, die Chemodiversität von *Clusia minor* in Bezug auf die SPM eingehender zu untersuchen. Zu diesem Zwecke wurden Wurzeln dieser Pflanzenart für eingehende phytochemische Analyse ausgewählt, wobei standardisierte chromatographische Techniken zur Auftrennung des Extrakts und zu Isolierung der SPM eingesetzt wurden. Die Identifikation der Strukturen isolierte Verbindungen erfolgte durch Interpretation von MS- und NMR- Daten. Insgesamt konnten fünf Verbindungen isoliert werden, von denen drei strukturell eindeutig identifiziert werden konnten. Zwei Stoffe erwiesen sich als Tocotrienol-Derivate (*Z*-δ-Garcinoic Acid und δ-Tocotrienol). Basierend auf NMR- und MS-Daten konnte die dritte Verbindung als ein neues Benzophenon-Derivat identifiziert werden, welches durch eine ungewöhnliche fünfteilige Ringstruktur gekennzeichnet ist und als Minoron bezeichnet wurde. Darüber hinaus wird die bisher bekannte Literatur in Bezug auf Chemodiversität der SPM ausgewertet und umfassend diskutiert.

8.1.1 Schlagwörter

Clusia minor, spezielle Pflanzenmetablite; Tocotrienol-Derivate, Benzophenon-Derivate

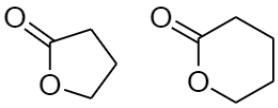
8.2 SPM reported in *Clusia* spp.

The compounds are divided according to the core structure of the molecule. The classes are listed in **Table 9** in order of increasing molecular mass of the core structure and subcategorization is in alphabetical order.

Table 9: SPM reported in genus *Clusia*

8.2.1 Lactones

Core structures

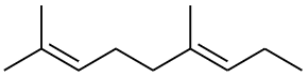


Compound	*Clusia* sp.	Organ	Reference
4-Hydroxy-5,5-dimethyldihydro-furan-2-one	*burle-marxii*	leaf	Ribeiro et al., 2011

8.2.2 Terpenoids

8.2.2.1 Monoterpenoids

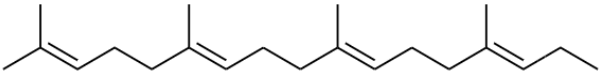
Core structure



Compound	*Clusia* sp.	Organ	Reference
α-Terpinene	*hilariana*	latex	da Camara et al., 2018
β-Pinene	*criuva*	latex	da Camara et al., 2018
E-Ocimenone	*grandiflora*	latex	da Camara et al., 2018
Z-Ocimenone	*grandiflora*	latex	da Camara et al., 2018
Camphene	*criuva* *hilariana*	latex	da Camara et al., 2018
Verbenone	*grandiflora*	latex	da Camara et al., 2018

8.2.2.2 Sesquiterpenoids

Core structure



Compound	*Clusia* sp.	Organ	Reference
1,10-Di-epi-cubenol	*spiritu-sanctensis*	latex	da Camara et al., 2018
1-Epi-cubenol	*nemorosa*	latex	da Camara et al., 2018
4-Hydroxy-3,5-di-tert-butyltoluene	*minor*	leaf	Mangas Marin et al., 2018
10-Epi-γ-eudesmol	*fluminensis* *weddelliana*	latex	da Camara et al., 2018
14-Hydroxi- α-muurolene	*paralicola*	latex	da Camara et al., 2018
α-Amorphene	*minor* *sadiensis*	fruit	Gonzalez et al., 1993
α-Aorfene	*minor*	leaf	Mangas Marin et al., 2018
α-Bulnesene	*weddelliana*	latex	da Camara et al., 2018
α-Cadinene	*criuva* *hilariana* *nemorosa* *panapanari* *spiritu-sanctensis*	fruit latex	de Oliveira et al., 2008; da Camara et al., 2018
α-Cadinenol	*lanceolata*	leaf	Ferreira et al., 2014
α-Cadinol	*grandiflora*	latex	da Camara et al., 2018
α-Calacorene	*grandiflora* *parviflora* *rosea* *sadiensis*	fruit latex	Gonzalez et al., 1993; da Camara et al., 2018
α-Caryophyllene	*lanceolata*	leaf	Ferreira et al., 2014
α-Cedrene	*eugenioides*	root	Gonzalez et al., 1993
α-Coapene	*criuva*	fruit	Mangas Marin et

	fluminensis *grandiflora* *hilariana* *lanceolata* *minor* *nemorosa* *paralicola* *spiritu-sanctensis*	latex leaf	al., 2008a; de Oliveira et al., 2008; Ferreira et al., 2014; da Camara et al., 2018
α-Cubebene	*hilariana* *minor* *nemorosa* *paralicola*	fruit latex	Gonzalez et al., 1993; de Oliveira et al., 2008; da Camara et al., 2018
α-Curcumene	*eugenioides* *hilariana* *rosea*	latex root stem	Gonzalez et al., 1993; da Camara et al., 2018; Rosado et al., 2020
α-Curcumene oxide	*eugenioides*	root	Gonzalez et al., 1993
α-Gurjunene	*weddelliana*	latex	da Camara et al., 2018
α-Humulene	*criuva* *fluminensis* *hilariana* *grandiflora* *lanceolata* *nemorosa* *panapanari* *rosea* *spiritu-sanctensis* *weddelliana*	fruit latex	de Oliveira et al., 2008; da Camara et al., 2018
α-Muurolene	*criuva* *hilariana* *minor* *nemorosa* *sadiensis*	fruit latex leaf	Gonzalez et al., 1993; de Oliveira et al., 2008; da Camara et al., 2018; Mangas Marin et al., 2018
α-Selinene	*fluminensis* *grandiflora* *weddelliana*	latex	da Camara et al., 2018

α-Ylangene	*criuva* *lanceolata* *sadiensis*	fruit latex	Gonzalez et al., 1993; da Camara et al., 2018
β-Bisabolene	*rosea*	latex	da Camara et al., 2018
β-Bourbonene	*lanceolata* *nemorosa*	fruit leaf	de Oliveira et al., 2008; Ferreira et al., 2014
β-Calacorene	*nemorosa*	fruit	de Oliveira et al., 2008
β-Caryophyllene	*criuva* *fluminensis* *grandiflora* *hilariana* *lanceolata* *nemorosa* *paralicola* *parviflora* *spiritu-sanctensis* *weddelliana*	fruit latex	de Oliveira et al., 2008; da Camara et al., 2018
β-Copaen-4α-ol	*criuva* *grandiflora*	latex	da Camara et al., 2018
β-Copaene	*lanceolata*	leaf	Ferreira et al., 2014
β-Cubebene	*minor*	leaf	Mangas Marin et al., 2008a
β-Elemene	*lanceolata* *minor* *panapanari* *paralicola* *spiritu-sanctensis* *weddelliana*	latex leaf	Mangas Marin et al., 2008a; Ferreira et al., 2014: da Camara et al., 2018
β-Guaiene	*minor*	leaf	Mangas Marin et al., 2008a
β-Gurjunene	*grandiflora* *nemorosa* *paralicola* *weddelliana*	fruit latex	de Oliveira et al., 2008; da Camara et al., 2018
β-Selinene	*fluminensis* *grandiflora*	fruit latex	Gonzalez et al., 1993; Mangas

	lanceolata *minor* *paralicola* *rosea* *sadiensis* *weddelliana*	leaf	Marin et al., 2008a; Ferreira et al., 2014; da Camara et al., 2018; Mangas Marin et al., 2018
β-Maaliene	*minor*	leaf	Mangas Marin et al., 2008a; Mangas Marin et al., 2018
γ-Cadinene	*criuva* *grandiflora* *hilariana* *lanceolata* *minor* *nemorosa* *panapanari* *parviflora* *spiritu-sanctensis*	fruit latex leaf	de Oliveira et al., 2008; Mangas Marin et al., 2008a; Ferreira et al., 2014; da Camara et al., 2018
γ-Muurolene	*grandiflora* *lanceolata* *nemorosa* *panapanari*	fruit latex leaf	de Oliveira et al., 2008; Ferreira et al., 2014; da Camara et al., 2018
δ-Amorphene	*lanceolata*	leaf	Ferreira et al., 2014
δ-Cadinene	*criuva* *grandiflora* *hilariana* *lanceolata* *minor* *nemorosa* *panapanari* *paralicola* *rosea* *spiritu-sanctensis* *weddelliana*	fruit latex leaf	de Oliveira et al., 2008; Mangas Marin et al., 2008a; Ferreira et al., 2014; da Camara et al., 2018
δ-Elemene	*lanceolata* *minor* *nemorosa* *panapanari*	fruit latex leaf	de Oliveira et al., 2008; Mangas Marin et al., 2008a; Ferreira

	paralicola *spiritu-sanctensis*		et al., 2014 Mangas Marin et al., 2018; da Camara et al., 2018
(-)-Spathulenol	*criuva* *fluminensis* *grandiflora* *minor* *nemorosa* *panapanari* *rosea* *spiritu-sanctensis*	latex leaf	Mangas Marin et al., 2008a; da Camara et al., 2018
E-α-Bergamotene	*criuva* *rosea* *panapanari* *spiritu-sanctensis*	latex	da Camara et al., 2018
E -β-Farnesene	*nemorosa*	latex	da Camara et al., 2018
E -β-Guaiene	*fluminensis*	latex	da Camara et al., 2018
E -γ-Bisabolene	*spiritu-sanctensis*	latex	da Camara et al., 2018
E-Cadina-1,4-diene	*grandiflora* *lanceolata* *weddelliana*	latex	da Camara et al., 2018
E-Nerolidol	*weddelliana*	latex	da Camara et al., 2018
Z-Caryophyllene	*lanceolata*	leaf	Ferreira et al., 2014
Allo-aromadendrene	*lanceolata* *nemorosa*	latex leaf	Ferreira et al., 2014; da Camara et al., 2018
Aromadendrene	*lanceolata* *minor* *panapanari* *paralicola*	latex leaf	Ferreira et al., 2014; Mangas Marin et al., 2018; da Camara et al., 2018
Bicyclogermacrene	*lanceolata* *panapanari* *spiritu-sanctensis*	latex leaf	da Camara et al., 2018; Ferreira et al., 2014

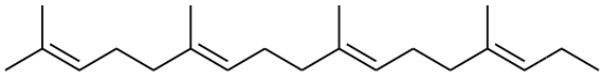
Cadalin	*minor*	leaf	Mangas Marin et al., 2018
Calamenene	*sadiensis*	fruit	Gonzalez et al., 1993
Caryophyllene	*minor*	leaf	Mangas Marin et al., 2008a
Caryophyllene oxide	*fluminensis* *grandiflora* *hilariana* *lanceolata* *minor* *nemorosa* *parviflora* *sadiensis*	fruit latex leaf	Gonzalez et al., 1993; de Oliveira et al., 2008; Ferreira et al., 2014; da Camara et al., 2018
Caryophyllenyl alcohol	*lanceolata*	leaf	Ferreira et al., 2014
Cedrane	*eugenioides*	root	Gonzalez et al., 1993
Cubenol	*grandiflora* *panapanari* *spiritu-sanctensis*	latex	da Camara et al., 2018
Cycloisolongifolene	*minor*	leaf	Mangas Marin et al., 2018
Cyclosativene	*nemorosa*	fruit	de Oliveira et al., 2008
Cyperene	*eugenioides* *lanceolata*	latex root	Gonzalez et al., 1993; da Camara et al., 2018
Dihydro-Aromadendrene	*spiritu-sanctensis*	latex	da Camara et al., 2018
Dihydro-curcumene	*hilariana*	stem	Rosado et al., 2020
Epi-α-cadinol	*nemorosa* *rosea*	latex	da Camara et al., 2018
Epi-α-muurolol	*lanceolata* *panapanari* *parviflora*	latex leaf	Ferreira et al., 2014; da Camara et al., 2018
Eudesmol	*lanceolata*	leaf	Ferreira et al., 2014
Germacrene B	*lanceolata*	fruit	de Oliveira et al.,

	nemorosa	leaf	2008; Ferreira et al., 2014
Germacrene D	*criuva* *fluminensis* *hilariana* *lanceolata* *nemorosa* *panapanari* *paralicola* *parviflora*	latex leaf	Ferreira et al., 2014; da Camara et al., 2018
Globulol	*lanceolata* *minor* *parviflora*	latex leaf	Ferreira et al., 2014; Mangas Marin et al., 2018; da Camara et al., 2018
Guaiol	*criuva*	latex	da Camara et al., 2018
Humulene epoxide II	*lanceolata* *nemorosa*	fruit leaf	de Oliveira et al., 2008; Ferreira et al., 2014
Isocaryophyllene	*grandiflora*	fruit	Gonzalez et al., 1993
Junenol	*lanceolata*	leaf	Ferreira et al., 2014
Khusimone	*panapanari*	latex	da Camara et al., 2018
Khusinol	*paralicola*	latex	da Camara et al., 2018
Ledol	*lanceolata*	leaf	Ferreira et al., 2014
Palustrol	*lanceolata*	leaf	Ferreira et al., 2014
Selin-4,7(11)-diene	*minor*	leaf	Mangas Marin et al., 2018
Spirolepechinene	*lanceolata*	latex leaf	Ferreira et al., 2014; da Camara et al., 2018
Valencene	*nemorosa* *sadiensis*	fruit	Gonzalez et al., 1993; de Oliveira et al., 2008

Vetivenene	*sadiensis*	fruit	Gonzalez et al., 1993
Viridiflorene	*lanceolata*	leaf	Ferreira et al., 2014
Viridiflorol	*lanceolata*	leaf	Ferreira et al., 2014
Vulgarol B	*minor*	leaf	Mangas Marin et al., 2008a

8.2.2.3 Diterpenoids

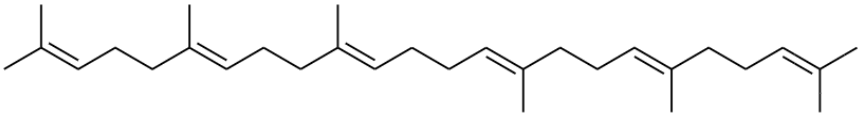
Core structure



Compound	*Clusia* sp.	Organ	Reference
Neophytadiene	*minor*	leaf	Mangas Marin et al., 2008a; Mangas Marin et al., 2018; Mangas Marin et al., 2019
Kauren	*coclensis* *cretosa* *multiflora* *triflora*	leaf	Medina et al., 2006
Phytol	*minor*	leaf	Mangas Marin et al., 2008a; Mangas Marin et al., 2019
Verticiol	*coclensis* *cretosa*	leaf	Medina et al., 2006

8.2.2.4 Triterpenoids

Core structure



Subclass	Compound	*Clusia* sp.	Organ	Reference
Cholestane	β-Sitosterol glucoside	*nemorosa*	bark fruit leaf	De Andrare et al., 1998; Ferreira et al., 2015; Ferreira et al., 2022
	β-Sitosterol	*burle-marxii* *criuva* *lanceolata* *melchiorii* *minor* *nemorosa* *obdeltifolia* *paralicola* *pernambucensis*	fruit leaf trunk bark	Mangas Marin et al., 2008a; Teixeira et al., 2006; Oliveira et al., 2012; Silva et al., 2013; Ferreira et al., 2015; Ferreira et al., 2016; Mangas Marin et al., 2018; Mangas Marin et al., 2019; Ribeiro et al., 2019; Marques et al., 2021
	Cholestane	*multiflora*	leaf	Medina et al., 2006
	Lanosterol	*criuva* *fluminensis* *minor* *pernambucensis*	flower fruit latex leaf trunk	de Oliveira et al., 1996; Mangas Marin et al., 2008a; Duprat et al., 2017; Marques et al., 2021
	Sitostenone	*burle-marxii* *criuva* *lanceolata* *melchiorii* *obdeltifolia*	leaf trunk	Teixeira et al., 2006; Ferreira et al., 2016; Ribeiro et al., 2019; Marques et al., 2021
	Stigmast-5-en3β,7α-diol	*burle-marxii*	trunk	Ribeiro et al., 2019
	Stigmast-5-en-3β,7β-diol	*burle-marxii*	trunk	Ribeiro et al., 2019
	Stigmast-7-en3β-ol-6-one	*burle-marxii*	trunk	Ribeiro et al., 2019
	Stigmastan-3,5-diene	*minor*	leaf	Mangas Marin et al., 2019

	Stigmastenone	*lanceolata melchiorii*	leaf trunk	Teixeira et al., 2006; Ferreira et al., 2016
	Stigmastenone acetate	*hilariana*	stem	Rosado et al., 2020
	Stigmasterol	*burle-marxii criuva lanceolata melchiorii minor nemorosa obdeltifolia paralicola*	fruit leaf trunk	Mangas Marin et al., 2008a; Teixeira et al., 2006; Oliveira et al., 2012; Ferreira et al., 2015; Ferreira et al., 2016; Mangas Marin et al., 2018; Mangas Marin et al., 2019; Ribeiro et al., 2019; Marques et al., 2021
	Stigmasterol glucoside	*nemorosa*	fruit	Ferreira et al., 2022
Euphane	Butyrospermol	*burle-marxii*	trunk	Ribeiro et al., 2019
	Euphane-8, 23-diene-3b, 25-diol	*columnaris*	branch+leaf	Compagnone et al., 2008
	Euphane-8, 25-diene-3b, 24-diol	*columnaris*	branch+leaf	Compagnone et al., 2008
	Euphane-8-ene-3b, 24, 25-triol	*columnaris*	branch+leaf	Compagnone et al., 2008
	Euphol	*burle-marxii columnaris flava melchiorii*	fruit trunk	Teixeira et al., 2006; Compagnone et al., 2008; Parker, 2017, Ribeiro et al., 2019
Friedelane	3-Oxo-friedelin	*hilariana*	stem	Rosado et al., 2020
	Epifriedelinol	*hilariana minor*	leaf stem	Mangas Marin et al., 2008a; Mangas Marin et al., 2018; Rosado et

				al., 2020
	Friedelin	*burle-marxii* *coclensis* *criuva* *liesneri* *melchiorii* *minor* *multiflora* *nemorosa* *obdeltifolia* *peninsulae* *portolandiana* *triflora* *uvitana*	bark fruit leaf trunk	Barrios et al., 1990; De Andrare et al., 1998; Teixeira et al., 2006; Medina et al., 2006; Mangas Marin et al., 2008a; Ribeiro et al., 2011; Ferreira et al., 2015; Parker, 2017; Mangas Marin et al., 2018; Mangas Marin et al., 2019; Ribeiro et al., 2019; Marques et al., 2021
	Friedelin-3β-ol	*nemorosa* *portolandiana*	bark fruit leaf	De Andrare et al., 1998; Ferreira et al., 2015; Parker, 2017
	Friedelinol	*criuva* *uvitana*	leaf trunk	Barrios et al., 1990; Marques et al., 2021
	Friedolane	*coclensis* *cretosa* *liesneri*	leaf	Medina et al., 2006
Linear triterpenoid	Squalene	*coclensis* *minor* *liesneri* *minor* *multiflora* *peninsulae* *stenophylla* *triflora*	leaf	Medina et al., 2006; Mangas Marin et al., 2008a; Mangas Marin et al., 2018; Mangas Marin et al., 2019
Lupane	Betulin	*osseocarpa*	leaf	Medina et al., 2006
	Betulinic acid	*burle-marxii* *criuva*	bark fruit	De Andrare et al., 1998; Teixeira et

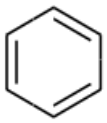
		melchiorii *nemorosa* *obdeltifolia* *pernambucensis*	leaf trunk	al., 2006; Ribeiro et al., 2011; Silva et al., 2013; Ferreira et al., 2015; Ribeiro et al., 2019; Marques et al., 2021
	Betulinic aldehyde	*burle-marxii* *obdeltifolia*	trunk	Teixeira et al., 2006; Ribeiro et al., 2019
	Betulonic acid	*burle-marxii* *obdeltifolia*	trunk	Teixeira et al., 2006; Ribeiro et al., 2019
	Lupenone	*hilariana*	stem	Rosado et al., 2020
	Lupeol	*criuva* *minor* *multiflora*	leaf trunk	Mangas Marin et al., 2008a; Mangas Marin et al., 2018; Marques et al., 2021
	Winchic acid	*criuva*	trunk	Marques et al., 2021
Oleanane	28-nor-olean-17-en-3-one	*hilariana*	stem	Rosado et al., 2020
	Friedo-olean-7,9(11)-diene-3- β-ol	*hilariana*	stem	Rosado et al., 2020
	Oleanoilic acid	*hilariana*	floral resin fruit	Kelecom et al., 2002
	Glutinol	*obdeltifolia*	trunk	Teixeira et al., 2006
Taraxane	Taraxerol	*stenophylla*	leaf	Medina et al., 2006
Ursane	α-Amyrin	*hilariana* *lanceolata* *liesneri* *minor*	leaf stem	Mangas Marin et al., 2008a; Ferreira et al., 2016; Mangas Marin et al., 2019; Rosado et al., 2020
	β-Amyrin	*hilariana*	fruit	Mangas Marin et

	lanceolata *liesneri* *minor* *paralicola*	leaf stem	al., 2008a; Oliveira et al., 2012; Ferreira et al., 2016; Mangas Marin et al., 2019; Rosado et al., 2020
Urs-12-en-28-al	*minor*	leaf	Mangas Marin et al., 2018

8.2.3 Phenolics

8.2.3.1 Simple benzene derivatives

Core structure

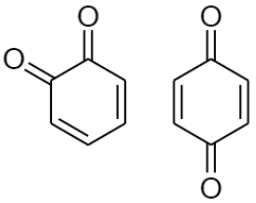


Compound	*Clusia* sp.	Organ	Reference
Benzaldehyde	*criuva*	latex	da Camara et al., 2018
Butylhydroxytoluene	*amazonica* *cylindrica* *minor* *multiflora* *osaensis* *rosea*	leaf	Weckwerth, personal comunication
Ethyl benzoate	*criuva* *parviflora*	latex	da Camara et al., 2018
Methyl benzoate	*hilariana* *paralicola*	latex	da Camara et al., 2018
p-Anisaldehyde	*criuva* *fluminensis* *grandiflora* *lanecolata* *paralicola* *spiritu-sanctensis*	latex	da Camara et al., 2018
Protocatechuic acid	*amazonica* *burle-marxii* *cylindrica* *minor* *multiflora*	leaf trunk	Ribeiro et al., 2011; Ferreira et al., 2022

	osaensis *rosea*		
Pyrogallol	*amazonica* *cylindrica* *minor* *multiflora* *osaensis* *rosea*	leaf	Weckwerth, personal comunication
Vanillic acid	*amazonica* *cylindrica* *minor* *multiflora* *osaensis* *rosea*	leaf	Schinnerl, personal comunication; Weckwerth, personal comunication

8.2.3.2 Benzoquinone derivatives

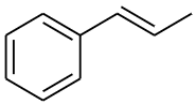
Core structures



Compound	*Clusia* sp.	Organ	Reference
2,6-Dimethoxy-1,4-benzoquinone	*arboricida*	N.A.	Nagem et al., 1993

8.2.3.3 Phenylpropanoids

Core structure

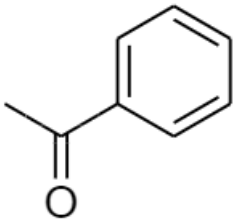


Compound	*Clusia* sp.	Organ	Reference
Caffeic acid	*amazonica* *cylindrica* *minor* *multiflora* *osaensis* *rosea*	leaf	Schinnerl, personal comunication; Weckwerth, personal comunication
Coumaric acid	*nemorosa*	fruit	Ferreira et al., 2022
Octacosanoyl ferulate	*nemorosa*	fruit	De Andrare et al.,

			1998

Core structure

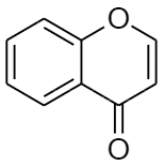
8.2.3.4 Acetophenone derivatives



Compound	*Clusia* sp.	Organ	Reference
Acetophenone	*criuva* *fluminensis* *grandiflora* *lanceolata* *nemorosa* *paralicola* *parviflora* *rosea* *weddelliana*	latex	da Camara et al., 2018
6S,8S,28S-nemorosic acid	*nemorosa*	fruit	Ferreira et al., 2015
Gundlachiione A	*gundlachii*	fruit	Zhang et al., 2018
Gundlachiione B	*gundlachii*	fruit	Zhang et al., 2018
Gundlachiione C	*gundlachii*	fruit	Zhang et al., 2018
Gundlachiione D	*gundlachii*	fruit	Zhang et al., 2018
Gundlachiione E	*gundlachii*	fruit	Zhang et al., 2018

Core structure

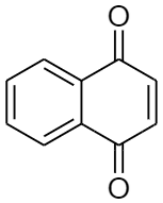
8.2.3.5 Chromone derivatives



Compound	*Clusia* sp.	Organ	Reference
5,7-Dihydroxy-2-(*n*-hep-taeicosanyl)chromone	*nemorosa*	bark leaf	De Andrare et al., 1998

8.2.3.6 Naphtaquinone derivatives

Core structure

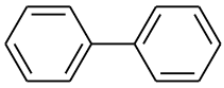


Compound	*Clusia* sp.	Organ	Reference
Xyloidone	*melchiorii*	trunk	Teixeira et al., 2006

8.2.4 Polyphenolics

8.2.4.1 Biphenyl derivatives

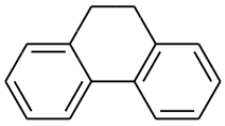
Core structure



Compound	*Clusia* sp.	Organ	Reference
2,2-Dimethyl-3,5-dihydroxy-7-(4-hydroxyphenylchro-mane	*burle-marxii*	trunk	Ribeiro et al., 2011
2,2-Dimethyl-5-hydroxy-7-phenylchromane	*burle-marxii*	trunk	Ribeiro et al., 2019
2,2-Dimethyl-5-hydroxy-7-phenylchromene	*criuva* *melchiorii*	trunk	Teixeira et al., 2006; Marques et al., 2021
Aucuparin	*criuva*	trunk	Marques et al., 2021
Clusiachromene D	*paralicola*	N.A.	Pubchem, viewed 2022
Clusiaparalycoline A	*paralicola*	root	Seo et al., 1999; Delle Monache et al., 2002
Clusiaparalycoline B	*paralicola*	root	Seo et al., 1999; Delle Monache et al., 2002
Clusiaparalycoline C	*paralicola*	root	Seo et al., 1999; Delle Monache et al., 2002
Clusiaparalycoline D	*paralicola*	root	Delle Monache et al., 2002

8.2.4.2 Dihydrophenantrene derivatives

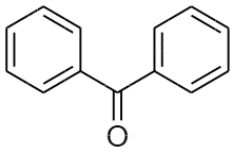
Core structure



Compound	*Clusia* sp.	Organ	Reference
Paralycoline A	*paralicola*	root	Delle Monache et al., 2002
Paralycoline B	*paralicola*	root	Delle Monache et al., 2002

8.2.4.3 Benzophenone derivatives

Core structure



Subclass	Compound	*Clusia* sp.	Organ	Reference
BBS	Clusiachromene C	*multiflora*	fruit	Gonzalez et al., 1995
	Clusiacitran A	*multiflora*	fruit	Gonzalez et al., 1995
	Clusiacitran B	*multiflora*	fruit	Gonzalez et al., 1995
	Clusiacycol A	*multiflora*	fruit	Gonzalez et al., 1995
	Clusiacycol B	*multiflora*	fruit	Gonzalez et al., 1995
	Clusiaphenone A	*ellipticifolia sadiensis*	fruit	Delle Monache et al., 1991a; Olivares et al., 1994
	Clusiaphenone C	*ellipticifolia*	fruit	Olivares et al., 1994
	Clusiaphenone D	*ellipticifolia*	fruit	Olivares et al., 1994
PPBS Type-A	1-benzoyl-5-(1-hydroxy-1-methylethyl)-6,6,13,13- tetrame-	*obdeltifolia*	trunk	Teixeira et al., 2005

thyltetracy-clo[7.3.1^{3,11}.0^{3,8}]tetradecane-2,12,14-trione			
7-*Epi*-nemorosone (II)	*flava*	fruit	Parker, 2017
7α-H-1-benzoyl-4-hydroxy-3-(3-hydroxy-3methylbutyl)-6,6,13,13-tetramethyl-11-(3-methyl-2-butenyl)-5- oxatetracy-clo[7.3.1.0^{3,7}0^{4,11}]tridecane-2,12-dione	*obdeltifolia*	trunk	Cruz & Teixeira, 2004
7β-H-11-benzoyl-5α-hydroxy-6,6,10,10-tetramethyl-1-(3-methyl-2-butenyl)tetracyclo [7.3.1.1^{3,11}0^{3,7}]tetradecane-2,12,14-trione	*obdeltifolia*	trunk	Cruz & Teixeira, 2004
8-benzoyl-4α-(1-hydroxy-1-methylethyl)-7,7-dimethyl1,3-di(3-methyl-2-bute-nyl)tricyclo[4.3.1.1^{3,8}]undecane-2,9,11-trione	*obdeltifolia*	trunk	Cruz & Teixeira, 2004
13-benzoyl6,6,8,14,14-pentamethyl-11,15-di(3-methyl-2-butenyl)-9- oxatet-racyclo[11.3.1.0^{1,10}.	*obdeltifolia*	trunk	Teixeira et al., 2005

$0^{3,8}$]heptadec-10-ene-12,17-dione			
15,16-Dihydro 16-hydroperoxy-plukenetione F	*havetioides*	fruit	Christian et al., 2001
28,29-Epoxy-plukenetione A	*havetioides obdeltifolia*	fruit trunk	Christian et al., 2001; Teixeira et al., 2005
33-Hydroxy-isoplukenetione C	*havetioides*	fruit	Christian et al., 2001
Chamone I	*grandiflora*	trunk latex	Lokvam et al., 2000
Clusiachromene A / Chamone II	*columnaris grandiflora*	fruit trunk latex	Lokvam et al., 2000; Compagnone et al., 2008
Garcinielliptone I	*burle-marxii minor*	fruit trunk	Mangas Marin et al., 2008b; Ferraz et al., 2021b
Goianone	*rosea*	floral resin fruit	Novais et al., 2016
Hyperibone B	*burle-marxii minor*	fruit trunk	Mangas Marin et al., 2008b; Ferraz et al., 2021b
Hyperisampsin E	*criuva*	trunk	Marques et al., 2021
Hyperisampsin F	*criuva*	trunk	Marques et al., 2021
Hypersampsone T	*burle-marxii*	trunk	Ferraz et al., 2021b
Insignone	*insignis*	floral resin	Porto et al., 2000
Nemorosone II	*grandiflora hilariana renggerioides rosea*	floral resin	de Oliveira et al., 1999; Lokvam et al., 2000; Porto et al., 2000
Obdeltifolione C	*burle-marxii*	trunk	Ferraz et al., 2019
Plukenetione A	*flava plukenetii*	fruit	Henry et al., 1996; Parker, 2017

Plukenetione B	*plukenetii*	fruit	Henry et al., 1999
Plukenetione C	*havetioides plukenetii*	fruit	Henry et al., 1999; Christian et al., 2001;
Plukenetione D	*plukenetii*	fruit	Henry et al., 1999
Plukenetione E	*plukenetii*	fruit	Henry et al., 1999
Plukenetione F	*flava havetioides plukenetii*	fruit	Henry et al., 1999; Christian et al., 2001; Parker, 2017
Plukenetione G	*flava havetioides plukenetii*	fruit	Henry et al., 1999; Christian et al., 2001; Parker, 2017
Propolone A	*criuva rosea*	trunk	Novais et al., 2016; Marques et al., 2021
Propolone B	*criuva*	trunk	Marques et al., 2021
Propolone C	*criuva*	trunk	Marques et al., 2021
Propolone D	*criuva minor*	fruit trunk	Mangas Marin et al., 2008b; Marques et al., 2021
Sampsonione B	*burle-marxii criuva obdeltifolia*	trunk	Cruz & Teixeira, 2004; Ferraz et al., 2021b; Marques et al., 2021
Sampsonione G	*havetioides obdeltifolia*	fruit	Christian et al., 2001; Cruz & Teixeira, 2004
Sampsonione N	*burle-marxii*	trunk	Ferraz et al., 2019
Sampsonione L / Hyperibone G	*burle-marxii*	trunk	Ferraz et al., 2021b
Scrobiculatone A	*scrobiculata*	floral resin	Porto et al., 2000
Scrobiculatone B	*scrobiculata*	floral resin	Porto et al., 2000

	Unnamed compound 5	*burle-marxii*	trunk	Ferraz et al., 2021b
PPBS Type-B	7-Epi-clusianone	*torresii*	fruit	Piccinelli et al. 2005
	Burlemarxione A	*burle-marxii*	trunk	Ferraz et al., 2019
	Burlemarxione B	*burle-marxii*	trunk	Ferraz et al., 2019
	Burlemarxione C	*burle-marxii*	trunk	Ferraz et al., 2019
	Burlemarxione D	*burle-marxii*	trunk	Ferraz et al., 2021a
	Burlemarxione E	*burle-marxii*	trunk	Ferraz et al., 2021a
	Burlemarxione F	*burle-marxii*	trunk	Ferraz et al., 2021a
	Clusianone	*burchellii* *congestiflora* *fluminensis* *lanceolata* *panapanari* *paralicola* *pernambucensis* *sadiensis* *spiritu-sanctensis* *torresii* *weddelliana*	bark+branches floral resin flower fruit	McCandlish et al., 1976; Delle Monache et al., 1991a; de Oliveira et al., 1996; Porto et al., 2000; Piccinelli et al. 2005; Dupret et al., 2017
	Gutiiferone E	*rosea*	leaf	Gustafson et al., 1992
	Isonemorosonol A	*portolandiana*	fruit	Parker, 2017
	Isonemorosonol B	*burle-marxii* *portolandiana*	fruit trunk	Ferraz et al., 2019
	Nemorosonol (A)	*multiflora* *nemorosa*	fruit	Delle Monache et al., 1988; Cerrini et al., 1993; Gonzalez et al., 1995
	Spiritone	*burchellii* *fluminensis*	floral resin	Porto et al., 2000

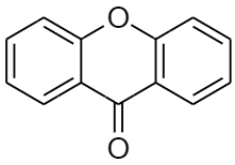
		panapanari *pernambucen-sis* *spiritu-sanctensis* *weddelliana*		
	Xanthocymol	*rosea*	leaf	Dreyer, 1974; Gustafson et al., 1992
	Xerophenone A	*portolandiana*	branch+leaf fruit	Henry et al., 1995; Henry et al., 1999; Parker
	Xerophenone B	*portolandiana*	branch+leaf fruit	Henry et al., 1995; Henry et al., 1999; Parker
PPBS Type-C	7-*Epi*-nemorosone	*insignis* *nemorosa* *renggerioides*	floral resin	de Oliveira et al., 1999; Porto et al., 2000
	13-Hydroxynemoroso-ne	*nemorosone*	floral resin	de Oliveira et al., 1996; de Oliveira et al., 1999
	Nemorosone	*grandiflora* *hilariana* *insignis* *nemorosa* *rosea*	floral resin flower fruit	de Oliveira et al., 1996; de Oliveira et al., 1999; Por-to et al., 2000; Kelecom et al., 2002
PPBS Type-D	2,2-Dimethyl-5-hydroxy-6-benzoyl-7-oxo-8-(3-methylbut-2-enyl)-8-[5-methyl-2-(1-methylvinyl) hexa-5-enyl]-2*H*-chromene	*portolandiana*	fruit	Parker, 2017
	2,2-Dimethyl-5-oxo-6-benzoyl-7-hydroxy-8-(3-methylbut-2-enyl)-8-[5-methyl-2-(1-	*portolandiana*	fruit	Parker, 2017

methylvinyl) hexa-5-enyl]-2*H*-chromene			
2-benzoyl-3,5-hydroxy-4,6- bis(3-methylbut-2-enyl) -4-[5-methyl-2-(1-methylvinyl) hexa-5-enyl]cyclohexa-2,5-dien-1-one	*portolandiana*	fruit	Parker, 2017
2-benzoyl-3,5-hydroxy-4,6- bis(3-methylbut-2-enyl) -6-[5-methyl-2-(1-methylvinyl) hexa-5-enyl]cyclohexa-2,5-dien-1-one	*portolandiana*	fruit	Parker, 2017
Clusiaphenone B	*sadiensis*	fruit	Delle Monache et al.,1991a
Grandone	*grandiflora*	floral resin	de Oliveira et al., 1996; de Oliveira et al., 1999
Isovismiaphenone B	*ellipticifolia*	fruit	Olivares et al., 1994
Machuone	*columnaris sadiensis*	fruit	Delle Monache et al., 1991a; Compagnone et al., 2008;
Nemorosinic acid A	*nemorosa portolandiana*	fruit	Delle Monache et al., 1991b; Parker, 2017
Nemorosinic acid B	*nemorosa portolandiana*	fruit	Delle Monache et al., 1991b; Parker, 2017
Lanceolatone	*burchellii fluminensis hilariana lanceolata panapanari paralicola*	floral resin	Porto et al., 2000

		pernambucen-sis		
	Vismiaphenone B	*ellipticifolia*	fruit	Olivares et al., 1994
	Weddellianone A	*burchellii fluminensis hilariana lanceolata panapanari paralicola pernambucen-sis weddelliana*	floral resin	Porto et al., 2000
	Weddellianone B	*panapanari weddelliana*	floral resin	Porto et al., 2000
Non-classifyable	Minorone	*minor*	root	Kerpan, current study

8.2.4.4 Xanthone derivatives

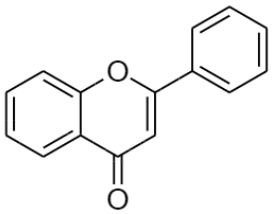
Core structure



Compound	*Clusia* sp.	Organ	Reference
1,7-Dihydroxy-3,4-dimethoxyxanthone	*paralicola*	root	Delle Monache et al., 2002
6-Deoxyisojacareubin	*burle-marxii criuva*	trunk	Ribeiro et al., 2019; Marques et al., 2021
8-Desoxygartanin	*nemorosa*	floral resin	de Oliveira et al., 1999
Brasilixanthone B	*criuva*	trunk	Marques et al., 2021
Clusiaxanthone	*pernambucensis*	bark	Silva et al., 2013
Clusone	*insignis*	flower	Ishiguro et al., 1998
Neriifolone C	*criuva*	trunk	Marques et al., 2021
Osajaxanthone F	*criuva*	trunk	Marques et al., 2021
Padiaxanthone	*burle-marxii*	trunk	Ribeiro et al., 2019

8.2.4.5 Flavonoids

Core structure

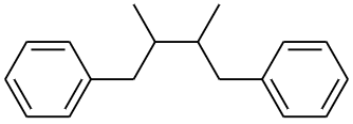


Subclass	Compound	*Clusia* sp.	Organ	Reference
Flavanol	(-)-Epicatechin	*paralicola* *uvitana*	fruit	Barrios et al., 1990; Oliveira et al., 2012
	Catechin	*amazonica* *cylindrica* *minor* *multiflora* *osaensis* *rosea*	leaf	Schinnerl, personal comunication; Weckwerth, personal comunication
Flavone	Apigenin	*nemorosa*	fruit	Ferreira et al., 2022
	Isoorientin	*lanceolata*	leaf	Ferreira et al., 2014; Ferreira et al., 2016
	Isovixetin	*columnaris* *criuva* *lanceolata*	branch+leaf leaf	Chedier et al., 1999; Compagnone et al., 2008; Ferreira et al., 2016
	Isovixetin-2''-O-α-L-rhamnopyranoside	*lanceolata*	leaf	Ferreira et al., 2014; Ferreira et al., 2016
	Orientin	*lanceolata*	leaf	Ferreira et al., 2014; Ferreira et al., 2016
	Vixetin	*columnaris* *criuva* *lanceolata* *uvitana*	branch+leaf leaf	Barrios et al., 1990; Chedier et al., 1999; Compagnone et al., 2008; Ferreira et al., 2016

	Vixetin-2''-O-α- L-rhamnopyranoside	*lanceolata*	leaf	Ferreira et al., 2014; Ferreira et al., 2016
	Vixetin-2''-xyloside	*columnaris*	branch+leaf	Compagnone et al., 2008
Flavonol	3-O-α- L-rhamnopyrano-sylkaempferol	*burle-marxii*	leaf	Ribeiro et al., 2011
	3-O-α- L- rhamno-pyranosylquercetin	*burle-marxii*	leaf	Ribeiro et al., 2011
	Kaempferol	*nemorosa*	bark fruit leaf	De Andrare et al., 1998; Ferreira et al., 2015
	Quercetin	*amazonica* *cylindrica* *minor* *multiflora* *nemorosa* *osaensis* *rosea*	leaf fruit	Ferreira et al., 2015; Schinnerl, personal comu-nication; Weck-werth, personal comunication

8.2.4.6 Lignan derivatives

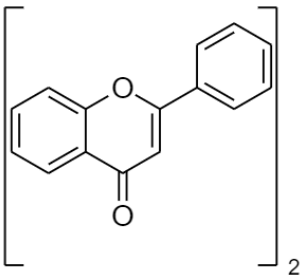
Core structure



Compound	*Clusia* sp.	Organ	Reference
Lyoniresinol	*burle-marxii*	trunk	Ribeiro et al., 2011

8.2.4.7 Biflavonoids

Core structure



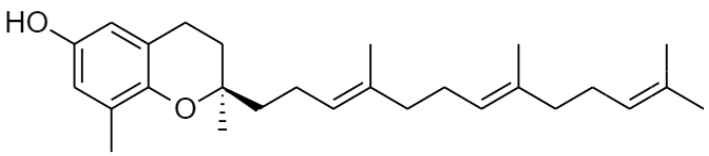
Subclass	Compound	*Clusia* sp.	Organ	Reference
Anthocyanidin-	Proanthocyanidin	*minor*	bark	Schinnerl, perso-

Anthocyanidin				nal comunication
Flavanone- Flavanone	2R, 3S, 2″R, 3,8″-binaringenin-7″-O-β-glucoside	*paralicola*	fruit	Oliveira et al., 2012
	GB-1a / I3,II8-Binaringenin	*columnaris*	branch+leaf leaf	Bittar et al., 2000; Compagnone et al., 2008
	GB-2a	*columnaris*	branch+leaf	Compagnone et al., 2008
	GB-2a-7''-O-glucoside	*columnaris*	branch+leaf	Compagnone et al., 2008
Flavanone-Flavanonol	*2R, 3S*, 2″R, 3″R-GB1-7″- O-β-glucoside	*paralicola*	fruit	Oliveira et al., 2012
Flavanone-Flavone	Morelloflavone / Fukugetin	*columnaris guavariensis*	branch+leaf fruit	Martinez et al., 1996; Compagnone et al., 2008
	Morelloflavone-7''-O-glucoside / Fukugiside	*columnaris guavariensis*	branch+leaf fruit	Martinez et al., 1996; Compagnone et al., 2008
	Volkensiflavone	*columnaris*	branch+leaf	Compagnone et al., 2008
	Volkensiflavone-7''-O-glucoside	*columnaris*	branch+leaf	Compagnone et al., 2008

8.2.5 Prenylated chromanols

8.2.5.1 Tocotrienol derivatives

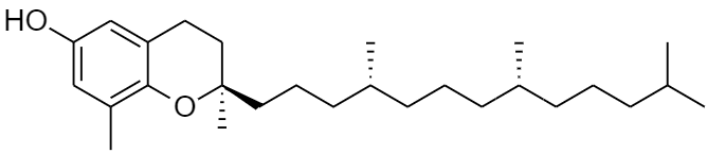
Core structure



Compound	*Clusia* sp.	Organ	Reference
δ-Tocotrienol	*minor* *pernambucensis*	bark root	Silva et al., 2013; Kerpan, current study;
2-Tocotrienol methyl ester	*pernambucensis*	bark	Silva et al., 2013
2*E*-Tocotrienolic alcohol	*obedltifolia* *pernambucensis*	bark trunk	Teixeira et al., 2006; Silva et al., 2013;
E-δ-Garcinoic acid / 2*E*-δ-tocotrienoloic acid	*burle-marxii* *criuva* *minor* *obdeltifolia*	leaf root trunk	Teixeira et al., 2006; Ribeiro et al., 2011; Marques et al., 2021; Kerpan, current study
Z-δ-Garcinoic acid / 2*Z*-δ-tocotrienoloic acid	*criuva* *obdeltifolia* *pernambucensis*	bark trunk	Teixeira et al., 2006; Silva et al., 2013; Marques et al., 2021

8.2.5.2 Tocopherol derivatives

Core structure

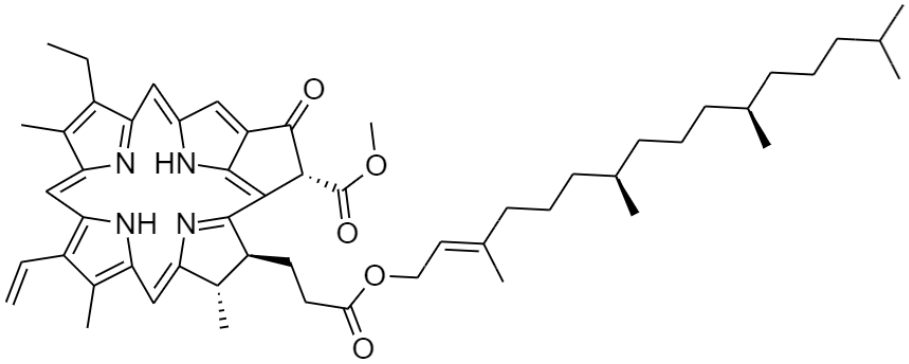


Compound	*Clusia* sp.	Organ	Reference
α-Tocopherol	*minor*	leaf	Mangas Marin et al., 2008a; Mangas Marin et al., 2018; Mangas Marin et al., 2019; Schinnerl, personal comunication

8.2.6 Prenylated porphyrins

8.2.6.1 Pheophytin derivatives

Core structure

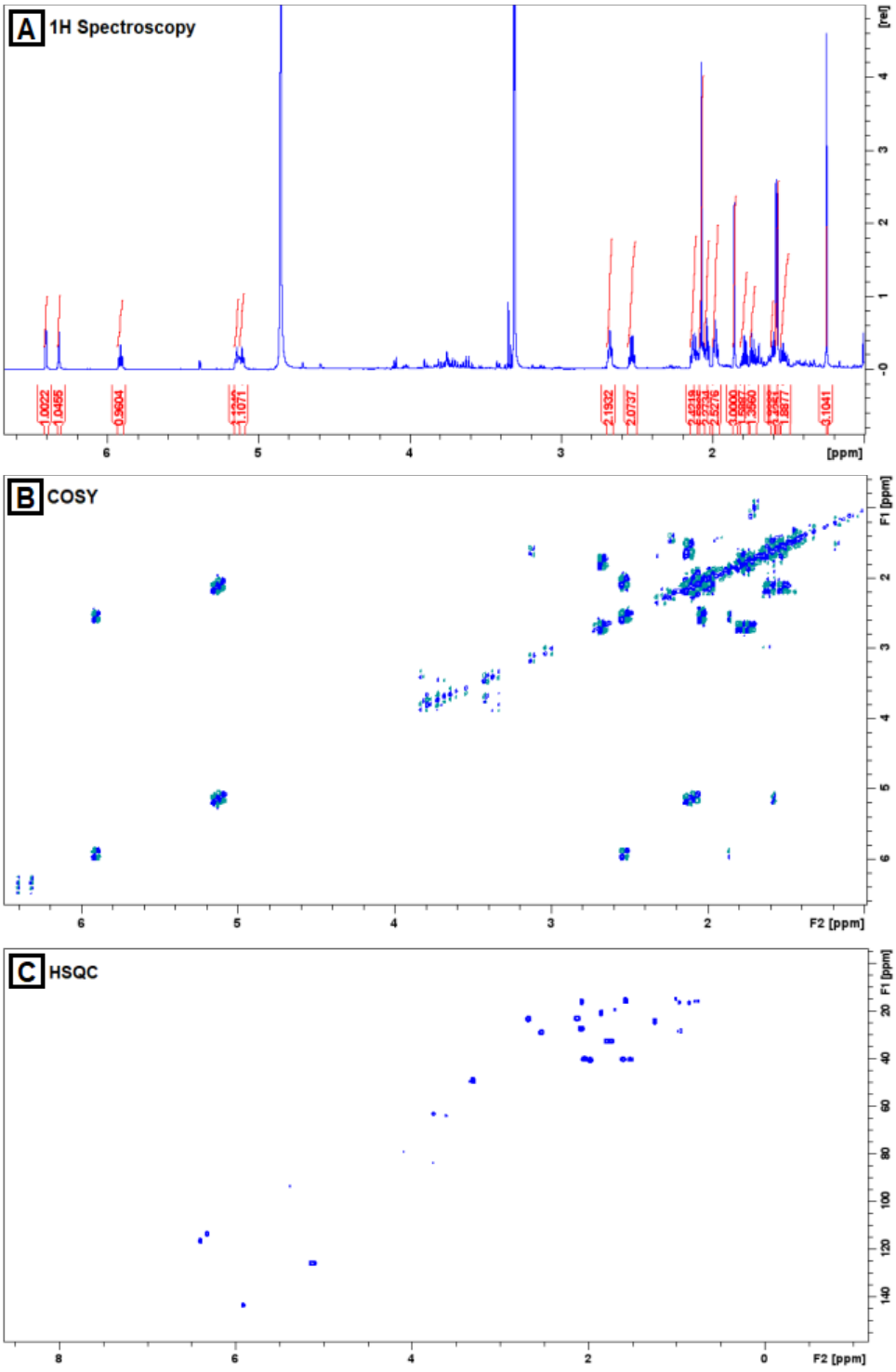


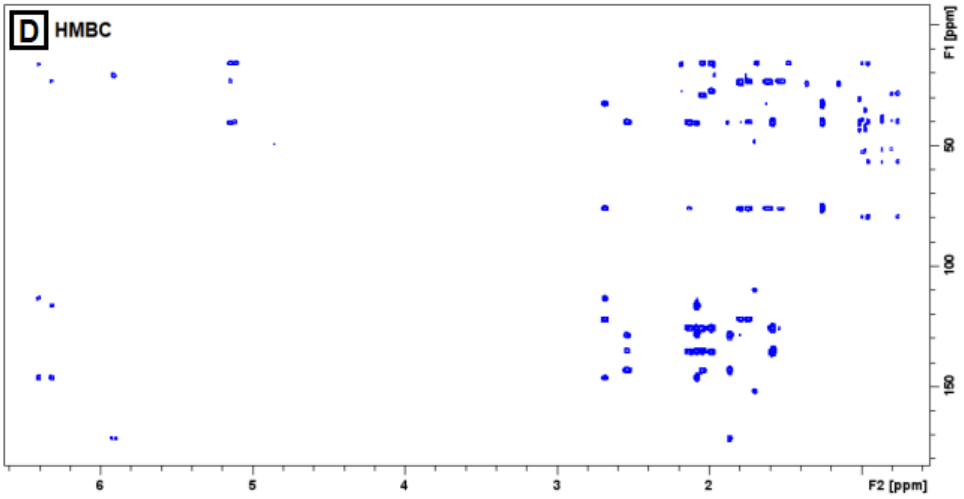
Compound	*Clusia* sp.	Organ	Reference
13^2-hydroxy-(13^2-*S*-)-phaeophytin a	*lanceolata*	leaf	Ferreira et al., 2016
13^2-hydroxy-(13^2-*R*)-phaeophytin a	*lanceolata*	leaf	Ferreira et al., 2016

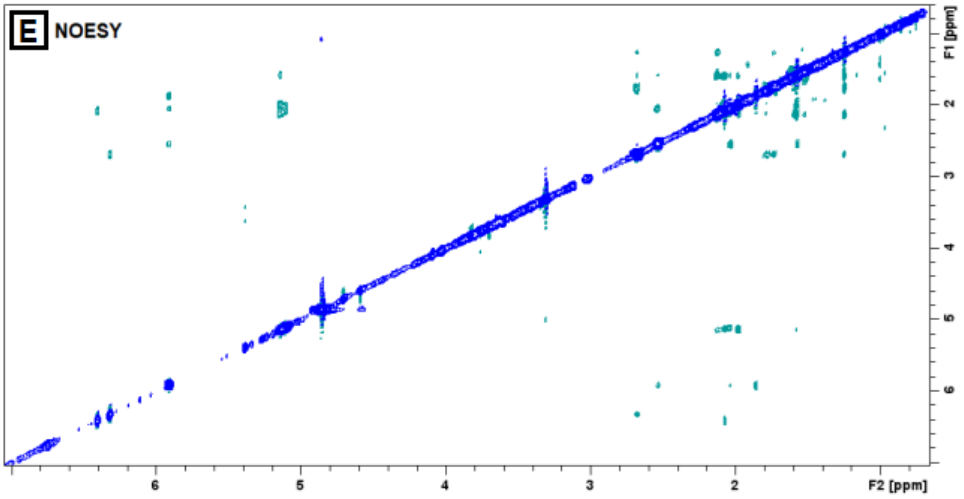
Incerta sedis: Clusiachromene B, Obdeltifolione A, Obdeltifolione B

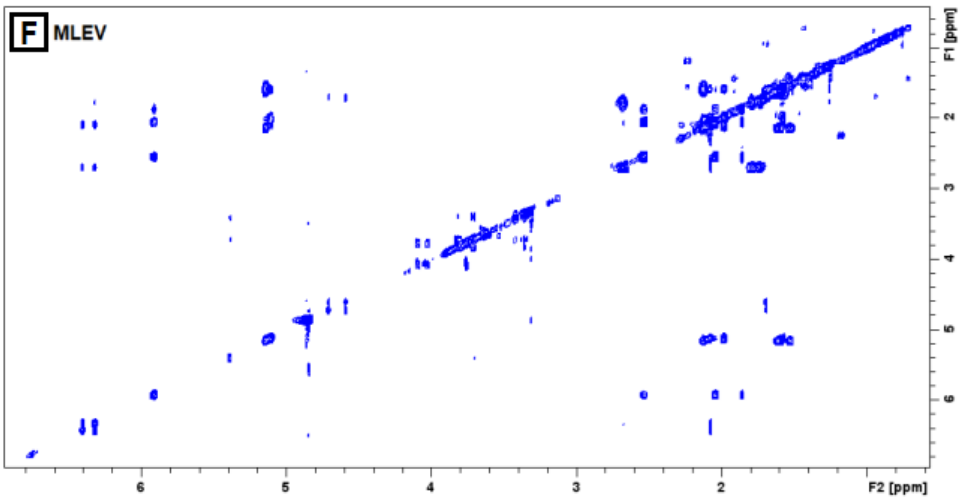
8.3 NMR data

8.3.1 PK-CM01




D
HMBC
F1 [ppm]
50
100
150
6
5
4
3
2
F2 [ppm]


E
NOESY
F1 [ppm]
2
3
4
5
6
6
5
4
3
2
F2 [ppm]


F
MLEV
F1 [ppm]
2
3
4
5
6
6
5
4
3
2
F2 [ppm]

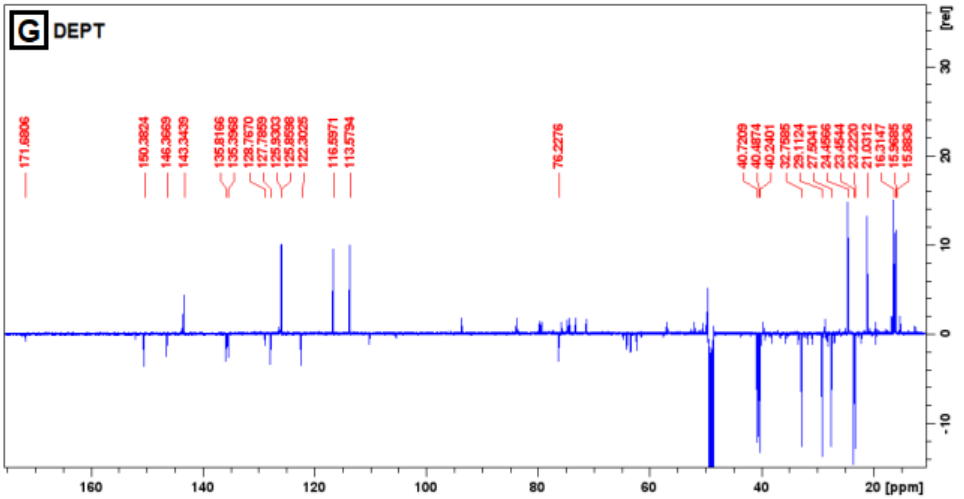
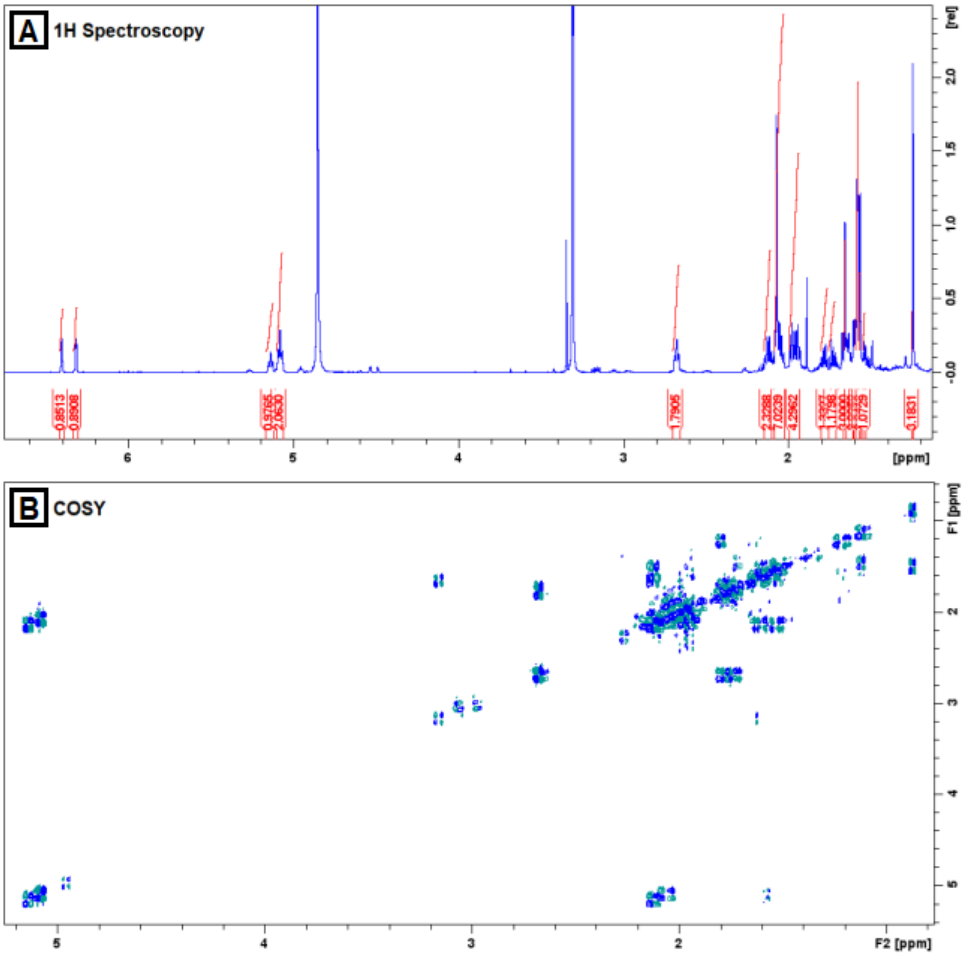
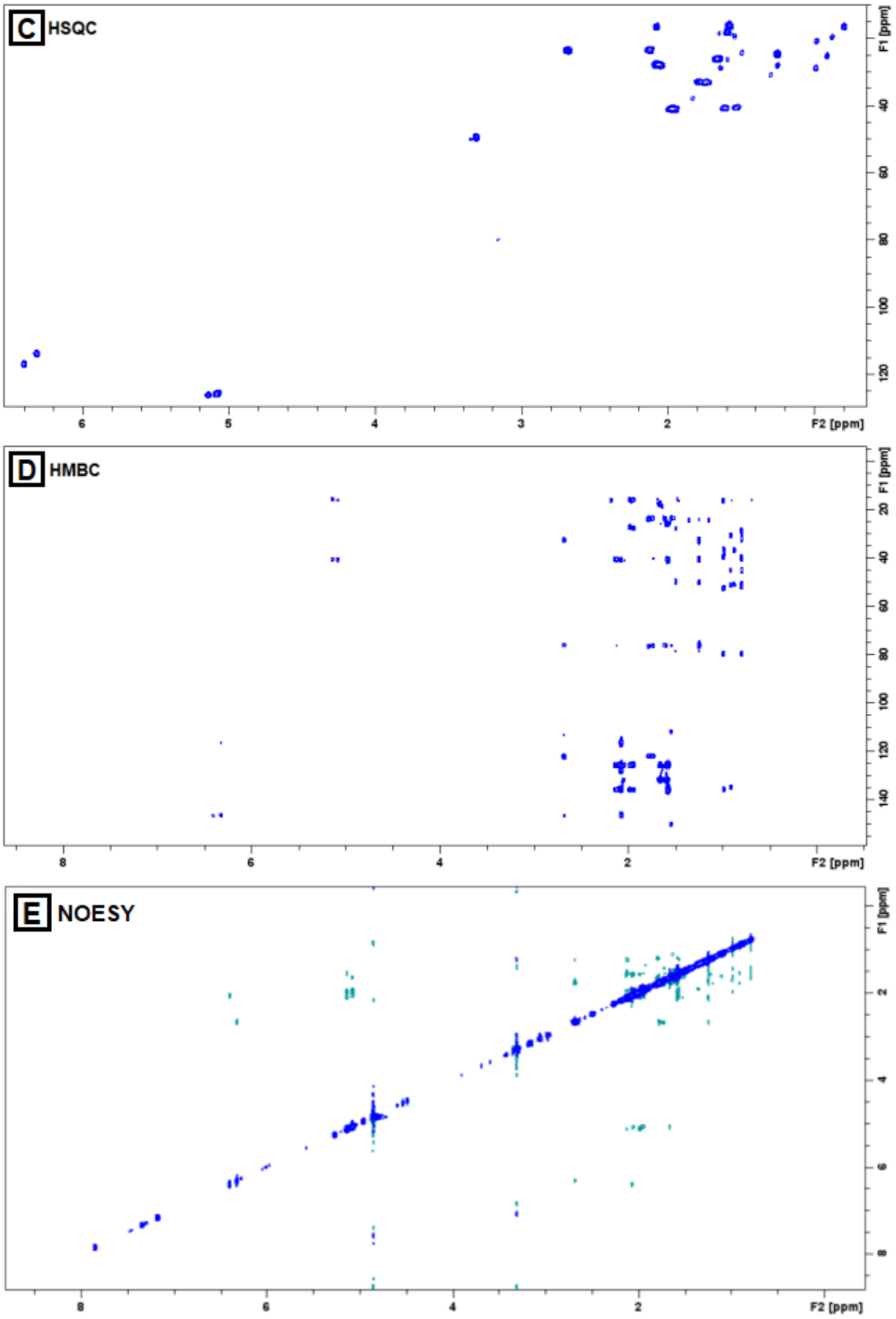


Figure 40: NMR analyses outputs of PK-CM01: A) 1H B) COSY; C) HSQC; D) HMBC; E) NOESY; F) MLEV; G) DEPTq

8.3.2 PK-CM02




C HSQC
F1 [ppm]
F2 [ppm]
D HMBC
F1 [ppm]
F2 [ppm]
E NOESY
F1 [ppm]
F2 [ppm]

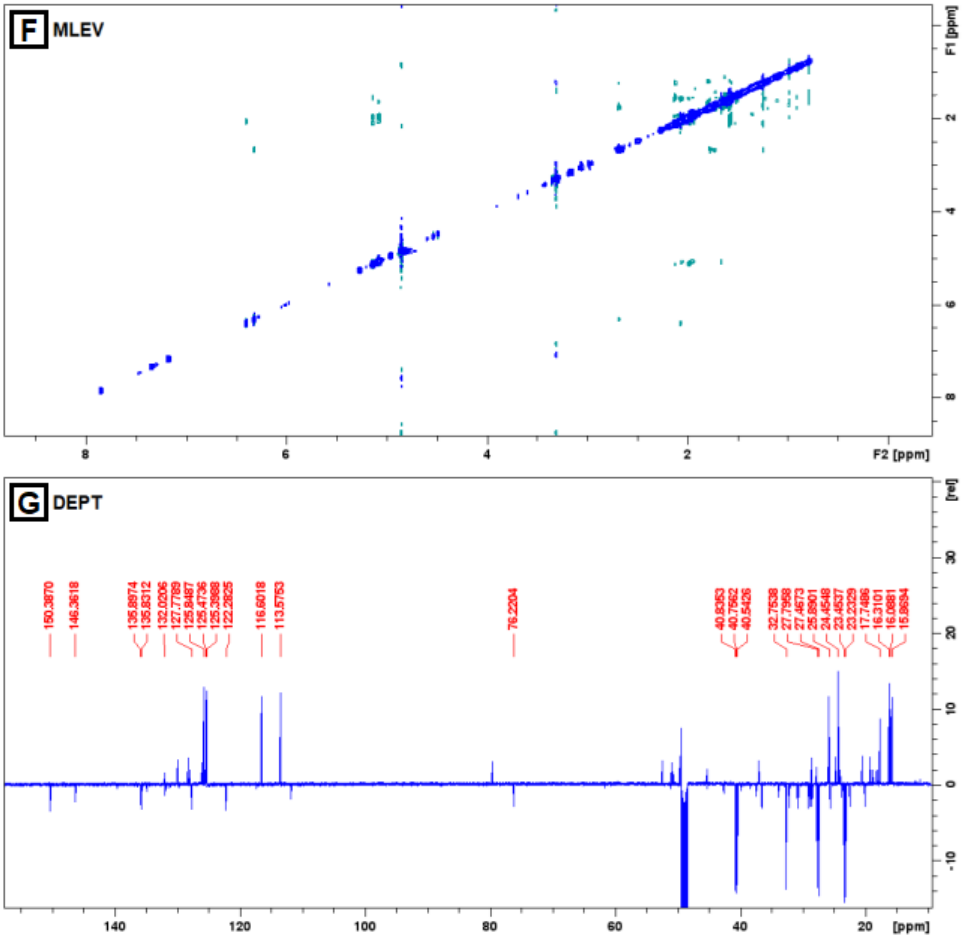
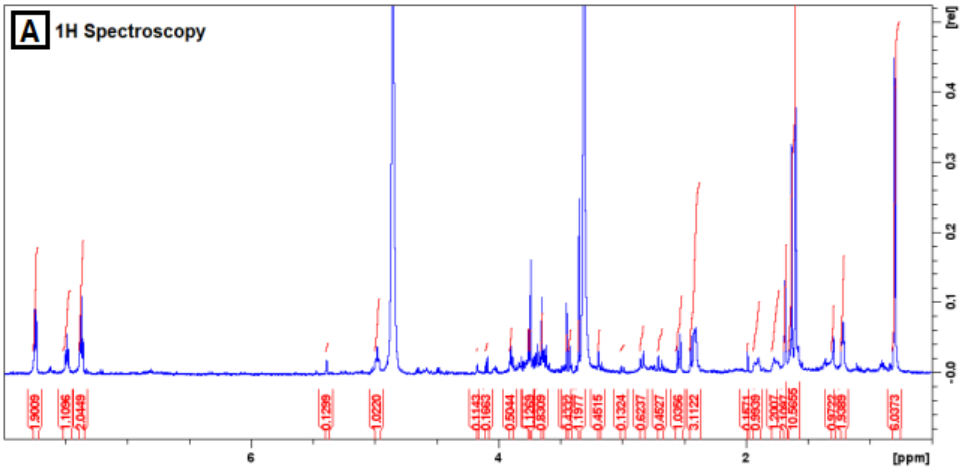
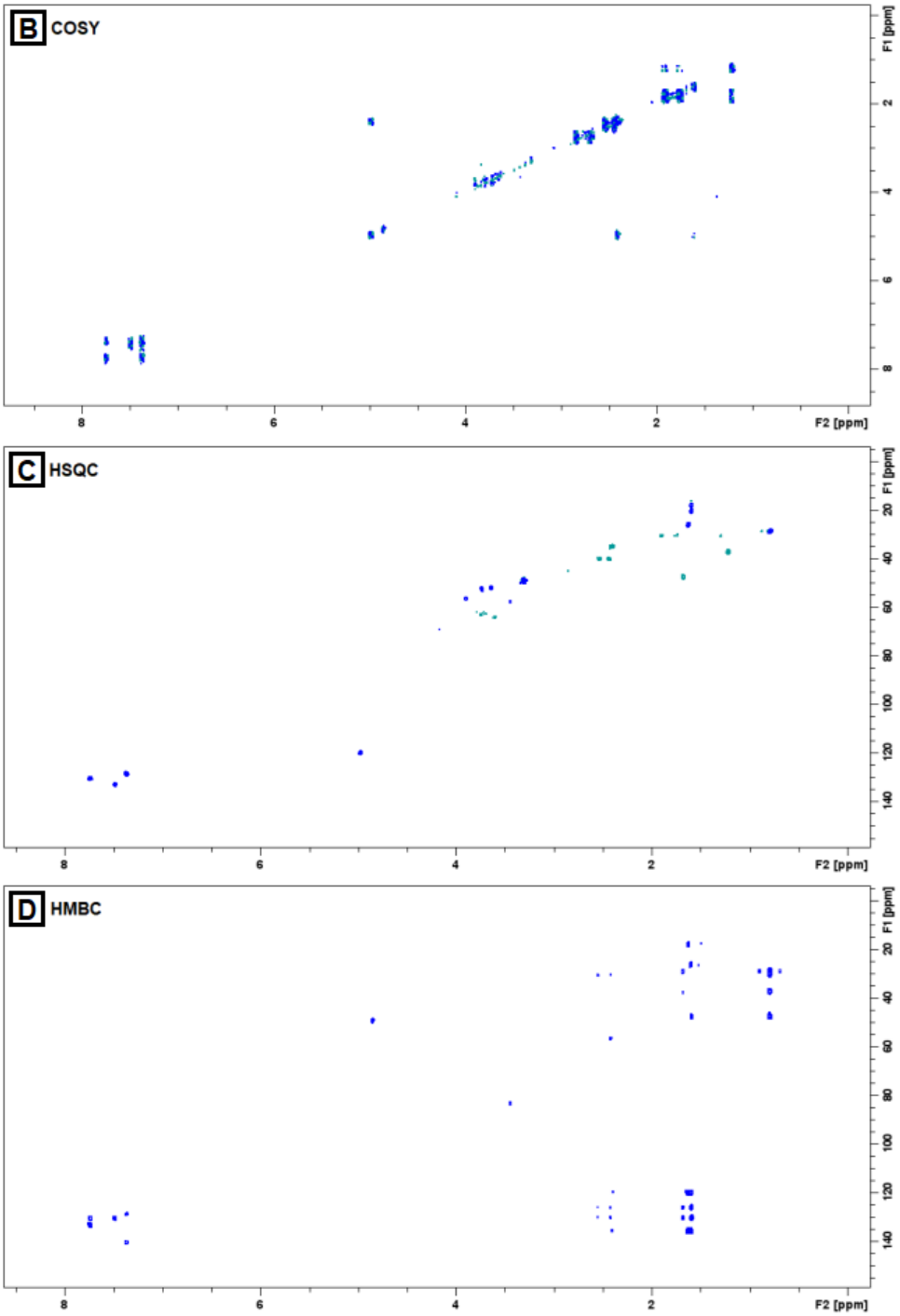


Figure 41: NMR analyses outputs of PK-CM02: A) 1H B) COSY; C) HSQC; D) HMBC; E) NOESY; F) MLEV; G) DEPTq

8.3.3 PK-CM05




B COSY
F1 [ppm]
F2 [ppm]
C HSQC
F1 [ppm]
F2 [ppm]
D HMBC
F1 [ppm]
F2 [ppm]

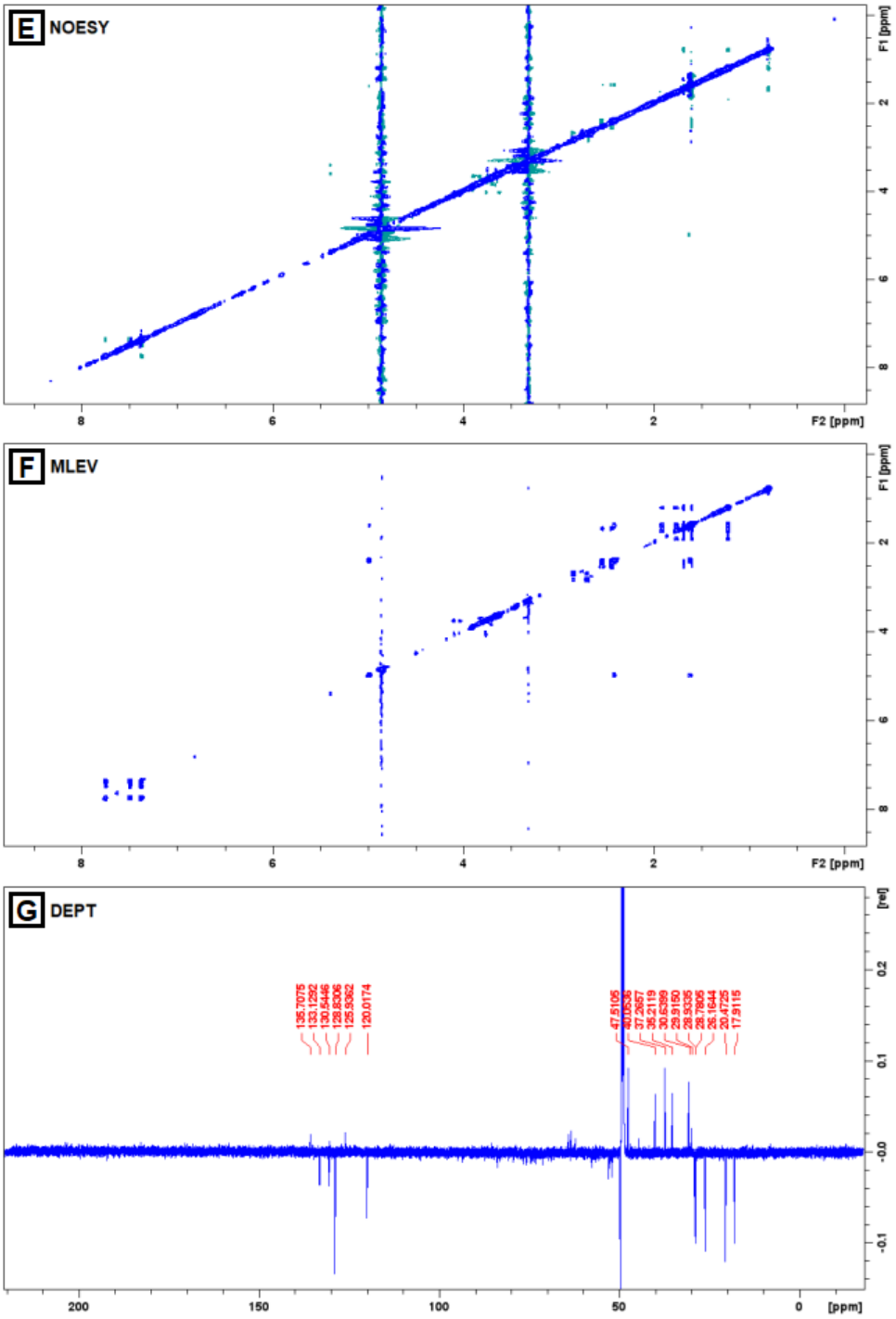


Figure 42: NMR analyses outputs of PK-CM05: A) 1H B) COSY; C) HSQC; D) HMBC; E) NOESY; F) MLEV; G) DEPTq