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"Chemical profiling of *Eumachia microdon* (D.C.) Delprete & J.H.Kirkbr: Searching for Alkaloids"

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INTRODUCTION

The Rubiaceae family is cosmopolitan, widely distributed in the tropics, and characterised by producing bioactive metabolites with diverse plant functions and pharmacological potential (Martins & Nunez, 2015). The classification of the different members within this family is confusing, and to date, there are gaps between the different genera and species, so morphological and genetic characters are insufficient for a clear separation of the respective taxa. Thus, the study of secondary metabolites diversification is suggested as a chemotaxonomical identification tool (Taylor et al., 2017).

In addition, the plants of this alliance have importance at the level of traditional medicine, as they are used in spiritual rituals and traditional Asian medicine, with high pharmacological potential (Berger et al., 2022a). Because of this pharmaceutical potential, studies have been conducted on the secondary metabolites of the *Psychotria* alliance plants. Most studies currently focus on the genera *Palicourea* and *Psychotria* (Calixto et al., 2016; Taylor et al., 2017). Due to the complex classification of species within the genus *Eumachia*, few studies have focussed on the secondary metabolites of this genus, especially the alkaloid compounds. Therefore, this study aims to isolate and identify alkaloids from leaf and stem extracts of *Eumachia microdon* (D.C.) Delprete & J.H.Kirkbr in specimens collected in Costa Rica.

1. SYSTEMATICS, TAXONOMY AND MORPHOLOGY

1.1. Rubiaceae

The Rubiaceae family is a member of the order Gentianales and shares characteristics of other families of the order, particularly the basic morphology of leaves and flowers, the presence of colleters and the lack of internal phloem (Davis et al., 2009). The family is the fourth largest within the angiosperm families, comprising approximately 13000 species and about 611 genera (Bremer & Eriksson, 2009; Davis et al., 2009). It has a cosmopolitan distribution, and the species diversity and biomass are well represented in the tropics and subtropics, especially in lowland rainforests. Although less diverse, some species may be found in temperate regions. Its habitat is vast, and it can be found as lianas, shrubs, and small to large trees up to 5 metres (Davis et al., 2009; Delprete & Kirkbride, 2015).

Rubiaceae genera and species assignments' classification and systematics are still problematic and partly unresolved. Thus, earlier classification based on morphological characters only was insufficient for definite species identification. Furthermore, using only one or a few characters as absolute markers of the different taxonomic groups would generate misleading identifications. Using molecular techniques for phylogenetic studies and comparative morphological analyses reduced the number of synonymous genera (Bremei, 2009). However, classification within the family remains a matter of debate. Bremer and Eriksson (2009) did phylogenetic analyses using molecular data, and according to their results, the family Rubiaceae is divided into three major subfamilies: Ruboideae, Ixoroideae and Cichonoideae (Figure 1). The Rubioideae are split into different tribes, and two other major clades, the *Psychotria* alliance and the Spermacoceae alliance, comprise several tribes each. The following focuses on the *Psychotria* alliance, as this group contains the species under study.

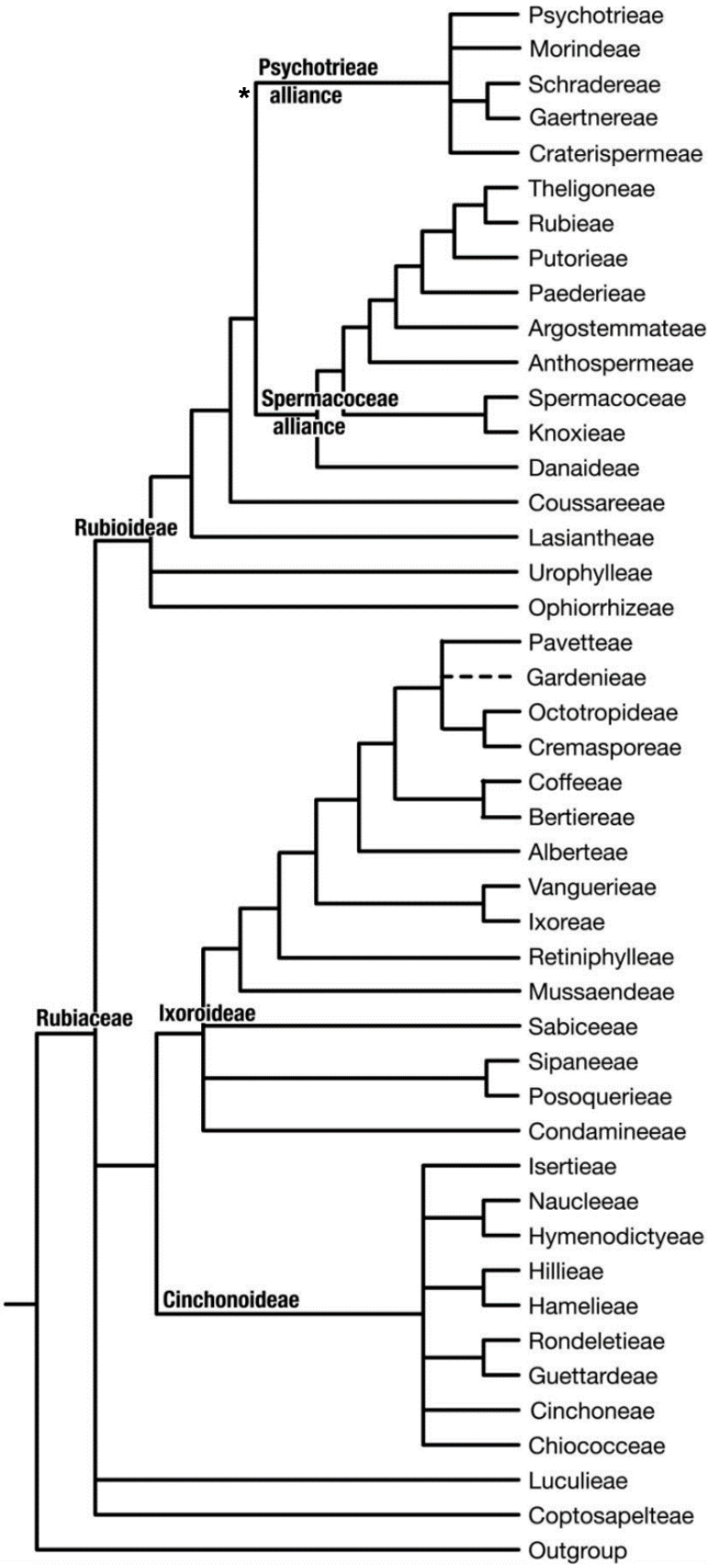

Figure 1. 90% majority-rule consensus tree of the Rubiaceae family from Bayesian analysis. Root portion with the outgroup, first major splits, and part of Rubioideae. * *Psychotria*e alliance clade. Adapted from Bremer & Eriksson, 2009.

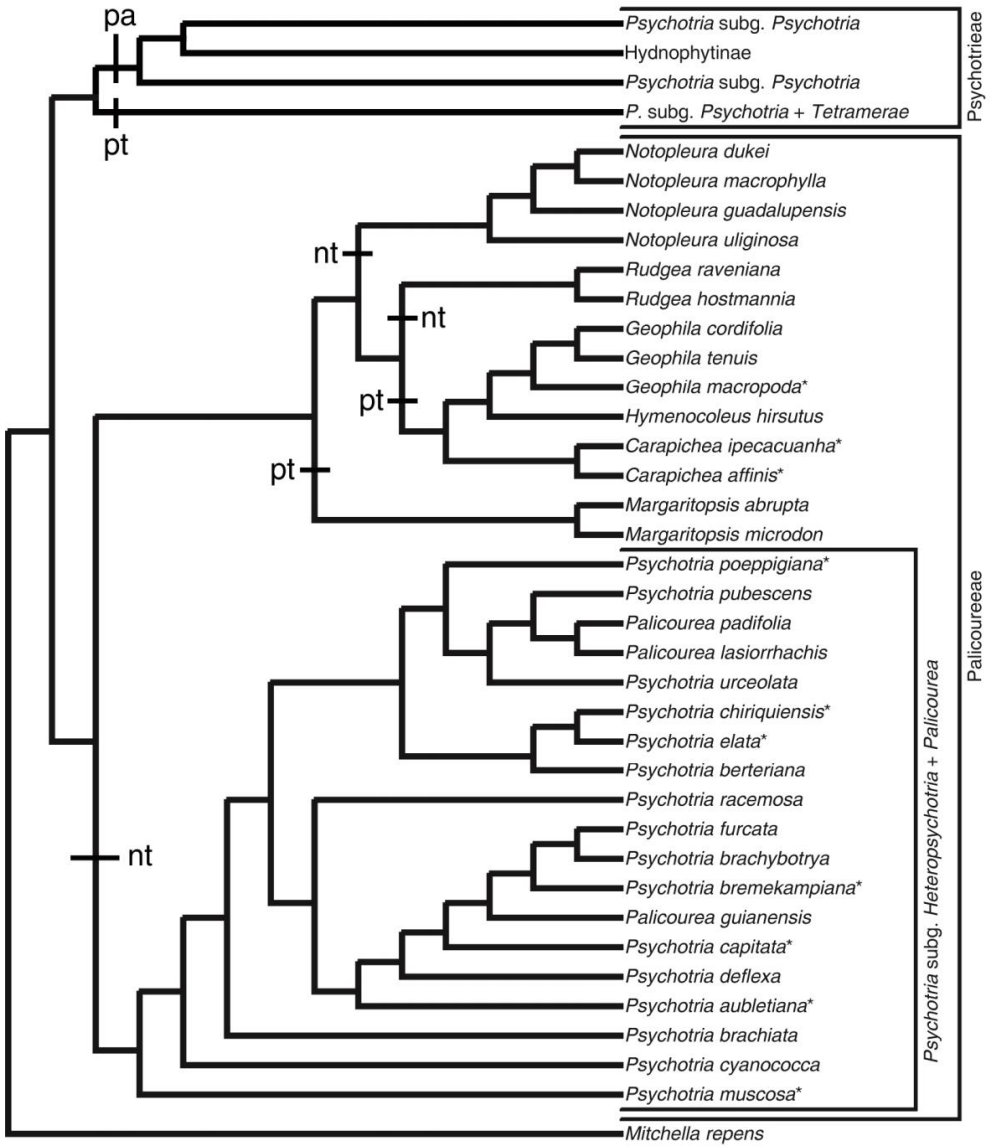

Figure 2. Maximum parsimony phylogenetic tree from analysis of ITS sequence data. Placement of the tribe Palicureeae and Psychotrieae, according to (B. Bremer & Manen, 2000). Clades are identified according to their distribution: (nt) Neotropics, (pa) Pacific and (pt) pantropical. (*) Species traditionally classified as Cephadelis. Adapted from Berger, 2012.

1.2. Psychotria alliance

The *Psychotria* alliance consists of two sister tribes, *Psychotrieae* and *Palicoureae* (Figure 2), conforming to a megadiverse group with the most extensive radiation within the Rubiaceae family (around 3000 species described in 54 genera), including around 24% of the Rubiaceae species. The group includes the genera *Psychotria* (tribe *Psychotrieae*), *Carapichea, Eumachia, Geophila, Notopleura, Palicourea* and *Rudgea* (tribe *Palicoureeae*), which are diverse in the Neotropics (Berger et al., 2022a; Razafimandimbison et al., 2008).

The species within the *Psychotria* alliance are essential ecological components; they contribute to rainforest understorey species diversity, abundance, and biomass (Berger & Schinnerl, 2019; Razafimandimbison et al., 2008). Additionally, many species are ethnobotanically important, and several studies demonstrated that the plants within this alliance are rich sources of structurally varied alkaloids (Berger et al., 2015, 2022b). The alliance is characterised by fleshy fruits (berries or drupes), nourishing several bird and bat species (Razafimandimbison et al., 2008).

1.2.1. Morphological characteristics

The partially persistent shrubs or bushes characterise the genus; the branches are cylindrical, usually lateral or flattened, smooth or pubescent. The leaves are yellow-green, opposite, petiolate, and patent veins; the stipules are five-merous and persistent (Taylor, 2005; Zappi et al., 2017). The flowers are bisexual, actinomorphic and five-merous; corolla valvar white to cream, tubular, equalling the hypanthium; four to 5 stamens, ovary bilocular (Taylor et al., 2017). The terminal inflorescences are funnel drupes orange to red and seeds with no rumanate endosperm (Taylor, 2005; Zappi et al., 2017). The morphological differences between *Margaritopsis* and *Emumachia* are evident in the corollas, which for *Margaritopsis* are reduced with short lobes, while Eumachia are funnel-shaped with long lobes. The stipules in *Margaritopsis* are reduced, truncated and triangular, and for *Eumachia*, they are calyptrate or lanceolate. In addition, the calyx of *Margaritopsis* is reduced and may or may not have teeth with rounded buds; in comparison, *Eumachia* has an elongated, inflated calyx with erect lobes and reduplicate buds (Barrabé, 2013; Taylor et al., 2017).

2. SECONDARY METABOLITES

Plants produce their primary metabolites for growth and development; with some of these pathways lead to secondary metabolites, which despite not being a first need for the plant, are essential for survival and reproductive fitness, functioning as intra- and interspecies communication signals, for pollinator attraction, for the establishment of symbiotic relationships and plant defence mechanisms (Hussein & El-Anssary, 2019; Wink, 2003; Yang et al., 2016).

In addition, many secondary metabolites show antiviral, antifungal and antibiotic activities, which accounts for plants producing them as traditional medicines to treat several diseases, as a source of vitamins and as part of indigenous rituals. This knowledge has been expanded to the pharmaceutical industry for drug development using alkaloids, flavonoids, and volatile oils as primary components of medicines (Srivastav et al., 2019).

Secondary metabolites are chemically diverse and heterogeneous, and they are classified according to the primary metabolite from which they are derived, the biosynthetic pathway through which they are derived, or the common structural elements present in them. These can be classified into three main groups: terpenoids, phenolics and nitrogen-containing compounds (Figure 3) (Thippeswamy et al., 2021).

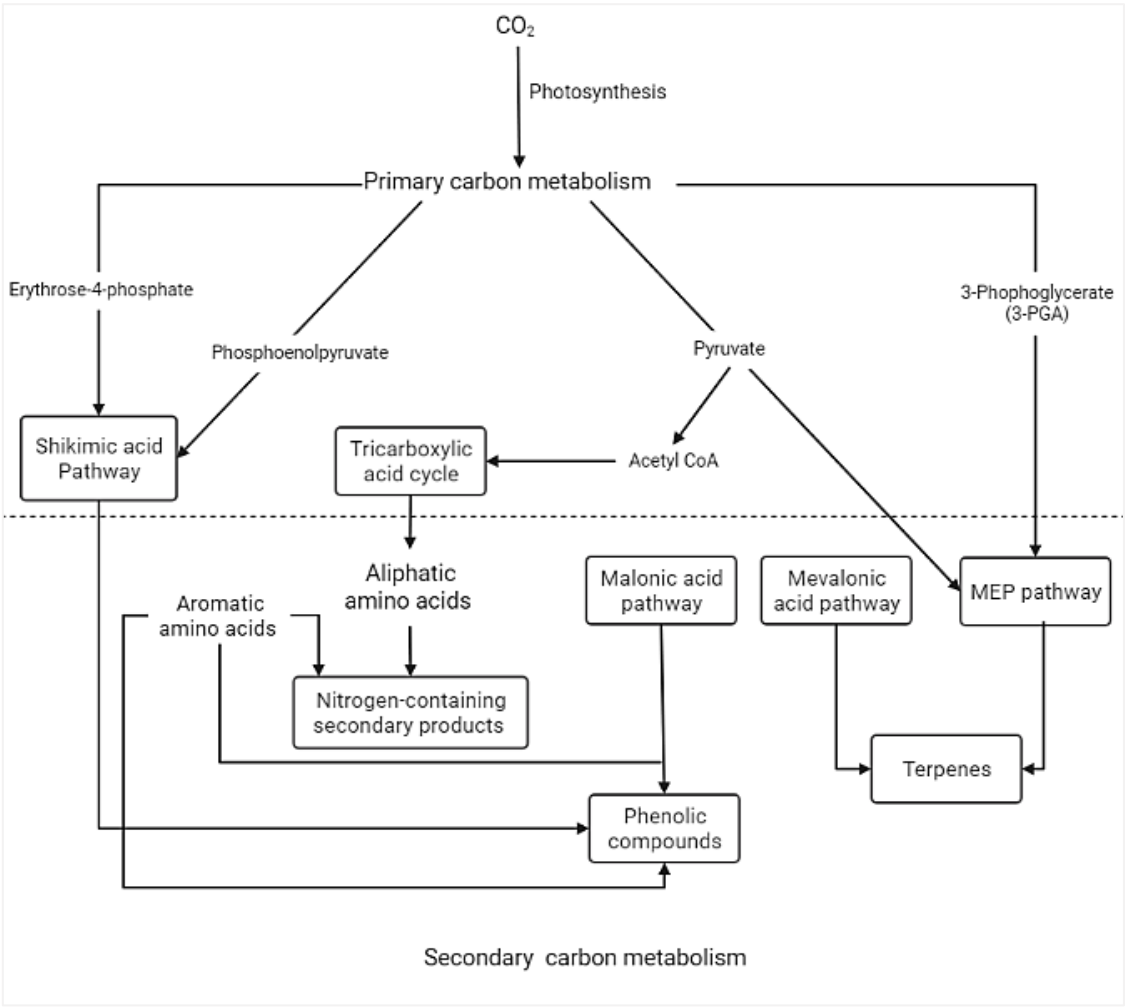

Figure 3. Simplified scheme of secondary metabolites biosynthesis and their interrelationship with primary metabolites. Adapted and modified from Thippeswamy et al., 2021

2.1. Terpenes

Terpenes belong to a large family of specialised metabolites containing some 30,000 identified compounds, the largest and most diverse group of secondary metabolites in nature. They are used as defence vehicles by plants in antagonistic and mutualistic interactions (Ninkuu et al., 2021). Among the functions of terpenes in plants are to counteract biotic (pathogenic attacks, herbivory, pests and weeds) and abiotic (water, temperature, light and salinity) stresses (Ninkuu et al., 2021) and as signal molecules to attract insects in pollination (Singh & Sharma, 2015).

Moreover, these compounds are important to the plant because they make floral fragrances that attract pollinators and are plant hormones involved in plant growth functions (gibberellic and abscisic acid). Additionally, they can act as toxins (e.g., drimane sesquiterpenes) to protect plants from predation (Katerova, 2012; Teoh, 2016).

Structurally, the basic building unit is five-carbon isoprene (C5H8) (Masyita et al., 2022) and can be classified as C5 hemiterpenes, C10 monoterpenes, C15 sesquiterpenes, C20 diterpenes, C25 sesterterpenes, C30 triterpenes, C40 tetraterpenes, and C>40 polyterpenes (Figure 4) (Abdallah & Quax, 2017; Aldred, 2008).

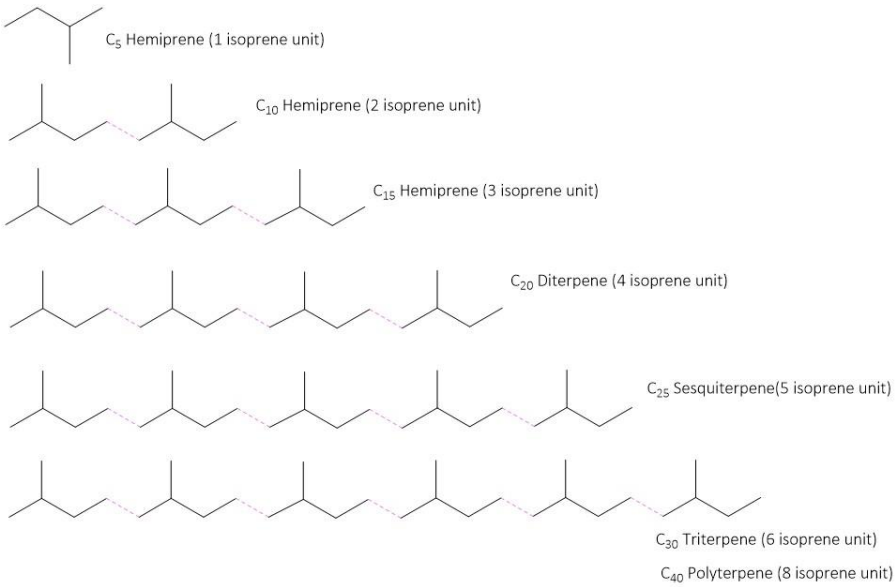

Figure 4 Terpenes classification according to the number of isoprene units. Adapted from Masyita et al., 2022

Isoprenyl diphosphate (IPP) and dimethylallyl pyrophosphate (DMAPP) are the biosynthetic precursors of terpenes and are derived from two different biosynthetic pathways: the classical mevalonate pathway (MEV) and the 2C-methyl-D-erythritol-4-phosphate (MEP) pathway (Figure 5). MEP occurs mainly in the plastids, while mevalonic in the cytosol; sterols (triterpenes) are produced via mevalonate, while (C5) hemi-, mono- and diterpenes and carotenoids (tetra) are produced via MEV(Oldfield & Lin, 2012). Both pathways are present in plants and have various rearrangements, repeats and cyclisation reactions, producing diverse terpene compounds (Abdallah & Quax, 2017; Ninkuu et al., 2021). On one side, the MEV pathway comprises seven enzymatic reactions to convert the precursors acetyl-CoA to IPP and DMAPP (Figure 5). A condensation of acetyl CoA occurs, synthesising 3-hydroxy-3-

methylglutaryl CoA, which later produces mevalonate. The last one is phosphorylated and decarboxylated until it produces (IPP) (Figure 5A) (Singh & Sharma, 2015).

Conversely, in the MEP pathway (Figure 5B), the prenyltransferases enzymes add the active isoprene unit (IPP) to DMAPP or a prenyl diphosphate in consecutive head-to-tail condensations leading to the production of a range of molecules with fixed lengths and stereochemistry (Abdallah & Quax, 2017).

Geranyl pyrophosphate synthase (GPPS) and farnesyl pyrophosphate synthase (FPPS) catalyse the condensation of IPP and DMAPP to produce GPP (C10 monoterpene) and FPP (C15 sesquiterpenes and triterpenes). Geranylgeranylgeranyl pyrophosphate synthase (GGPPS) and farnesyl geranylgeranyl pyrophosphate synthase (FGPPS) are responsible for the formation of GGPP (C20 diterpenes) and FGPP (C25 Sesterterpene). The precursors GPP, FPP, GGPP and FGPP are cyclised and rearranged by different terpene synthase enzymes (Figure 5B) (Abdallah & Quax, 2017; Ninkuu et al., 2021; Oldfield & Lin, 2012).

Several *in vivo* and *in vitro* studies have reported the health benefits of terpenes and terpenoids as anticancer, antimicrobial, anti-inflammatory, antioxidant, anti-allergenic, neuroprotective, sedative, and analgesic agents. Thus, due to the activity of monoterpenes, sesquiterpenes, diterpenes, triterpenes, tetraterpenes and glycosylated compounds (Masyita et al., 2022).

Furthermore, some plant extracts containing terpenoids are used in traditional Chinese medicine due to their therapeutic properties to promote health and well-being. The sesquiterpene artemisinin, isolated from *Artemisia annua,* is used in the natural remedy “Qinghao” as an antimalarial treatment (Elshafie et al., 2023; Tu, 2016). Its discovery by the pharmaceutical chemist Youyou Tu gave her the Nobel Prize in Physiology or Medicine in 2015 (Tu, 2016).

Many studies are taking place in the pharmaceutical field to use the diverse bioactivity of the terpenoids for drug development due to their anticancer and antiviral activity. One of the most

extensively studied terpenes is paclitaxel, known commercially as Taxol™, an anticancer drug isolated from the bark of the pacific yew tree (*Taxus breviflora*) and elucidated structurally in 1971 (Bergman et al., 2019; Wani et al., 1971). Other beneficial properties of terpenes under research include antimicrobial, anti-inflammatory, antioxidant, and antiparasitic activities (Bergman et al., 2019).

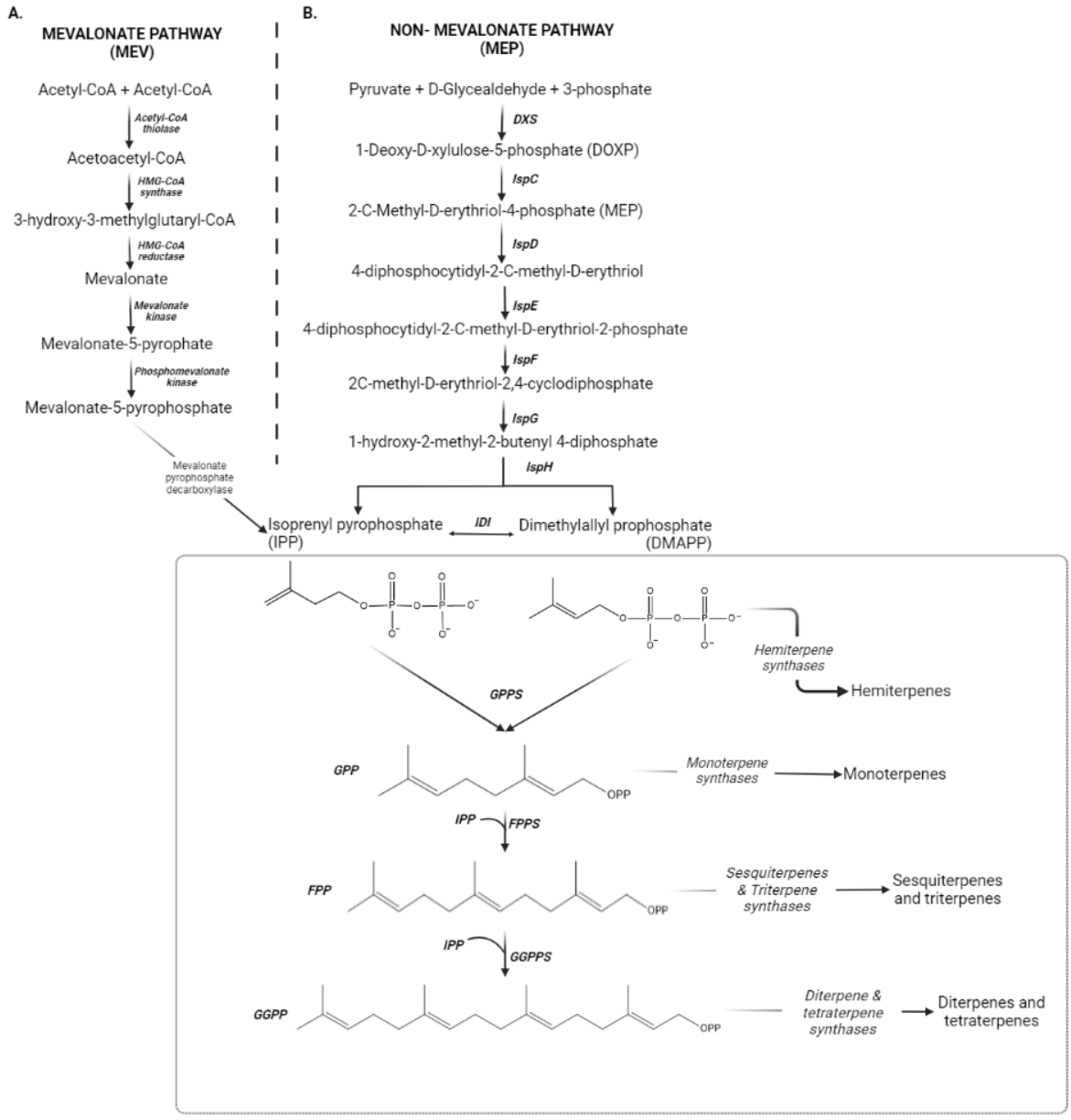

Figure 5 Biosynthetic pathways of terpene production. Left: Mevalonate pathway. Right: Non-mevalonate pathway (MEP). The rectangle shows the downstream biosynthesis of the various classes of terpenes. Structures were drawn and analysed with ChemDraw professional software, version 15.0.0.106. Adapted and modified from Abdallah & Quax, 2017; Ninkuu et al., 2021; Singh & Sharma, 2015

2.2. Phenolics

Phenolic compounds are one of the largest groups of secondary plant metabolites. They are derived from the shikimate/phenylpropanoid pathway, giving rise to phenylpropanoids. The acetate/malonate pathway can produce simple phenols (Figure 3), thus producing monomeric and polymeric phenols, which serve various physiological functions in plants (Aldred, 2008; Lattanzio, 2013).

The phenolic compounds have at least one aromatiC-ring with one hydroxyl group in their structure and are classified according to these structural features into several groups, being the main subgroups phenolic acids and flavonoids (

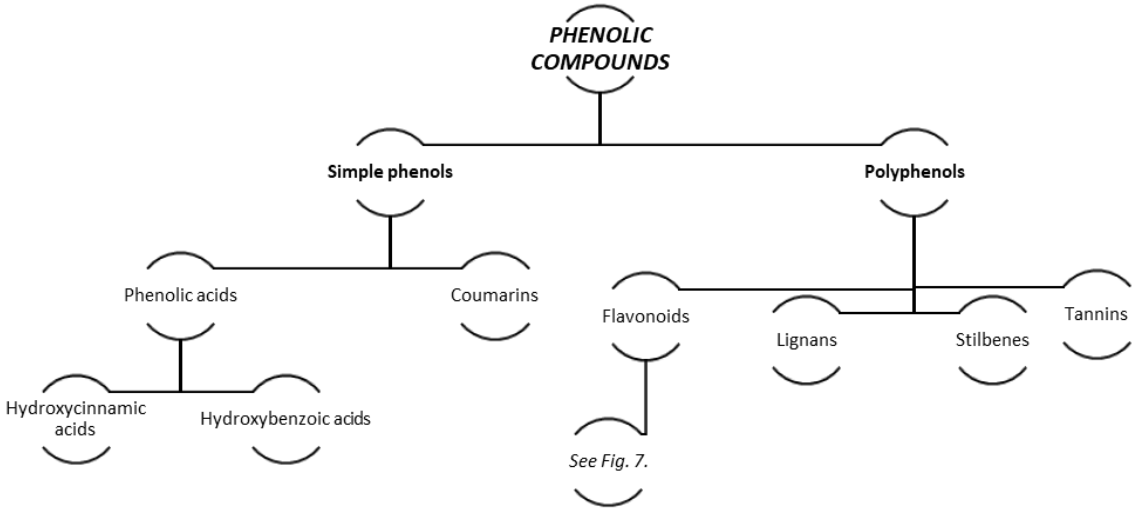

Figure **6**). They have different roles in the plant, such as structural functions like lignin and suberin. They protect the plant from ultraviolet radiation; and are responsible for forming the different pigments in the plant (e.g., anthocyanins, flavones and flavonols) (Aldred, 2008).

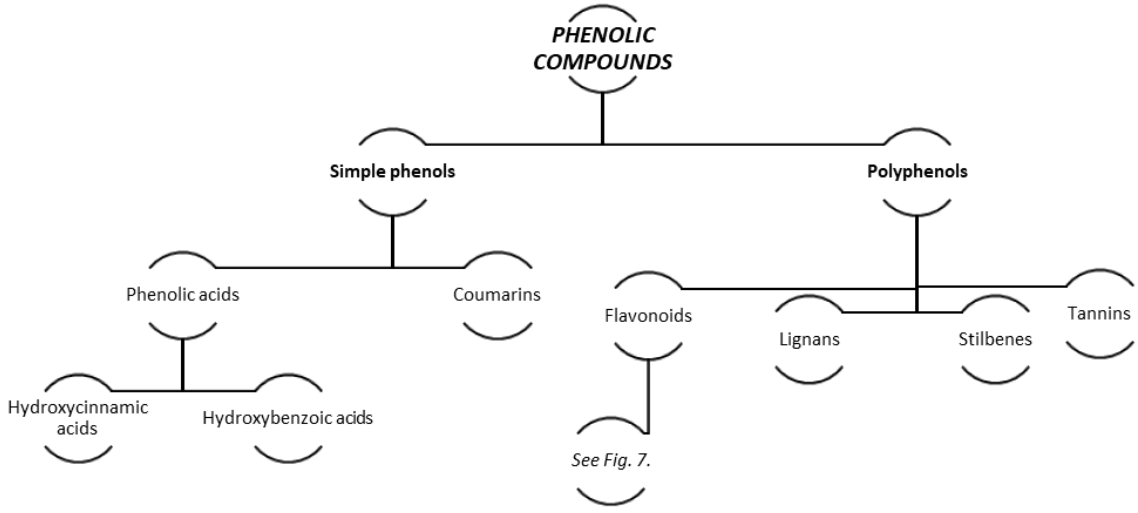

Figure 6. Classification scheme of phenolic compounds according to their structure. Adapted from Soto et al., 2015.

Some regulatory functions are described in plant-microorganism interactions (e.g. *Rhizobia, Agrobacterium*) (Bhattacharya et al., 2010). Other compounds have allelopathic effects (e.g. ferulic acid) and pollinator-attracting effects (Lattanzio, 2013). Additionally, some phenolic compounds are consumed by humans, such as vanillin, a simple phenol used as a food flavouring, and salicylic acid, the precursor of aspirin (Teoh, 2016).

These secondary metabolites benefit plants and play an essential role in preventing human diseases, like cancer, cardiovascular diseases, or even neurodegenerative diseases such as Alzheimer's; this is due to the anti-oxidative activities of these compounds (Gimeno Creus, 2004). In following, the focus is on some subclasses of flavonoids, such as flavone-*C*-glycosides and flavones.

2.2.1. Flavonoids

These types of polyphenols have a small molecular weight and are formed by two phenyl rings that are connected to phenolic hydroxyl groups via three carbon atoms found in the centre of the polyphenol (Miao et al., 2022), and thus they have a basic C6-C3-C6 skeletal structure (Badshah et al., 2021). Based on their chemical structure, the flavonoids can be classified into flavones, isoflavones, flavonols, flavanols (flavan-3-ols or catechins), flavanonols, flavanones, neoflavonoids, anthocyanidins, and chalcones (Figure 7) (Panche et al., 2016; Veitch & Grayer, 2011).

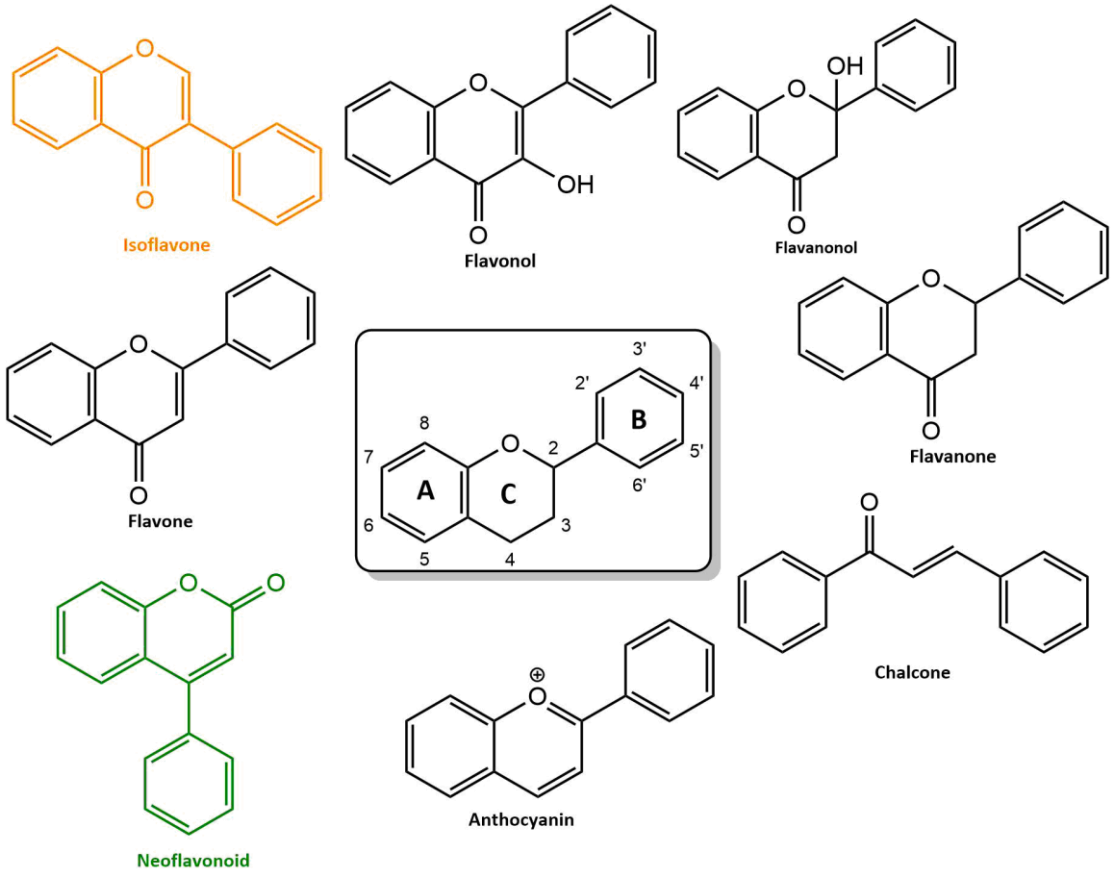

Figure 7 Classification of flavonoids: basic skeleton structure and their classes. In centre: core structure of flavonoids. For the isoflavones (orange) and neoflavonoids (green), the B ring is connected at the C_3 and C_4 position, respectively. The B ring is attached to the position C2 of the C-ring for the rest of the flavonoids. Adapted and modified from Farombi et al., 2018; Miao & He, 2021; Panche et al., 2016. Figure created with ChemDraw professional software, version 15.0.0.106

Flavonoids hold great importance in plants as they serve diverse biological functions and are responsible for colours and their nectar guide; in fruits, their function is to attract pollinators and fruit dispersion to help with seed and spore germination (Céspedes et al., 2006; Panche et al., 2016). Additionally, they can protect the plant against abiotic stress by adding UV filters or functioning as signal molecules, allelopathic compounds and antimicrobial defensive compounds (Panche et al., 2016). These compounds are also well known for their antioxidant activity due to their ability to reduce free radicals formation and scavenge and neutralise harmful free radicals (Pietta, 2000; Shen et al., 2022). Fresh fruits and vegetables rich in flavonoids can protect against chronic disease, inflammation and oxidation (Shen et al., 2022). Research has indicated that flavonoid-enriched foods can regulate the functioning of pancreatic β-cells, hepatocytes and adipocytes. This regulation helps prevent the progression from insulin resistance to type 2 diabetes (Procházková et al., 2011). Moreover, flavonoid compounds can enhance blood vessel dilation, treat endometrial dysfunction and insulin resistance, safeguard against atherosclerosis, and reduce blood pressure (Pietta, 2000; Shen et al., 2022).

2.2.1.1. Biosynthesis of flavonoids

Flavonoids can be biosynthesised via the phenylpropanoid pathway (Figure 8), in which three molecules of malonyl-CoA and one molecule of *p*-coumaroyl-CoA produce naringenin chalcone in a reaction catalysed by chalcone synthase (CHS). Chalcone is the intermediate product from which the two phenyl rings (A- and B-ring) form the backbone of all flavonoids (Falcone Ferreyra et al., 2012; Shen et al., 2022). The enzyme chalcone isomerase (CHI) catalyses the cyclisation of the chalcones into flavanones, forming the heterocyclic C-ring in the flavonoid pathway (Lou et al., 2021; Shen et al., 2022). Moreover, flavanone is catalysed by the flavone synthase (FNS) to yield flavone, forming a double bond between the C_2 and C_3 positions of the C-ring (Lou et al., 2021)

Additionally, flavanone can be converted into isoflavone with the help of isoflavone synthase (IFS) (Liu et al., 2021). The oxidation of the latter compound by flavanone 3-hydroxylase (F3H) yields flavanonol (also known as dihydroflavonol), which is the common precursor of flavonol, anthocyanin and protoanthocyanin (Petrussa et al., 2013). Next, flavonol synthase (FLS) can oxidise flavononols to flavonols, producing kaempferol and quercetin (W. Liu et al., 2021; Petrussa et al., 2013) and the dihydroflavonol-4-reductase (DFR) reduces leucosntocyanidins (flavan-3,4-diols), forming a hydroxyl group at position C_4 of the C-ring. These flavan-3,4-diols can be transformed into anthocyanidins by anthocyanidin synthase (ANS) and also into protoanthocyanidin by the leucoanthocyanidin reductase (LAR), which drives the C-4 dehydroxylation of the C-ring (W. Liu et al., 2021; Lou et al., 2021).

Finally, for the production of anthocyanins, the activity of UDP-glucose:flavonoid 3-*O*-glucosyl transferase (UFGT) is required, where the unstable anthocyanidins are converted to stable anthocyanins (Petrussa et al., 2013).

2.2.1.2. Health benefits of Flavonoids

Many flavonoids are part of the human diet, found in many fruits and vegetables, and provide associated health benefits such as reducing the risk of developing chronic diseases. Among the foods consumed that contain flavonoids are berries (blackberries, cranberry, blueberry, raspberry and strawberry), pomegranate, plum, red grape, and apple; among vegetables, spinach contains the highest content of these phenolic compounds (Ballard & Maróstica, 2019; Liu, 2013). Epidemiological studies have investigated the health effects of flavonols, flavones and flavanones. Studies have shown that consuming flavonoids reduces the risk of cardiovascular and cerebrovascular disease, cancer and other chronic diseases, including

asthma, cataracts, diabetes and rheumatoid arthritis (Ballard & Maróstica, 2019; Graf et al., 2005).

2.2.2. Flavonoid glycosides

Glycosylated flavonoids occur widely in nature and are found predominantly as *O*-glycosides of flavones and flavonols and less frequently, as *C*-glycosylated derivatives, primarily as flavones and more rarely as isoflavones, flavonols, flavanones and chalcones (Rauter et al., 2007; Veitch & Grayer, 2011).

There are distinct differences between *C*-glycosyl flavonoids and *O*-glycosyl flavonoids in addition to those mentioned before. First, the C-glycosyl flavonoids are non-hydrolysable "aglycones", whilst the *O*-Glycosides are hydrolysable derivatives (Seigler, 1998). Also, flavone *C*-glycosides contain a C-C covalent bond between the aglycone flavonoid backbone and a sugar moiety: generally, monomer glucose or galactose (Veitch & Grayer, 2011), unlike flavone *O*-glycosides that are formed by attaching sugar to the hydroxyl (Seigler, 1998). The properties of the flavonoid glycosides include antioxidant, anticancer, anti-inflammatory and neuroprotective effects (Shukla & Gupta, 2010). These can be found and extracted from several plants: cabbage, celery, carrot, chrysanthemum flowers, onions, sweet bell peppers, broccoli, artichoke, apple and parsley (Carbajal-Valenzuela et al., 2022).

Flavonol *O*-glycosides are fairly widespread in nature. For example, studies on several *Vinca* species (Apocynaceae) revealed the presence of four flavonol glycosides from *Vinca minor* L. from the methanolic leaf extract, identified as two kaempferol derivatives (Figure 9) and two quercetin derivatives Szostak and Kowalewski (1975). Decades later, one of the kaempferol derivatives described in the study mentioned above (Kaempferol-3-rhamnogluco-7-glucoside) was found in the same species, which showed high antioxidant activity and a potent radical scavenging activity (Grujic et al., 2014). In recent studies, phytochemical analyses were done for diverse *Vinca* species to isolate different phenolic compounds from the leaves and study their antibacterial activity against *E. coli* and *S. aureus* and their cytotoxic effects against keratinocytes and skin melanoma human cell lines (Ciorîță et al., 2021). For the studied species, kaempferol was found in *V. major,* and *V. major* var. *variegata* contained kaempferol with considerable antibacterial activity.

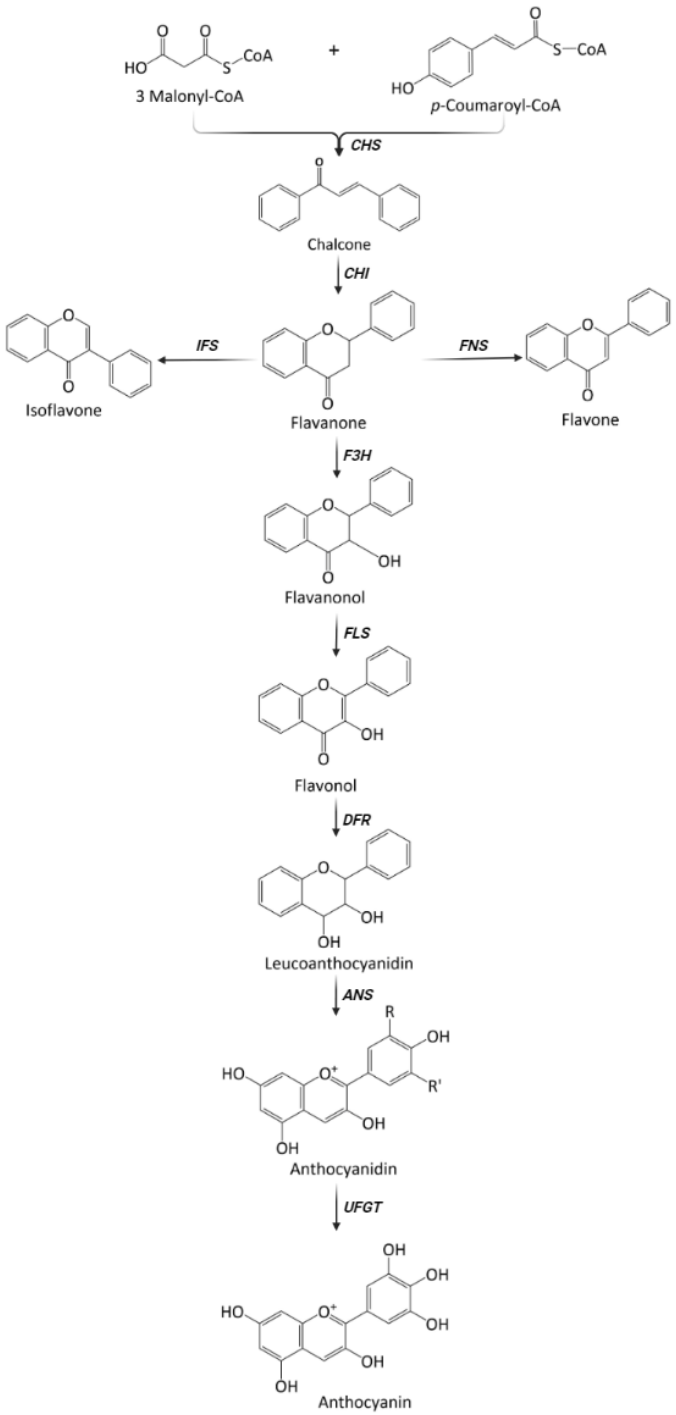

Figure 8 Scheme of the flavonoid biosynthetic pathway. The enzymes are abbreviated as follows: CHS, chalcone synthase; CHI, chalcone isomerase; IFS, isoflavone synthase; FNS, flavone synthase; F3H, 3-hydroxylase; FLS, flavonol synthase; DFR, dihydroflavonol-4-reductase; ANS, anthocyanidin synthase; UFGT, 3-O-glucosyl transferase.

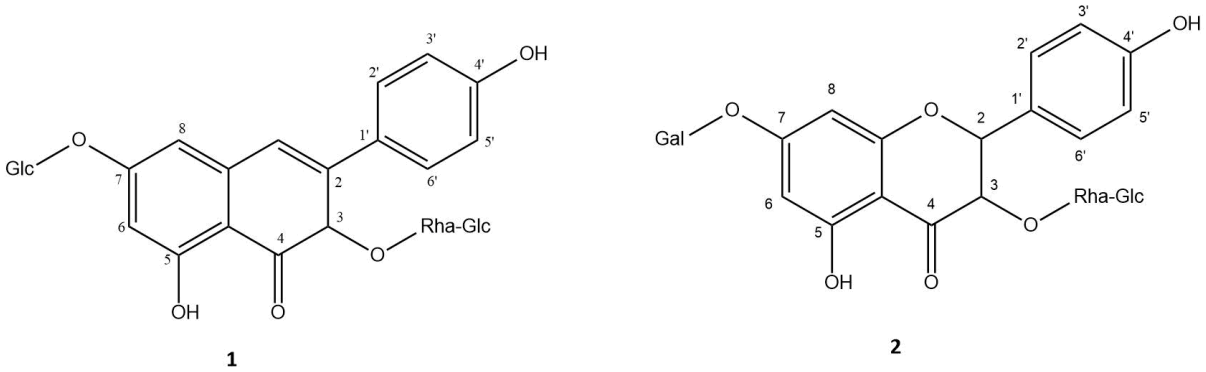

Figure 9 Chemical structure of kaempferol and derivatives. 1 Kaempferol-3-rhamnogluco-7-glucoside 2 Kaempferol-3-rhamnogluco-7-galactoside. Modified and redrawn from Szostak & Kowalewski, 1975. Figure created with ChemDraw professional software, version 15.0.0.106

2.2.3. Flavones: apigenin and luteolin

A flavone is an important flavonoid subgroup usually found in leaves, flowers, and fruits. They are characterised by a unique molecular structure that includes forming a double bond between the C_2 and C_3 positions of the C-ring and a carbonyl group (Panche et al., 2016) (Figure 10). Flavones, along with flavonols, are the main pigments found in white and cream-coloured flowers, and they serve as co-pigments alongside anthocyanins in blue flowers. These compounds can absorb ultraviolet radiation in 280-315 nanometers (Hostetler et al., 2017). Apigenin and luteolin are well-known flavones with antioxidant and anti-inflammatory properties and potential therapeutic applications. (Panche et al., 2016).

Apigenin is commonly found in fruits and vegetables such as parsley, celery, and chamomile and has been shown to inhibit cancer growth in several types of cancers (Farombi et al., 2018). Similarly, luteolin is found in various vegetables, fruits and herbs, such as carrots, cabbage, artichoke, tea, celery, and apple. It has been shown to induce apoptosis, cell cycle arrest, and inhibit metastasis and angiogenesis in multiple cancer cell lines (Imran et al., 2019).

Both flavones have the same basic structure consisting of two benzene rings connected by a three-carbon bridge, but they differ in their substituent groups and overall arrangement. The main difference between the two structures is the presence of a hydroxyl group (OH) at the 5' position of luteolin (Figure 10A), which is absent in apigenin (Figure 10B) (Imran et al., 2019; Salehi et al., 2019; Shukla & Gupta, 2010).This difference gives luteolin a more hydrophilic character and may affect its biological activity (Imran et al., 2019). Studies have found apigenin and luteolin in several *Psychotria* species, demonstrating their presence within the genus (Berger et al., 2016; Fu et al., 2015).

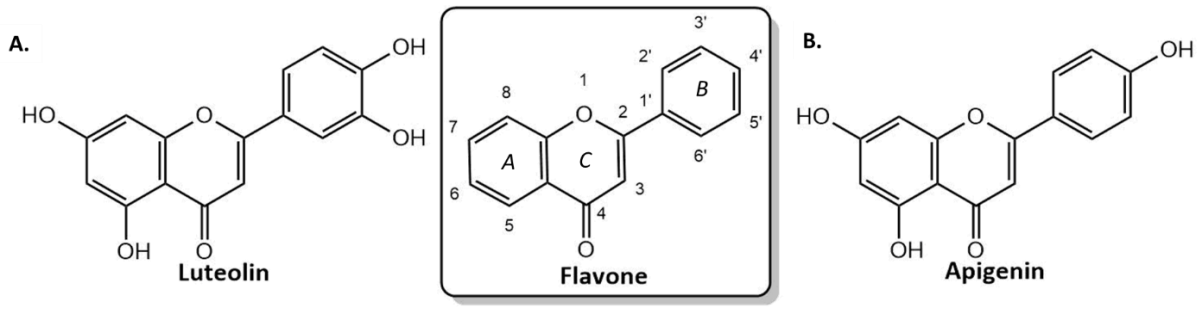

Figure 10. Chemical structure of flavones. The basic structure of flavone with position labels in the centre. A. luteolin. B. Apigenin Modified from Veitch & Grayer, 2011. Figure created with ChemDraw professional software, version 15.0.0.106

In leaves from *Psychotria nervosa* collections from Costa Rica, the flavone apigenin 7-*O*-rhamnoglucose was isolated (Berger et al., 2016), and in *Psychotria straminea,* this compound was isolated as well from samples from China, where it was described as the first study together luteolin and quercetin (Fu et al., 2015). In 2016, the allelopathic effect of *Psychotria viridis* on the germination and initial growth of *Lactuca sativa* was studied. From leaves of *P. viridis,* several alkaloids and flavonoids were isolated, among them luteolin and apigenin (Andrade et al., 2016). Additionally, two luteolin derivatives were isolated from Psychotria rubra's roots and identified: 6-hydroxy-luteolin-7-o-rutinoside and luteolin-7-*O*-rutinoside (Lu et al., 2014).

2.3. Alkaloids

Alkaloids are a broad group of natural compounds derived from secondary metabolism and can be chemically defined as organic compounds with at least one nitrogen atom in a heterocyclic C-ring derived from amino acids or transaminations. They are mostly isolated from plants and found in around 20% of plant species. However, some animals, fungi, invertebrates, and microorganisms can produce them (Aniszewski, 2007; Gutiérrez-Grijalva et al., 2020; Hussein & El-Anssary, 2019).

Alkaloids can be divided according to their structure and origin into true alkaloids, protoalkaloids and pseudoalkaloids. The first two are derived from amino acids; however, the nitrogen in protoalkaloids is not part of the heterocycle, as typical of true alkaloids, the amino acid-derived nitrogen is located in a heterocycle. On the other hand, pseudoalkaloids are derived from other biosynthetic pathways, excluding amino acids (Aniszewski, 2007; Berger et al., 2022b). Alkaloids may be accumulated in different cell organelles and plant organs (Gutiérrez-Grijalva et al., 2020).

The biosynthetic pathways of alkaloids are diverse, as seen in

Figure **11**. The aromatic amino acids phenylalanine, tyrosine and tryptophan are precursors of certain alkaloids originating from chorismate, the final product of the shikimate pathway (Jan et al., 2021; Maeda & Dudareva, 2012). The conversion of chorismate into any of the three proteinogenic aromatic amino acids is accompanied by the loss of its carboxylate group at position 1. The loss of its carboxylate group at position 1, the biosynthetic origin of which can be traced back to a phosphoenol pyruvate building block. The co-transfer of the carboxylic carbon to a downstream product and the other two carbons introduced into shikimate from the precursor phosphoenol pyruvate provides convincing evidence for a preprephenate or pre-anthranilate branch point (Jan et al., 2021).

This pathway is also involved in the biosynthesis of the precursor for vitamins K_1 and B_9 (Maeda & Dudareva, 2012). The shikimate pathway may be activated under stress conditions, thus producing the previously mentioned amino acids, which give rise to indoles and isoquinolines alkaloids (

Figure **11**) (Jan et al., 2021; Maeda & Dudareva, 2012).

Due to their bioactive properties, plant alkaloids have been used in different cultures as medicine since ancient times; they are essential in the ethnobotanical context and have been widely used as anaesthetics, cardioprotective, and anti-inflammatory agents (Heinrich et al., 2021). Currently, 900 new natural alkaloids were reported between 2014 and 2020 (Heinrich et al., 2021), and some have pharmacological importance; some are used as chemotherapy agents, and others are under study for their antidiabetic and neuroprotective capacity (Gutiérrez-Grijalva et al., 2020).

2.3.1. Monoterpene indole alkaloids (MIAs)

The monoterpenes indole alkaloids (MIAs) are one of nature's largest families of secondary metabolites, with more than 3000 identified compounds and more than 40 structural types (Dudley et al., 2022; X. Y. Liu & Qin, 2022). These alkaloids are characterised by having a bicyclic structure of a benzene ring fused to a five-membered pyrrole ring (Mohammed et al., 2021). The subsequent discussion will outline the MIAs' biosynthetic origin, biological activity, and pharmacological uses.

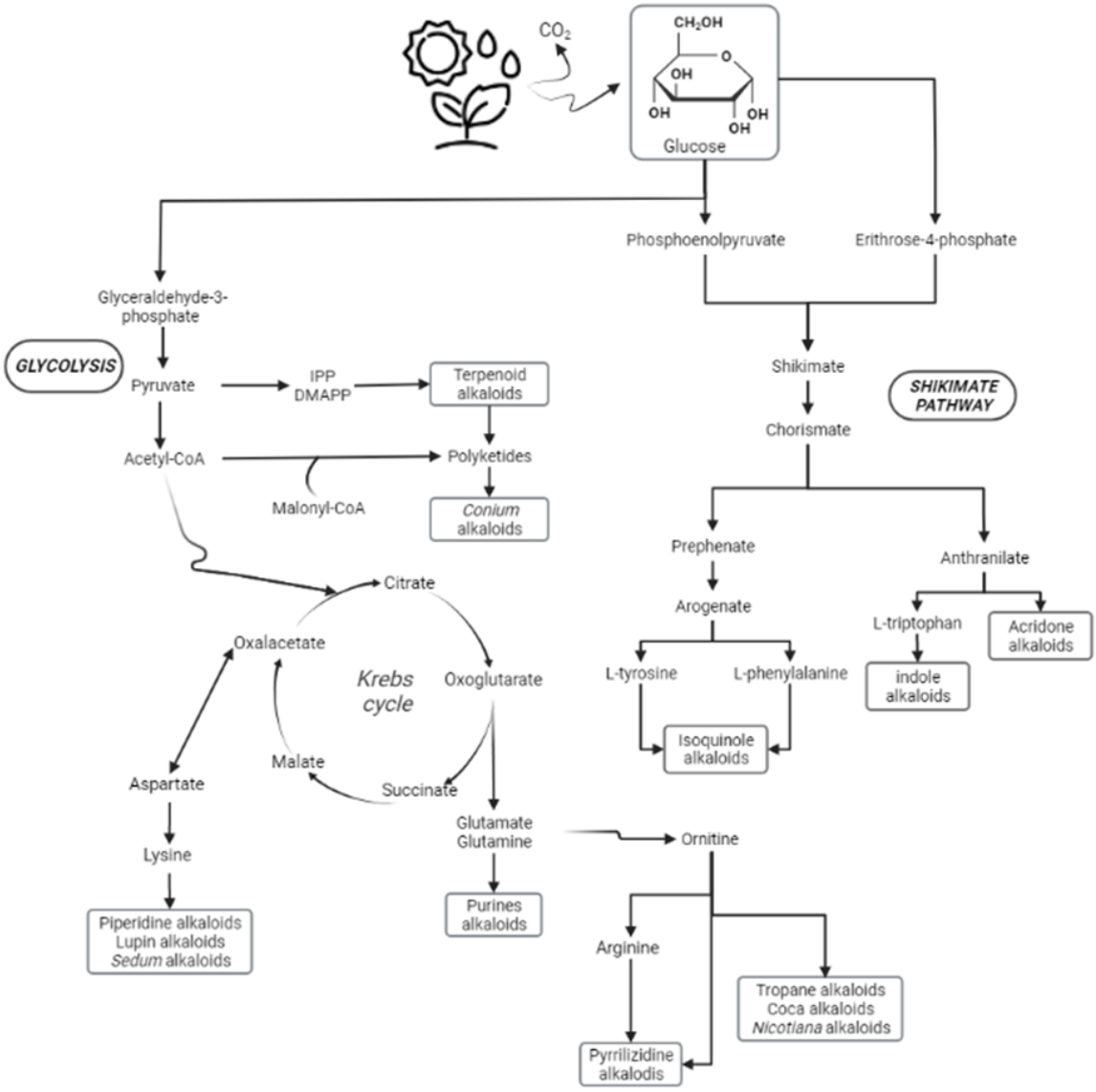

Figure 11 Metabolic pathway of alkaloid biosynthesis. On the right is the Shikimate pathway and the origin of indole alkaloids. The metabolic route of the purine alkaloids and terpenoid alkaloids is also shown. IPP: isopentenyl diphosphate, DMAPP: dimethylallyl diphosphate. Modified from Gutiérrez-Grijalva et al., 2020. Created with BioRender.com

2.3.1.1. Biosynthesis of MIAs

According to the biosynthetic pathway described for *C. roseous*, the MIAs' biosynthesis occurs in different leaf cell types (De Luca et al., 2014; Kulagina et al., 2022). MIAs originate from tryptophan and the iridoid monoterpene secologanin, and the whole reaction occurs within the vacuoles in the leaf epidermal cells (Liu & Qin, 2022; O'Connor & Maresh, 2006). On the one hand, the precursor tryptamine is formed, where the pyridoxal-dependent enzyme tryptophan decarboxylase converts tryptophan to tryptamine. Studies in suspended cell cultures of *Catharantus roseus* and C-glucose suggest that secologanin is derived from the non-mevalonate pathway (Figure 12) (Peebles et al., 2006).

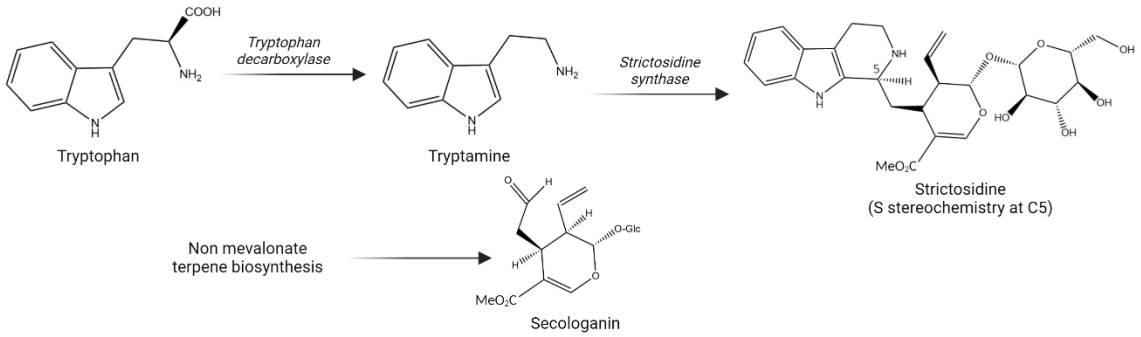

Figure 12 Overview of the first steps of monoterpene indole alkaloid biosynthesis (Structures created with ChemDraw professional software, version 15.0.0.106) Adapted from O'Connor & Maresh, 2006

Strycytosidine goes through an additional deglycosylation process with the help of strycytosidine-β-D-glucosidase (SGD), which is transported to the nucleus to ensure an optimal enzyme/substrate breakdown. This leads to the production of an aglycone, which serves as the starting point for the formation of several MIAs (Figure 13) (Kulagina et al., 2022)

During a predatory attack, the sudden disruption of the compartmentalisation of strictosidine and SGD leads to massive production of strictosidine aglycon that induces protein cross-linking, conferring toxic properties to the aglycon when ingested by the predator. The compartmentalisation of this nuclear enzyme-vacuolar substrate is a defence mechanism known as "the nuclear time bomb" and is similar to the mustard oil bomb in the myrosinase-glucosinolate system found in Brassicaceae (Courdavault et al., 2014).

2.3.1.2. Biological activity and pharmaceutical importance of MIAs

The diverse biological activities of MIAs have made them attractive targets for drug development. The antitumour properties of the monoterpene indole alkaloids vinblastine and vincristine have been extensively studied and used to treat various types of cancer.

In *Catharantus roseus* (Apocynaceae), the dimeric alkaloids vinblastine and vincristine have been isolated and studied for use in the treatment of different types of cancer, such as acute leukaemia, Hodgkin's and non-Hodgkin's lymphoma, neuroblastoma, rhabdomyosarcoma, Ewing's sarcoma, thyroid cancer, brain tumours, among other cancers and is also used to treat blood disorders (Keglevich et al., 2012; Moha, 2017).

Another MIA of importance is ibogaine, which is extracted from the root bark of *Tabernanthe iboga* (Apocynaceae) and used by indigenous people in small doses to combat fatigue, hunger and thirst. In the pharmaceutical field, it has been used and studied since the 1960s as a treatment for drug addiction (Mačiulaitis et al., 2008).

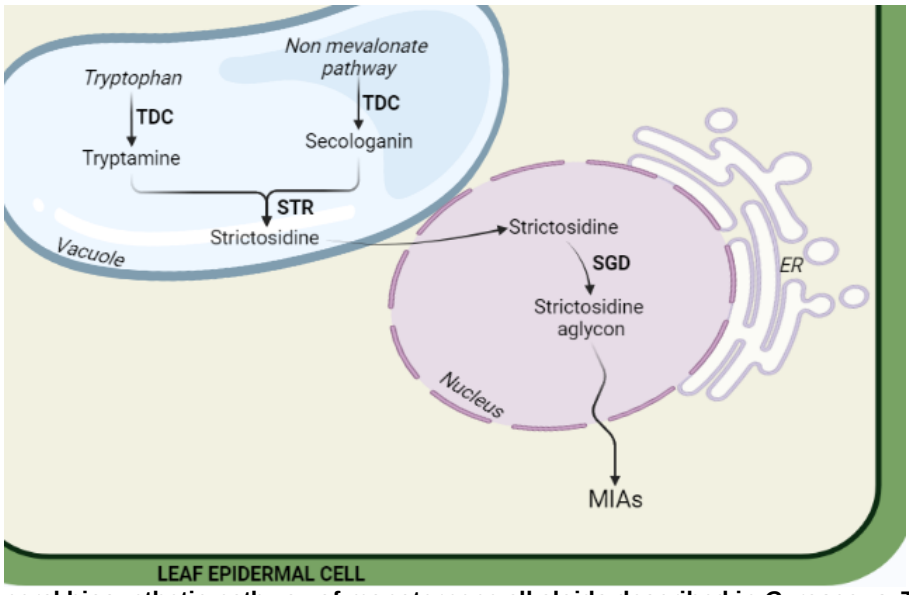

Figure 13 General biosynthetic pathway of monoterpene alkaloids described in *C. roseous.* The enzymes are abbreviated as follows: TDC, tryptophan decarboxylase; STR, strictosidine synthase; SGD, strictosidine β-glucosidase. Adapted from (Kulagina et al., 2022)**.Created with BioRender.com**

2.4. Secondary metabolites within the Rubiaceae family

Many bioactive secondary metabolites characterise the megadiverse Rubiaceae family with pharmacological potential. The compounds present within the family are iridoids, indole alkaloids, anthraquinones, terpenoids, and derivatives (Figure 14) (Martins & Nunez, 2015). Characteristic derivatives appear partly taxon-specific and are thus used as phytotaxonomic markers. Some secondary metabolites produced by the Rubiaceae family are of economic importance, such as caffeine extracted from the coffee plant *Coffea arabica* (subfamily *Ixoroideae*) and acting as a nervous system stimulant (Martins & Nunez, 2015). Within the *Cinchonoideae* subfamily, *Cinchona* species are the source of quinine, which is used as a treatment against malaria (Aniszewski, 2007).

Furthermore, in the subfamily *Ruboideae*, species of the *Psychotria* tribe yield substances used in indigenous religious ceremonies, known as "ayahuasca", which affects the central nervous system and produces hallucinogenic effects (de Carvalho et al., 2016). In the order Gentianales, only the families Loganiaceae, Rubiaceae, Apocynaceae and Naucleaceae contain monoterpene indole alkaloids, and for Rubiaceae, this is the only chemical marker present in the family (Martins & Nunez, 2015). Being of great importance to the Rubiaceae family, the monoterpene indole alkaloids will be described in detail as follows.

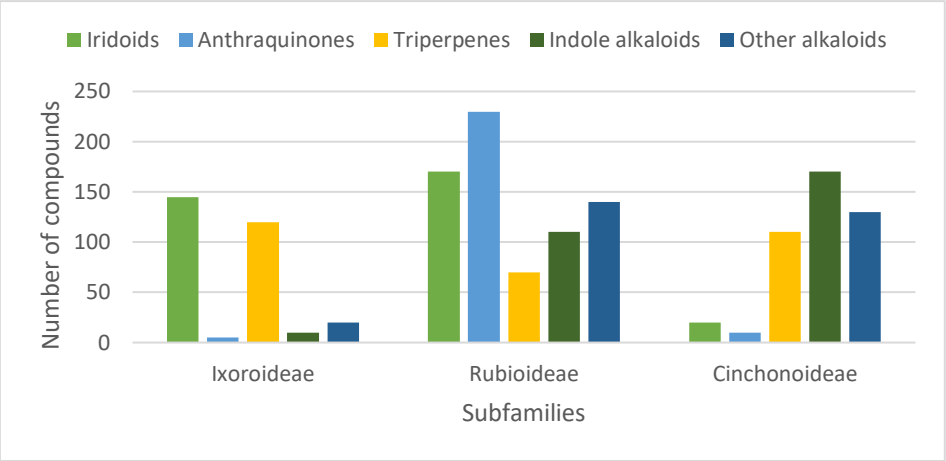

Figure 14. Chemical diversity and major secondary metabolites distribution among Rubiaceae subfamilies. Adapted from Martins & Nunez, 2015

Within the Rubiacaeae family, monoterpene indole alkaloids with pharmaceutical properties such as yohimbine and reserpine have been found. Yohimbine, isolated from the bark of the African tree *Corynanthe johimbe* (Rubiaceae), has been used as a treatment for erectile dysfunction and weight loss (Bourgeois & Eggleston, 2023) and is also used as a treatment for dementia, diabetic complications, insomnia, leprosy and low blood pressure (Anadón et al., 2016). Another compound is reserpine, found in the *Rauwolfia serpentina* and *Rauwolfia vomitoria* plants, and is mainly used to treat high and low blood pressure and as a sedative or tranquilliser (Adedokun et al., 2023).

2.4.1. Flavonoids within the Psychotria alliance

Berger and collaborators (2016) reported flavonoid glycosides from leaf extracts for seven species within the *Psychotria* alliance, the most abundant group of flavonoids described for this alliance. For *P. racemosa,* the quercetin glycosides rutin and quercetin 3,7-di-*O*-α-L-rhamnopyranosid were found; from *P. mortoniana* kaempferol 3-*O*-α-L-rhamnopyranosyl-(1/6)-β-D-glucopyranoside was identified.

Additional studies have been done on other species of the genus *Psychotria*. From leaves of *P. spectabilis*, quercetin was isolated, whilst, from *P. serpens*, the isolation of the flavonoids rutin, quercetin, tamarixetin-3-*O*-rutinoside and kaempferol was reported (Calixto et al., 2016). Further investigations dealt with flavonoids isolated from P. serpens to analyse their efficiency in traditional medicine. The plant extract from different organs showed robust activity against different tumoral cells without toxic effects on healthy cells (Lin et al., 2015).

2.4.2. Alkaloids in the Psychotria alliance: Indole alkaloids

Species of the tribes, *Psychotrieae* and *Palicoureae* produce structurally diverse alkaloids derived from indole. These indole alkaloids are among the largest and most diverse alkaloid groups, representing essential traits for species identification groups (Berger et al., 2022b). Secondary metabolites, particularly indole alkaloids, are relevant for chemosystematic interpretation within the *Psychotria* alliance since its species lack differentiating morphological features (Porto et al., 2009).

Figure 15 shows the alkaloids characteristic of each genus within the alliance and its phylogenetical relationships. It should be noted that some alkaloids are restricted to specific genera of the tribe *Palicoureeae* and may be completely absent in *Psychotrieae* (Berger & Schinnerl, 2019). Such is the case of N, N-dimethyltryptamine (DMT), which has been found and identified only in leaf extracts of *Psychotria viridis*, which is a hallucinogenic compound that, together with the stem bark of the vine *Banisteriopsis caapi*, is used in indigenous ceremonies to produce the ritual beverage "ayahuasca" (Matsuura et al., 2013).

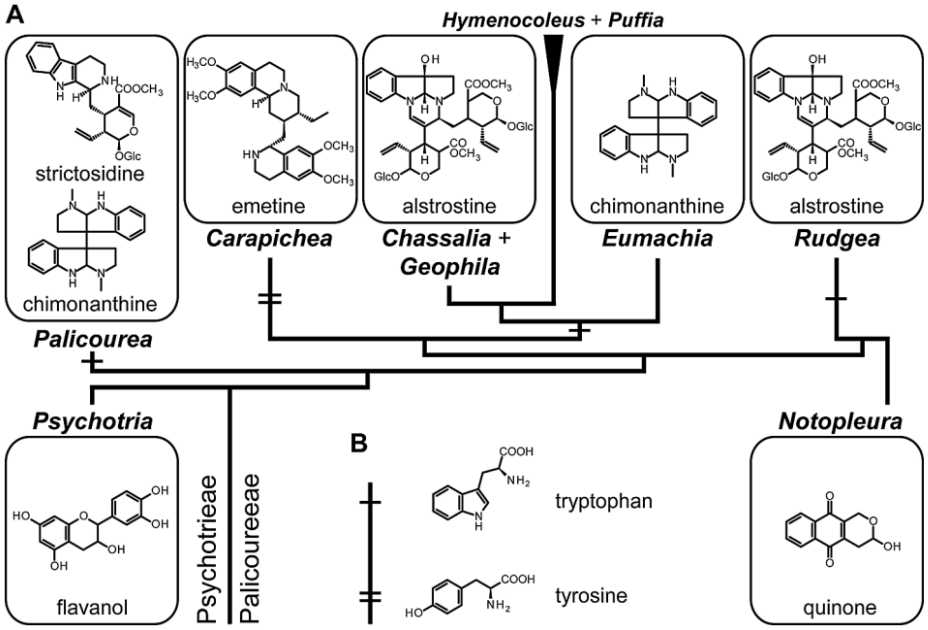

Figure 15. Alkaloids characterising the genera of the sister tribes Palicoureeae (except *Psychotria*) and Psychotrieae (*Psychotria*) plotted on a simplified phylogeny of the group. (a) Boxes with representative structures illustrate the compound groups characterising each genus. (b) In alkaloid-accumulating clades, crossbars on the phylogenetic tree indicate the amino acid building blocks incorporated in the alkaloids of the respective genera. Adapted from Berger et al., 2022b

2.5. Characteristic compounds of the genus Eumachia

Chemical studies have been done for nine known *Eumachia* species from Asia, Australia and the Pacific, with only one species from the Neotropics (Berger et al., 2022b). The indole alkaloids are distinct compounds in *Eumachia*, especially the polypyrroloindoline alkaloids

(Jamison et al., 2017). Polypyrroloindoline alkaloids are cyclotryptamines, hexahydropyrrolo indole alkaloids, or cis-pyrrolidino[2,3-β]indoline alkaloids, and they are structurally characterised by multiple cyclotryptamine subunits connected via sterically clustered and synthetically demanding quaternary stereocentres. The biosynthesis of these alkaloids is known to be via oxidative coupling of tryptamine monomers (Berger et al., 2022b; Jamison et al., 2017). Table 1 lists the synonyms of *Eumachia* species and references to studies on their secondary metabolites. For a detailed list of compounds, see Tables 4 and 5, respectively. Brand and collaborators (2012) reported the presence of Hodgkinsine and Quadrigemine B for *Margaritopsis cymuligera (*synonym of *Eumachia cymuligera),* which are pyrrolidinoindoline alkaloids occurring in *P. olieoides* (Jannic et al., 1999). Studies indicated the cytotoxicity of pyrrolidinoindoline alkaloids, making them good candidates for being used as anticarcinogens. Trials in human cancer lines were performed, and it was observed that compounds such as hodgkinsine showed effective actions against cancer cells (Nascimento, et al., 2015).

Table 1. *Eumachia* phytochemically studies species. [a] Synonyms: = heterotypic synonyms, ≡ homotypic synonyms. Adapted from Berger et al., 2021

Accepted species	Reported under [a]	References
Eumachia cymuligera	*Eumachia cymuligera*	Brand et al. (2012)
Eumachia depauperata	≡ *Margaritopsis carrascaoana*	Nascimento et al. (2015)
Eumachia forsteriana	≡ *Psychotria forsteriana*	Adjidabé et al. (1985, 1986, 1989,1992), Roth et al. (1985, 1986)
Eumachia fructescens	≡ *Hodgkinsonia fructescens*	Anet et al. (1961), Fridrichsons et al. (1967,1974), Parry et al. (1978)
Eumachia leptothyrsa	*Psychotria beccarioides*	Hart et al. (1974)
Eumachia lyciiflora	≡ *Psychotria lyciiflora*	Jannic et al. (1999)
Eumachia oleoides	≡ *Psychotria oleoides*	Guéritte-Voegelein et al (1992), Jannic et al. (1999), Libot et al. (1987)
Eumachia rostata	≡ *Psychotria rostata*	Lajis et al. (1993), Mahmud et al. (1993), Takayama et al. (2004)
Eumachia straminea	≡ *Psychotria straminea*	Fu et al. (2015)

3. MATERIALS AND METHODS

3.1. Plant Material

From the species *Eumachia microdon (*sub. *Margaritopsis microdon* (D.C.) C. M. Taylor) (Rubiaceae) leaves (BL 1535) and stem bark (SR 1535) were collected in March 2013 by A. Berger and J. Schinnerl. The plant material was collected in the Carara National Park west of Costa Rica at the Garavito canton, Puntarenas. The herbarium specimen is deposited at the herbarium of the University of Vienna (WU 0088141).

3.2. Extraction and isolation

Methanol was added to the dry and ground stem bark (177 g) and ground leaves (74.0 g) and sonicated for 20 minutes at room temperature. The solutions were filtered after three days in darkness, and the plant material was repeatedly soaked in methanol for another three days. After the filtration, the pooled extracts were evaporated using a rotary evaporator at 38°C, obtaining 6.5 g and 8.6 g from the crude extract of stem bark and leaves, respectively.

3.3. Analytical Methods

3.3.1. Thin-layer chromatography

The thin-layer chromatography (TLC) separates compounds based on a stationary phase attached to a solid surface and an eluent (mobile phase) that migrates through the plate by capillary forces. Pre-coated silica gel 60 TLC aluminium plates (0.2 mm, Sigma Aldrich) were used for monitoring separations done by CC, and the detection wavelength was set at 254 nm. Subsequently, the plates were sprayed with anisaldehyde reagent (85 mL MeOH, 10 mL acetic acid, 8 mL sulfuric acid and 0.5 mL p-anisaldehyde), followed by heating with a hot air gun and with Dragendorf reagent (Bismuth subnitrate (oxynitrate; 1.7 g) H_2O (80 mL) and acetic acid (20 mL); potassium iodide solution (50% w/v, 100 mL)) for alkaloid detection. TLC plates were developed in mixtures consisting of ethyl acetate (EtOAc), methanol (MeOH) and ammoniac at different concentrations.

3.3.2. High-performance liquid chromatography

High-performance liquid chromatography (HPLC) is based on the retention of the components of a sample dissolved in the mobile phase when going through a densely packed reverse phase column (stationary phase). The sample components are delayed depending on the physical and chemical interactions with the stationary phase as they pass through the column.

HPLC analyses were performed on Agilent 1100 Series equipped with a UV diode array detector (detection wavelength 230 nm), a hypersil column BDS-C18, 250 × 4.6 mm, 5 µm particle size. The mobile phase consisted of 10 mM ammonium acetate (A) and methanol (B) in a 10% to 100% gradient for 15 minutes with an injection volume of 10 µL at a flow rate of 1.0 mL/min.

3.3.3. Nuclear magnetic resonance spectroscopy

These analyses were performed at the Department of Organic Chemistry of the University of Vienna

3.4. Preparative Methods

3.4.1. Liquid-Liquid extraction

Liquid-liquid extraction (LLE) is a classical method widely used to separate compounds into two immiscible phases: aqueous and organic. Several LLE were accomplished for the extracts obtained from both plant organs (Figure *16*).

For the stem bark, the separation was done with chloroform ($CHCl_3$), ethyl acetate (EtOAc) and water (H_2O). For the resulting lipophilic phase ($CHCl_3$), an additional LLE was performed using petrol ether, ethyl acetate (PE: EtOAc 95:5), and H_2O.

The first extraction from the aqueous phase from stem bark was done with H_2O and EtOAc; from the resulting H_2O phase, an additional LLE was done using water and butanol (BuOH) as solvent. For the BuOH phase, a different separation was carried out with diethyl ether 5% and water. The leaf extract was partitioned in PE: EtOAc (95:5), $CHCl_3$, EtOAc and H_2O. An acid-basic LLE was done for the aqueous phase, using an aqueous ammonia solution with a pH of 9 and chloroform.

3.4.2. Acidic hydrolysis

Acidic hydrolysis was performed to split off sugars attached to the flavonoid compounds. This procedure was done following the protocol of Mabry et al., (1970). HCl 2M hydrochloric acid (pH 1.5) and methanol were added to the crude leaf extract. Then, it was heated in a steam bath at 100°C for two hours with reflux. The sample was then allowed to cool and was fully extracted. Subsequently, an LLE was performed with chloroform and water. The obtained fractions were analysed by HPLC.

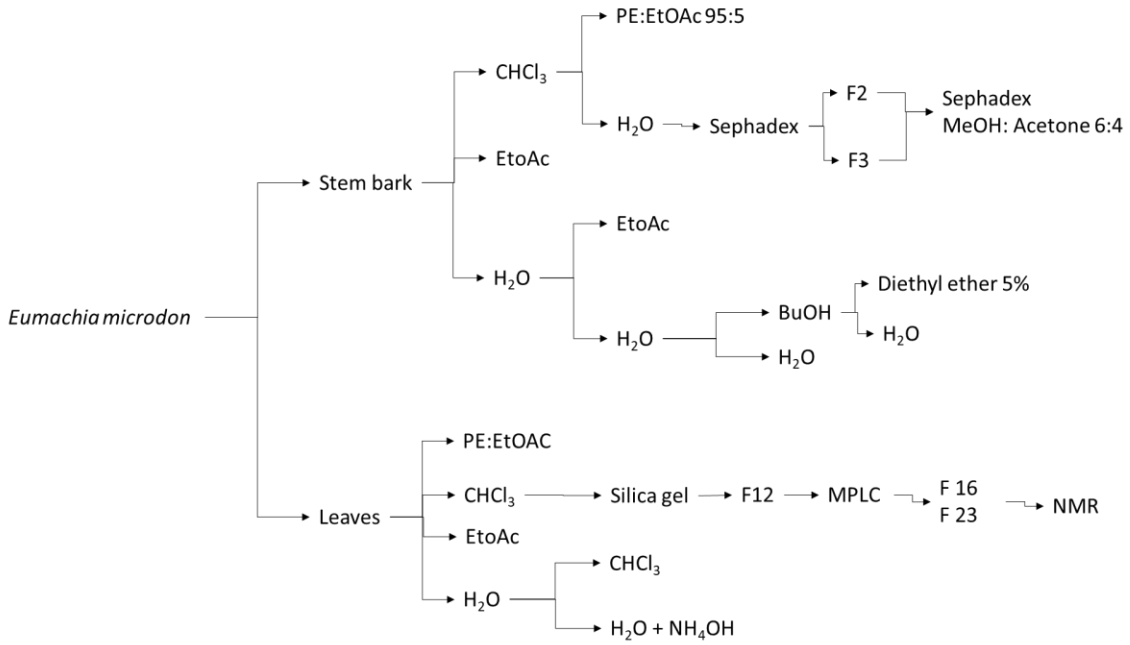

Figure 16. Liquid-liquid extraction scheme of *Eumachia microdon* (D.C.) Delprete & J.H.Kirkbr.

3.4.3. Column chromatography (CC)

This technique is used for the separation and purification of compounds. The stationary phase was placed in a glass column and filled with eluent (mobile phase), which, due to gravity, caused the sample to move through the column. For the lipophilic phase of the stem bark, the sample was subjected to size exclusion chromatography using Sephadex LH 20 as the stationary phase and eluted isocratically with pure methanol. In total, 19 fractions were collected, from which two of them were subjected to a silica gel 60 column (0.2–0.5 mm, Merk) with a solvent mixture of *acetone*: MeOH (70:30) for fraction 2 (15 fractions collected) and Acetone: MeOH (60:40) for fraction 3 (16 fractions collected) respectively (Table 3).

Several CCs were done to the aqueous and lipophilic phases resulting from the LLE from the leaf crude extract. A Sephadex LH 20 was eluted with pure MeOH for the aqueous phase. An additional LLE was done for the aqueous phase with a pH value of 9, adjusted by adding NH_4OH, and from the resulting aqueous phase, a CC with Sephadex LH. 20 was done (Table 3). For the lipophilic phase, a silica gel 60 column was used with solvent mixtures of P.E., EtOAc and MeOH with increasing polarities (Table 2), obtaining 35 fractions.

Table 2. Solvent mixtures used for column elution for Column chromatography for $CHCl_3$ phase of leaves of *Eumachia microdon.*

Petroleum ether (P.E.)	*Ethyl acetate (EtOAc)*	*Methanol (MeOH)*
80	20	
60	40	
50	50	
40	60	
20	80	
	100	
	75	25
	50	50
	20	80
		100

3.4.4. Ion exchange chromatography

Ion exchange chromatography (IEC) is a method that allows the separation of molecules based on their electric charge properties. 400 mg of the aqueous phase from the LLE NH_4OH to the aqueous phase of the leaves (see Figure 16) were subjected to a solvent mixture of H_2O containing 5% NH_4OH.

Table 3. General description of column chromatographies organised by sample, method, solvent mixture used and the number of fractions collected.

	Sample	Phase	Method	Solvent mixture
STEM BARK	$CHCl_3$	H_2O	Sephadex LH20	MeOH pa.
		F3	Silica gel	MeOH:*acetone* (60:40)
		F2	Silica gel	MeOH:*acetone* (70:30)
	H_2O	H_2O_BuOH	Sephadex LH20	MeOH pa.
LEAVES	Crude extract	$CHCl_3$	Silica gel	Table 2.
		H_2O	Sephadex LH20	MeOH pa.
		NH_4OH - H_2O phase	1.Sephadex LH20 (x 2)	MeOH pa.
			2.Sephadex (x3)	MeOH pa.

3.4.5. Medium Pressure Chromatography

Medium pressure chromatography (MPLC) is a preparative technique to separate complex mixtures. The MPLC column used was a Lobar 240-10 LiChroprep Si60 (40-60 µm) (Merck), and a UA6 UV/VIS Detector monitored the separation set at a different wavelength with a mixture of PE: EtOAc. A reversed-phase MPLC using a C-18 column (240 × 10 mm) separated aqueous phases. The eluents, in this case, were mixtures of MeOH and water.

3.4.6. Preparative thin-layer chromatography (pTLC)

A pTLC is used to separate small amounts of compound mixtures. The sample is spread as a streak onto the glass plate (silica gel 60 F_{254} glass plates with 0.5 mm layer thickness, Merk) and developed in a mixture of EtOAc : MeOH: NH_4OH (25% in water) in a ratio of 90:5.5:0.5. The plates were developed three times and afterwards detected under the UV detector. The band of interest was scratched from the plate and extracted with MeOH. This solution was filtered through a glass suction filter (G5).

4. RESULTS AND DISCUSSION

Comparative phytochemical profiling of leaf and stem extracts of *Eumachia microdon* indicated the presence of various phenolic compounds, concluded mainly from UV-spectra and retention times from HPLC profiles. Liquid-liquid extraction was applied, yielding an aqueous phase and the two phases, chloroform and ethyl acetate, respectively. Isolation of single compounds, particularly those suspected to be of an alkaloid nature, proved to be particularly difficult. The following chapters discuss the organ-specific diversity of specialised metabolites in relation to the few literature data from related taxa.

4.1. Composition of Leaf extract

By comparative HPLC profiling, a major and prominent peak was detected in the crude leaf extract (8.6 g), at a retention time of 7.2 minutes and with a UV-spectrum maximum of 210 and 294 nm. Profiles of those phases derived from liquid-liquid extraction revealed this major peak with several peaks at lower concentrations (Figure 17 B-D). The more lipophilic compounds remained in the chloroform phase (Figure 17B), whereas the ethyl acetate and water phases contained hydrophilic compounds. Various phenolic compounds were suspected, and a more detailed analysis of these fractions was carried out.

An unknown compound peak was detected at around the retention time of 7.2 minutes (Figure 17♦) and did not compare to any data from our database. From the characteristic UV spectra of catechins, showing UV maxima at 204 and 280 nm, it is assumed that this compound would correspond to a catechin derivative. In experiments done in leaves from *Uncaria gambir* (W.Hunter) Roxb. (Cinchonoideae), three different extraction methods were used, among them, methanolic extraction, obtaining high amounts of catechins (86.35 mg/g) in comparison with the other two (hexane and dichloromethane).

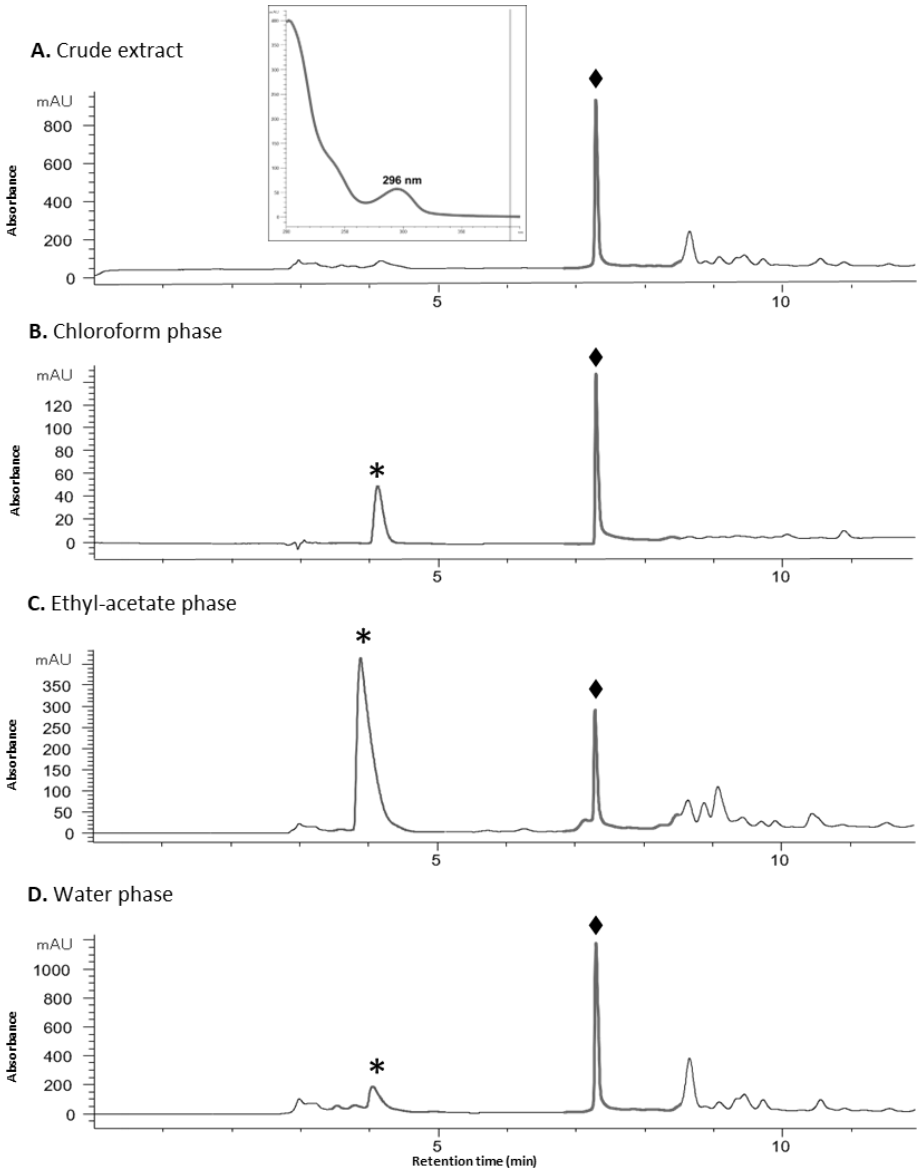

Figure 17 HPCL profile at 230 nm, amplification of the crude leaf extract obtained by liquid-liquid extraction at 230 nm up approximately 12 minutes. A. crude extract. B. chloroform phase. C. ethyl-acetate phase. D. Water phase. (*) traces of acetone. An unknown compound was detected at a retention time of 7.2 minutes (♦). A zoom-in of its UV spectrum (rectangle) and compared with the database, shows similarities with catechins (296 nm)

4.1.1. Phenolic compounds in leaf extracts

The HPLC profile of Figure 18 is a zoom-in of Figure 17 at the retention time between 8 and 12 minutes. Most of the U.V. spectra of the peaks detected here suggest the presence of glycosidic derivatives of flavonoids, and they are marked as **a1–a7** (Figure 18A). These compounds appeared in the ethyl acetate and water phases, so one can infer they are glycosides. This result is supported by comparing their UV-spectral data against compounds from the database.

3-*O*-glycosides of quercetin and kaempferol have been reported in other species of the *Psychotria* alliance in leaf extracts. These kaempferol derivatives have also been found in leaves from *Psychotria straminea* (Fu et al., 2015) from specimens from China. For Neotropical species from Costa Rica, Berger and collaborators (2016) reported five flavonoid glycosides from leaf extracts for seven species within the *Psychotria* alliance, the most abundant group of flavonoids described for this alliance. For *P. racemosa,* the quercetin glycosides rutin and quercetin 3,7-di-*O*-α-L-rhamnopyranosid (6.1 mg) were found; *P. mortoniana* accumulated Quercetin 3-*O*-α-L-rhamnopyranosyl-(1/6)- β-D-glucopyranoside (1.2 mg), and kaempferol 3-*O*-α-L-rhamnopyranosyl-(1/6)-β-D-glucopyranoside (1.2 mg).

Furthermore, compounds with similar UV spectra to the ones found here were reported in leaf extract of *Vinca minor L.* (Apocynaceae) (Ciorîță et al., 2021; Grujić et al., 2014; Szostak & Kowalewski, 1975). The finding of kaempferol-like compounds using methanol extraction can be compared with the results of Puff (1975), who used the same extraction procedure to obtain 15 flavonoids from the leaves of *Gallium* L. (Rubiaceae), among them two kaempferol derivatives, this would be in line with current results on *Eumachia.*

Two phenylpropanoids denoted as **b1-b2**, with UV-spectra similar to caffeic acid, were detected in the HPLC profile (Figure 18C). Caffeic acid is classified as a hydroxycinnamic acid widely distributed in plant tissues (Kumar & Goel, 2019; Magnani et al., 2014). This compound type has been isolated in other plant species from the Ruboideae subfamily. From the leaf extract of *Coussarea hydrangeifolia* (Benth.) Benth. & Hook. f. ex Müll.Arg., five phenylpropanoid glycosides were isolated from the aqueous phase, with antioxidant activity (Hamerski et al., 2005). Also, for four species within the *Galium L*. genus (Rubiaceae), caffeic acid derivatives were found in the extract of the aerial parts of the plant (Vlase et al., 2017).

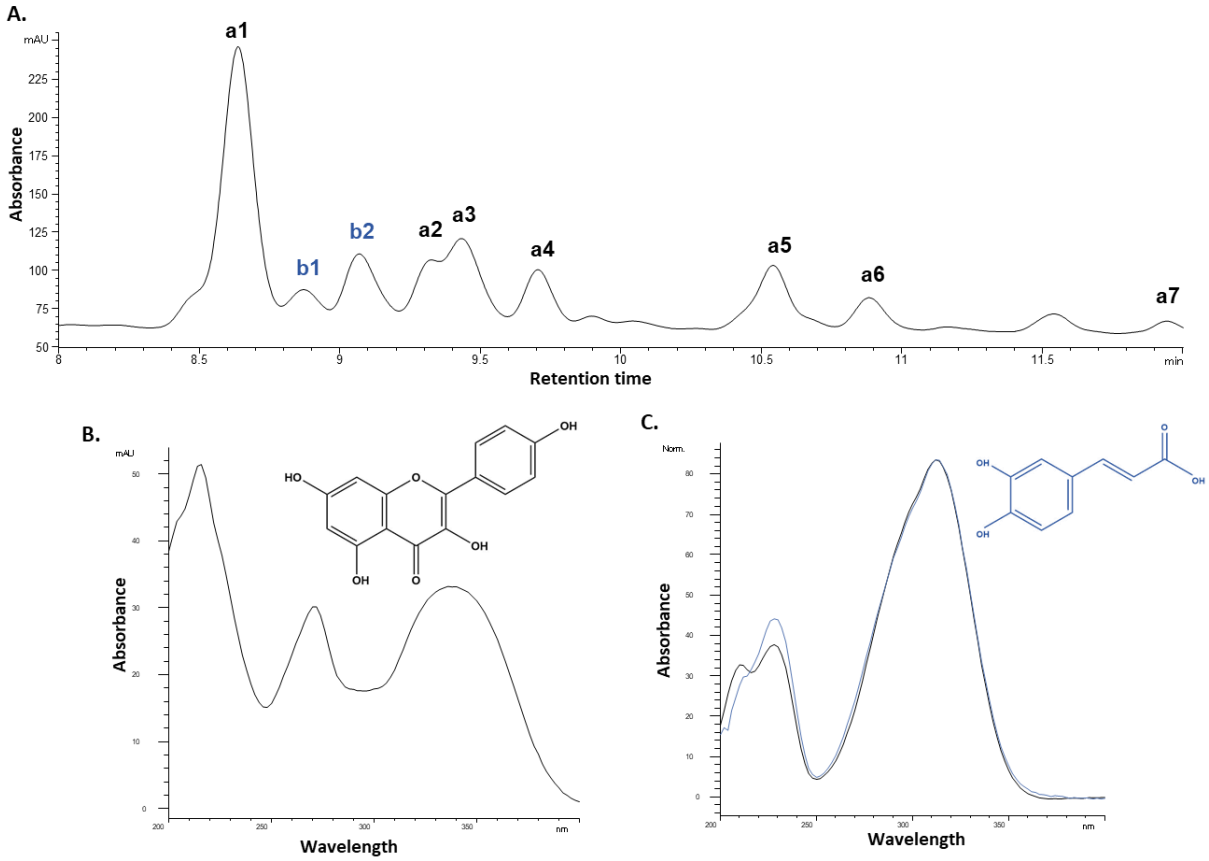

Figure 18 Zoom in on the HPLC profile at 230 nm from the liquid-liquid extraction of the methanolic leaf crude extract between 8 and 12 minutes. A. Compounds marked with a (a1-a7) have a UV spectrum similar to flavonoids (kaempferol derivatives); compounds marked with b (b1 and b2) are speculated to be phenylpropanoids. B. UV spectrum of the seven compounds derived from kaempferol (a1-a7). C. Suspected phenylpropanoids (b1-b2) due to their similarity to the UV spectrum of caffeic acid (in blue).

4.1.2. Analysis of leaf extract hydrolysates

To obtain more information on flavonoid composition, hydrolysis was applied to check for the presence of respective aglycones. Figure 19 compares the crude leaf extract (Figure 19A) with the lipophilic and hydrophilic phases (Figure 19B-C) obtained from the acidic hydrolysis. A peak at a retention time of 7.2 minutes is observed in all phases. The found compound has maximum peaks at 210 and 296 nm, and compared with the database, one can infer that this compound corresponds to a catechin derivative (Figure 19♦).

When zooming in within the retention times of 7.5 and 14.5 minutes (Figure 20), compounds with UV spectra almost identical to apigenin (denoted as **b**) are observed in the aqueous phase, with peaks at 216, 272 and 336 nm (Figure 20D) at retention times 8.4, 9.2 with a shoulder at 9.28, and 9.6 minutes (Figure 20C).

In the crude leaf extract, two compounds (denoted as **c**) were observed with similar UV spectra (Figure 20E). **c1** can be identified in the crude extract (Figure 20A), while **c2** is found in the

$CHCl_3$ and water phases (Figure 20B-C); both have UV maxima of 228 and 310nm (Figure 20E). Compared to the database, the UV spectra of isolated compounds have a similar shape to caffeic acid; however, the UV maxima of caffeic acid are 218, 238 and 324 nm, so it is speculated that they may be derived from the phenolic compound.

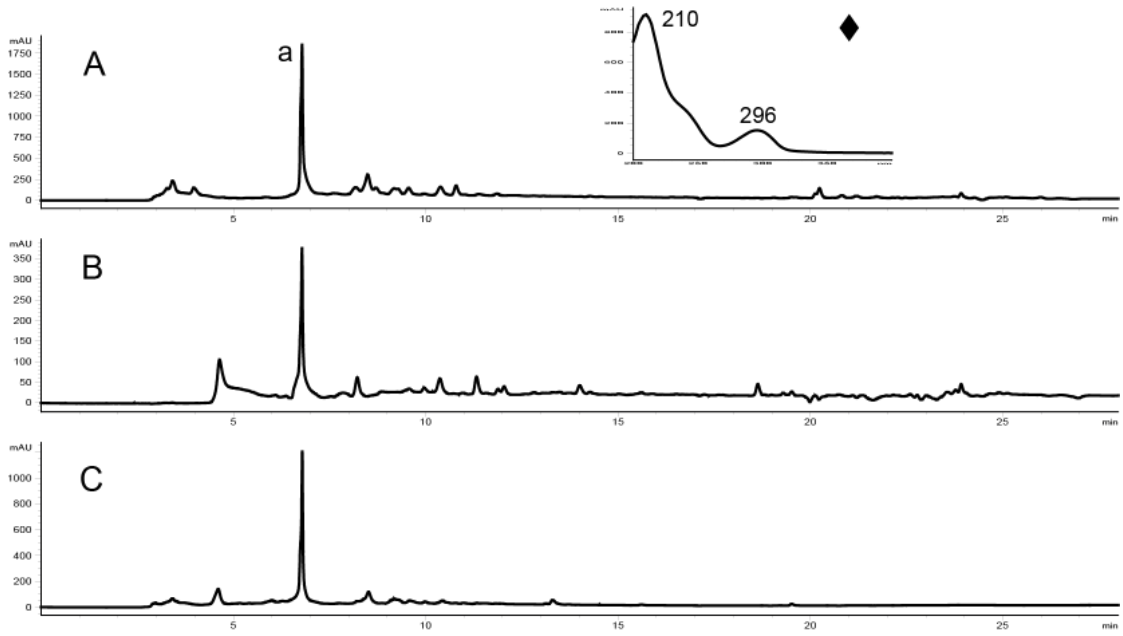

Figure 19. HPLC chromatograms at 230 nm from the acidic hydrolysis extractions of leaf extract. A. Crude leaf extract. B. Chloroform phase. C. Water phase. At the retention time of 7.2 minutes, a high peak was observed and marked as ♦. The UV spectrum was similar to a catechin derivative compared to the database.

According to the results obtained from the acidic hydrolysis extraction, a hypothesis emerged about the presence of C-glycosylflavones, probably based on apigenin. These glycosides are characterised by having one or more sugars connected to the flavonoid moiety at C6 or C8, and additionally, these glycosides do not hydrolyse due to the carbonic bonding with the sugars (Li et al., 2014).

When the acidic hydrolysis is performed, the Wessely-Moser rearrangement occurs, thus, seeking the equilibrium of C6 and C8 of the glycoside; when one sugar molecule is cleaved, the other remains attached to the flavonoid at either the C6 or C8 (Dou et al., 2015). Although these compounds are not commonly found, they have been found in other members of the *Psychotria* alliance, specifically in the genus *Psychotria* (Andrade et al., 2016; Berger et al., 2016; Fu et al., 2015).

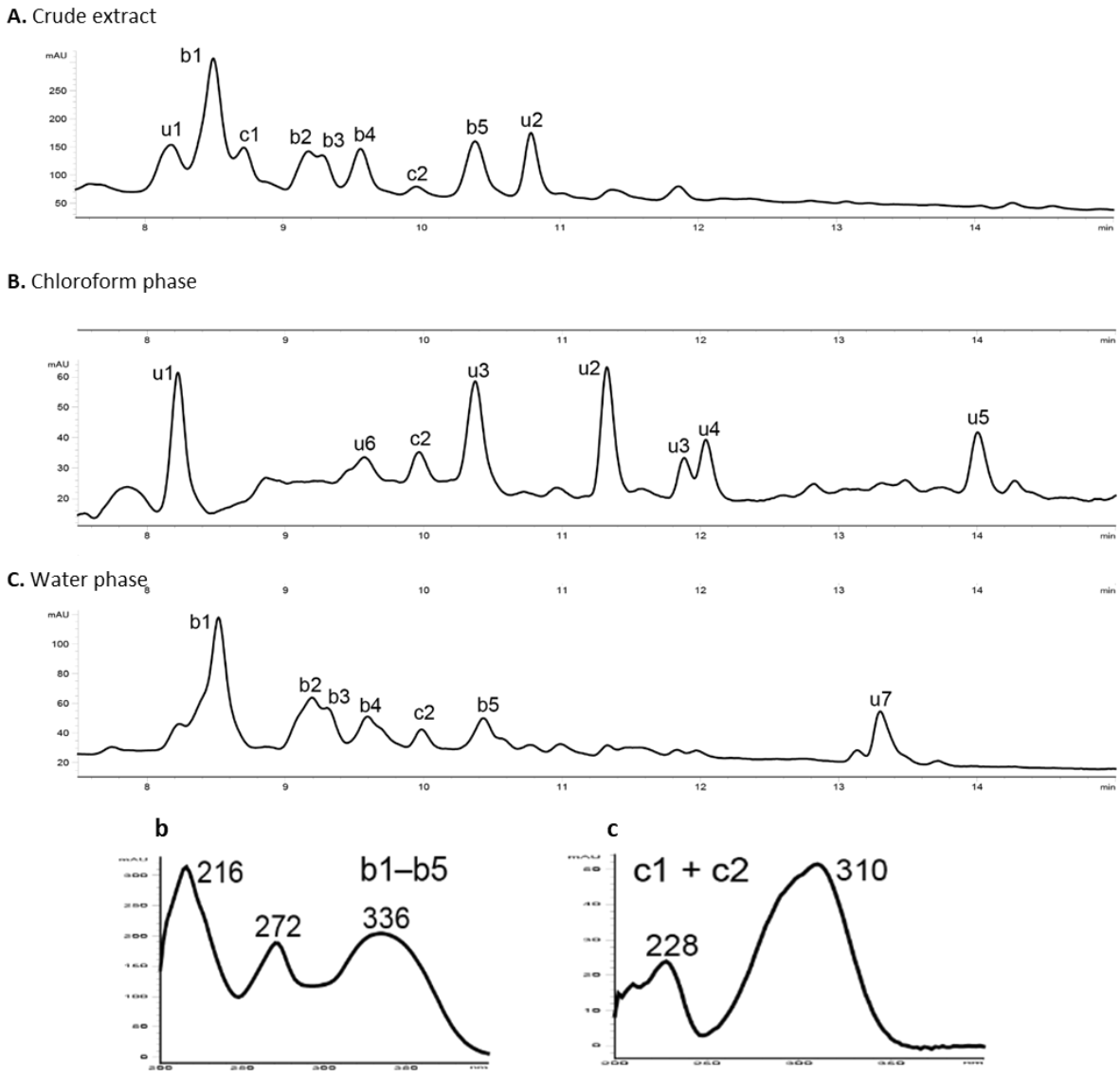

Figure 20 HPLC chromatograms at 230 nm from the acidic hydrolysis of the leaf extract zoom in within the retention times of 7.5 to 14.5 minutes. A. Crude leaf extract. B. Chloroform phase. C. in the aqueous phase, five compounds were identified as b1-b3. Bellow on the left side, the zoom-in of b1-b5 compounds with UV spectra almost identical to apigenin with peaks at 216, 272 and 336 nm. Bellow on the right side, are compounds with a shape similar to caffeic acid; however, this compound has UV max of 218, 238 and 324 nm. Compounds denoted by an u (u1-u7) correspond to unknown compounds.

4.1.3. Search for alkaloids

The aqueous phase obtained from the liquid-liquid extraction was purified by preparative column chromatography using Sephadex-LH20 eluting with methanol. A total of 17 fractions were obtained from the separation; when sampled on a TLC plate (ethyl acetate: methanol: NH_4OH (90:10.5:0.5)), the fractions 6–11 were found to be positive for the Dragendorff reagent (Figure 21). The other Dragendorff-positive spots with a Rf value of approximately 0.8 suggest the presence of artefacts. In the follow-up analysis, however, no nitrogen-containing compound could be detected.

Generally, false positives might be due to the formation of active compounds from inactive natural products, influencing their yield (Maltese et al., 2009). Tomassini and collaborators (1995) evaluated the presence of artefacts in the ethanolic plant extract of *Lonicera japonica* (Thunb.) (Caprifoliaceae) to isolate iridoid components from leaves and branches. They found that the presence of 7-O-butylsecologanic acid and secologanin dimethyl acetal and dibutyl acetalsecologanin could be due to the interaction of the secologanin aldehydes with the alcoholic solvents used in the extraction process.

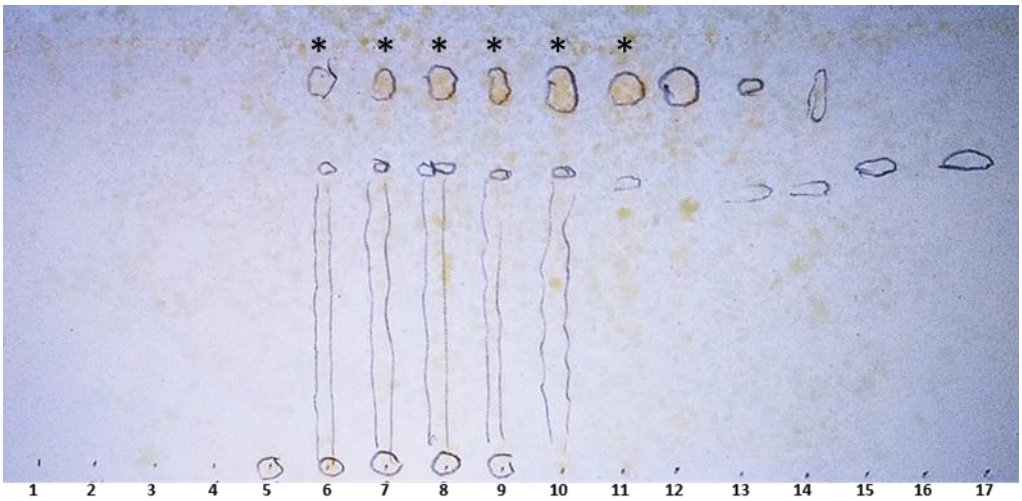

Figure 21. TLC plate from the Sephadex-LH2 column (methanol) developed in the solvent system ethylacetate: methanol:NH_4OH (90:10.5:0.5). (*) shows the Dragendoff positive fractions.

4.2. Composition of stem bark extract

The crude extract chromatogram revealed a pronounced peak at a retention time of 13 minutes (Figure 22A). Interestingly, the UV spectrum shown in this peak exhibited an absorption maximum of 214 nm, which suggested a limited number of conjugated bonds in the compounds present. Notably, this compound was found exclusively in the stem bark extract, not in the leaf extract.

In the other phases analysed, two compounds denoted as **d** were observed (Figure 22B-D). When compared to a database of known compounds, it was found that their UV spectra closely resembled derivatives of the iridoid daphyloside or other closely related compounds. Daphyloside iridoids are a subclass of secoiridoid glycosides with a disaccharide sugar moiety attached to the iridoid aglycone via a glycosidic linkage (Lopes et al., 2004). Although no reports of daphyloside iridoids have been found, other studies have focused on other iridoid compounds within *Psychotria* species. In different species belonging to the *Psychotriaeae* tribe native to Brazil, three iridoid glucosides (loganin, asperuloside, and deacetyl asperuloside) were identified in the species *Palicourea rigida, P. kleinii, and P. leiocarpa*, respectively (Lopes et al., 2004).

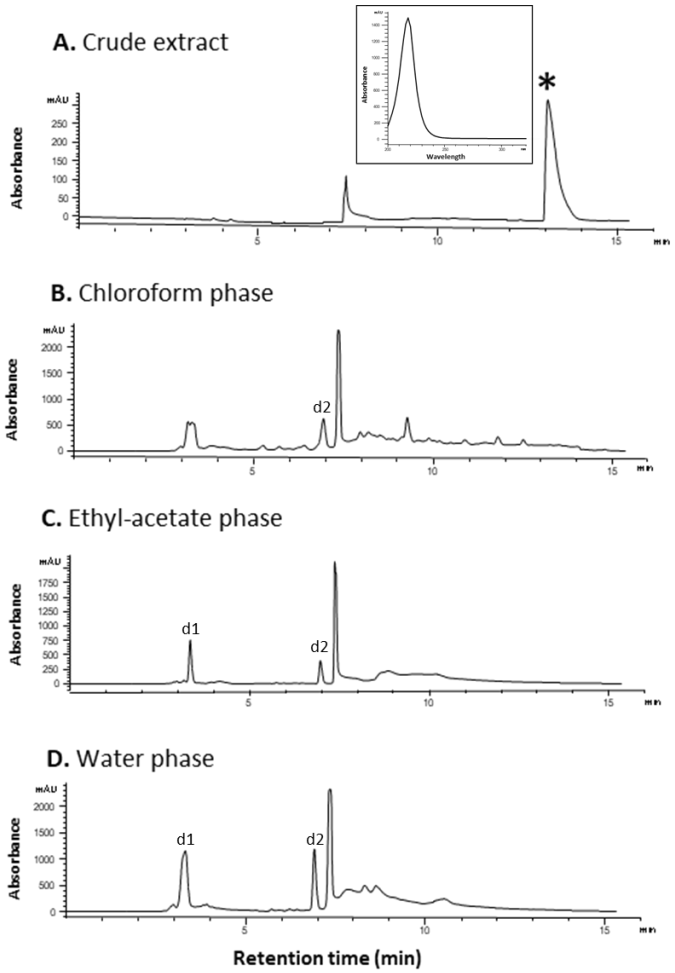

Figure 22 HPCL chromatograms at 230 nm of the crude stem bark extract obtained by liquid-liquid extraction at 230 nm up approximately 15 minutes. A. crude extract. B. chloroform phase. C. ethyl-acetate phase. D. Water phase. An unknown compound was detected at a retention time of 13 minutes (*) in the crude extract. A zoom-in of its UV spectrum (rectangle) at an absorption maximum of 214 nm, no similar compounds were found in the database. Compounds marked as c1 and c2 have UV spectra similar to the iridoid daphyloside derivatives or closely related compounds.

4.3. Comparison of the leaf and the stem bark extracts

When comparing crude leaf and stem extracts, differences are observed between the two extracts. In the leaf extract, a prominent peak is observed, presumably corresponding to a catechin derivative (Figure 23A). In the stem extract, traces of acetone and a prominent peak are observed, suggesting the presence of conjugated double bonds present in this extract.

Both extracts showed Dragendorff's positive spots on silica gel TLC plates, which remained at the starting point even when developed in solvent systems such as $CHCl_3$:MeOH (80:20). This solvent system was selected because of its previous use to separate tryptamine-derived alkaloid glucosides like strictosidine and related compounds in the *Psychotria* alliance (Berger et al., 2016). HPLC profiles performed for the *Eumachia* extracts show no similarity with related

species belonging to the *Psychotria* alliance. Moreover, none of the peaks detectable with this method showed similarities to tryptamine-derived alkaloids.

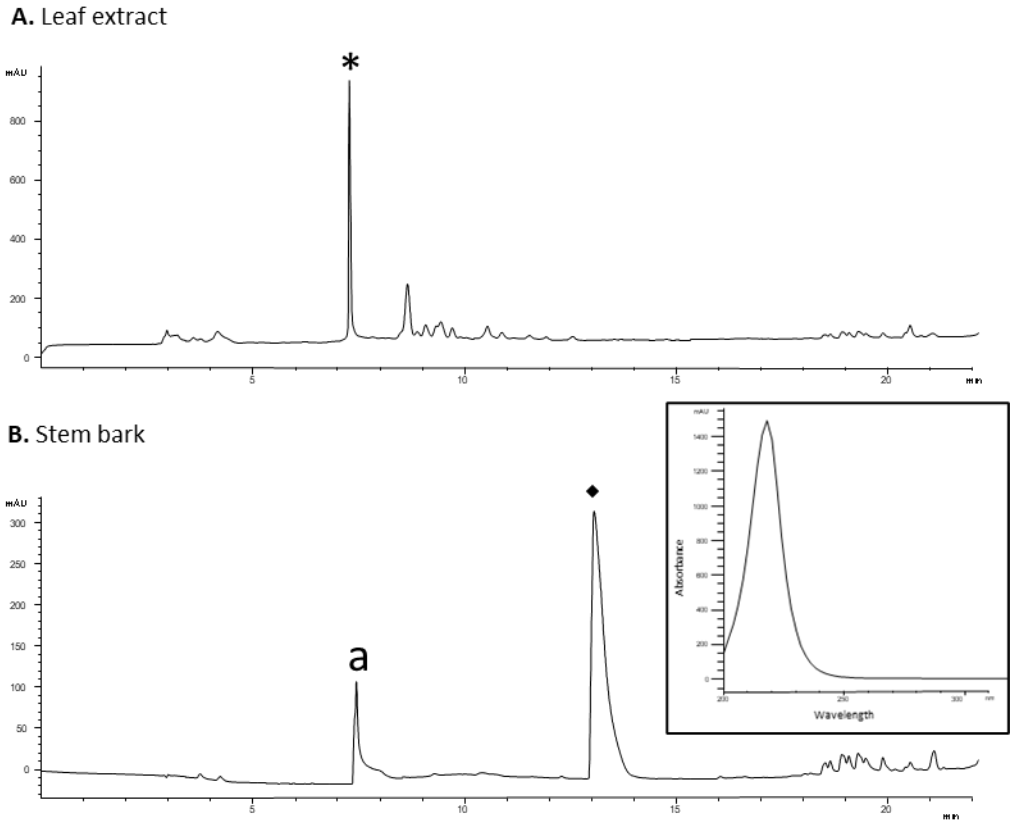

Figure 23 Comparative HPLC chromatograms at 230 nm from 0 to 20 minutes approximately of leaf and stem bark methanolic liquid-liquid extraction. A. Leaf crude extract. The peak at 7.2 minutes corresponds to the presumable catechin derivative (*) (See Figure 18) B. Stem bark crude extract. Traces of acetone (a) were detected, and the zoon in of the major peak (•) observable in the chromatogram (rectangle) shows a UV max of 218 nm, suggesting a limited number of conjugated double bonds present in these compounds.

4.4. Discussion of results compared to literature data

The *Psychotria* alliance, a group of plants within the Rubiaceae family, is known for its diverse array of phenolic compounds and flavonoids (Panche et al., 2016). In this study, catechin-derived compounds were observed in the leaf extract of *Eumachia,* which is consistent with studies conducted on *Psychotria* species. Research on *Psychotria nervosa* leaves revealed high concentrations of catechins or related polyphenols and the isolation of the flavone apigenin 7-*O*-rhamnoglucoside (Berger, 2012; Berger & Schinnerl, 2019). Chromatographic analyses performed by Berger and Schinnerl (2019) have also detected peaks corresponding to catechins and their derivatives in several *Psychotria* species. As a result of previous analyses, it has been determined that tannins are present within the compounds. Still, finding no nitrogen does not ensure that alkaloids are absent within the compounds (Berger &

Schinnerl, 2019). It is evident from these findings that catechins and related compounds are present in plants of the Rubiaceae family but are not necessarily present in all of them.

Limited studies on *Eumachia*'s secondary metabolites have been done, and until now, there is only one report on the isolation and identification of flavonoids within the species. In their study, Nascimiento and collaborators (2015) isolated six flavonols from *E. depauperata* (formerly *Margaritopsis carrascoana*), four of them from the leaves and the remaining two from the stem (Table 4). The compounds isolated from the stem correspond to flavonoids of the flavone glycoside class, which in their structure have luteolin as an aglycone and a sugar attached by glycosidic bonds to the hydroxyl group in the seventh position of the luteolin molecule.

Table 4 Flavonoid compounds found in different *Eumachia* species reported by Nascimento and collaborators (2015). The species name is shown as reported and as accepted

Accepted species	Reported under	Geographic Location	Plant organ	Crude extract amount (g)	Compound isolated	Amount (mg)
E. depauperata	*Margaritopsis carrascoana*	Brazil	Stems	295.5	Dehydrodiconiferyl alcohol 4-O-β-D-glucopyranoside	6,7
					Luteolin 7-O-[β-L-rhamnopyranosyl-(1→2)-β-D-glycopyranosyl]	5,3
			Leaves	118.5	Luteolin 7-*O*-[β-L-rhamnopyranosy-(1→2)-β-D-glycopyranosyl]	11,2
					Luteolin 7-O-[β-D-apiofuranosyl-(1→6)]-β-D-glucopyranoside	13,3
					Luteolin 7-O-[β-D-apiofuranosyl-(1→6)]-β-D-glucopyranoside	5,1
					Luteolin 7-O-{α-L-rhamnopyranosyl-(1→6)-[β-L-rhamnopyranosyl- (1→2)]-β-D-glucopyranosyl}	2,6

In the few studies conducted on *Eumachia* chemodiversity, it has been found to contain indole alkaloids such as polypyroloindoline alkaloids (see Table 5). These compounds accumulate as dimers such as (+)-chimonanthine and (-)-calycanthine (Berger et al., 2021b; Jamison et al., 2017). According to the results obtained here, the presence of tryptamine-derived alkaloids like strictosidine derivatives or chimonanthine derivatives cannot be confirmed. Studies have been done on different *Eumachia* species native to Malaysia, New Caledonia, and Vanuatu, and only two reports were found for *Eumachia cymuligera* and *Eumachia depauperata,* both indigenous species from Brazil (Table 5).

Pyrrolidinoindolines are also found in a few *Palicourea* and *Psychotria* species from Asia and the Pacific (Berger et al., 2021b). From the methanolic extract of leaves and stem bark of *Palicourea acuminata* (Benth.), it was able to isolate seven compounds, among them lagamboside, a novel compound that is characterised by the unusual pattern of *N*-glycosylation; and the co-occurrence of the rare alkaloid bahienoside, which comprises two

iridoids structures. The specific distribution of these alkaloid derivatives within the *Psychotria* alliance indicated chemotaxonomic significance (Berger, 2012; Calixto et al., 2016).

Table 5 *Eumachia* species from which alkaloid compounds were isolated. The different species names are shown as reported and as accepted. (*) Amounts not given. (★) amounts are given as w/w%. (♦)Location not reported.

Accepted species	Reported under	Geographic Location	Plant organ	Crude extract amount (g)	Compound isolated	Amount (mg)	References
E. cymuligea	*Margaritopsis cymuligea*	Brazil	Leaves	215	Quadrigemines B	*	(Brand et al., 2012)
					Hodgkinside	*	
E. depauperata	*Margaritopsis carrascoana*	Brazil	Aerial parts	295.5	Calycosidine	50,6	(Nascimento et al., 2015)
					Hodgkinsine	30,2	
					N*8 "formyl calycosidine	4,2	
					N-8"-methyl-N1'-dimethyl iso-calycosidine	5,6	
E. forsteriana		Vanuatu	Stem bark	*	Calycanthine	*	(Adjibade et al., 1985)
		Vaté Island (Vanuatu)	Fruits	*	Hodgkinsine	*	(Adjibade et al., 1989)
		Vanuatu	Dried stem bark	*	(-)-calycanthine	0,12%★	(Adjibade et al., 1992)
					Iso-calycanthine	0,036%★	
					Meso-chimonanthine	0,0097%★	
			Dried fruits	*	(-)-calycanthine	0,0036%★	
					Iso-calycanthine	0,004%★	
					Meso-chimonanthine	0,0097%★	
E. frutescens	*Hodgkinsonia frutescens*	Queensland	Leaves	*	Hodgkinsine	*	(Anet et al., 1961)
E. laptothyrsa	*Psychotria lyciiflora*	♦	Leaves	60	Hodgkinsine	*	(Fridrichsons et al., 1974)
E. lyciiflora	*Psychotria lyciiflora*	New Caledonia	Leaves	13	Meso-chimonanthine	*	(Jannic et al., 1999)
					Nb-desmethyl-*meso*-chimonanthine	*	
					Hodgkinsine	*	
E. oleoides	*Psychotria oleoides*	New Caledonia	Leaves		Hodgkinsine	90	
					Quadrigemine C	*	
					Isopsychotridine B	*	
					Psychotridine	*	
					Quadrigemine I	*	
					Oleoidine	*	
					Caledonine	*	
E. straminea		Malaysia	Dried bark and twinks	*	(-)-calycanthine	90	(Lajis et al., 1993)
					(+)-chimonanthine	33	
					Calycosidine	15	
					Hodgkinsine	10	
E. rostata	*Psychotria rostata*	Malaysia	Leaves	23	Psychotrimine	21	(Takayama et al., 2004)
					Psychopentamine	5,4	

One of the reasons why it was not possible to isolate alkaloids from *Eumachia microdon* may be due to the species is native to Costa Rica and does not face predators that can attack the plant, which means it does not require producing defence compounds regionally (Brand et al., 2012; Jannic et al., 1999; Nascimento et al., 2015). Additionally, the production of secondary metabolites may be linked to a specific region's available resources and environmental conditions (Levin, 1976). This suggests that the absence of these compounds in *E. microdon* may be due to regional differences, as the species studied in another research were collected in the South Pacific region. In fact, previous studies by Brand et al. (2012), Jannic et al. (1999), and Nascimento et al. (2015) analysed species from the South Pacific region, further supporting the idea of regional chemotypes.

In addition, the presence of indole alkaloids could be seasonal and related to ontogenesis. Such a phenomenon has been observed in *Psychotria brachyceras* in Brazil, where bioactive indole alkaloids are present in lower concentrations in summer compared to the other seasons, and the highest accumulation is found in the flowers. This accumulation may be related to the defence role of the alkaloids against predators and contribute to the success of fertilisation and seed production (Gregianini et al., 2004).

The extraction and isolation methods of alkaloid compounds are the same as those used in several studies for the same purpose. However, isolating alkaloids following the same methodology described by other authors was not possible. It could be inferred that such compounds are not present in *Eumachia microdon*, and further studies should be done to expand the knowledge of secondary metabolites among *Eumachia* species.

4.5. Recommendations for further studies

Although the information on secondary metabolites in species within the genus *Eumachia* is limited, this study has provided valuable initial information on compounds that can be found in *Eumachia microdon*, and further research could be carried out to expand on these findings.

For example, other different organs of the plant, such as roots or seeds, could be analysed. In *Psychotria succulenta* 13 secondary metabolites were isolated, some of them with antibacterial activity, including four alkaloids, three triterpenes, one steroid, four phenolic compounds and one iridoid (Jouogo et al., 2022).

In addition, comparisons could be made between crude extracts of individuals within a population and between populations to compare different individuals/populations of the same species to determine if there are differences in the production of secondary metabolites. In this

study, only one specimen of *E. microdon* was studied, so there were no comparisons with other individuals.

Furthermore, the production of secondary metabolites, especially alkaloids, may be linked to the developmental stages of the plant, so studies of the different stages of development could be relevant, such as flowering or fruiting.

The present study on *Eumachia* species has shed some light on their secondary metabolites. However, there are still avenues for further research that could contribute to a more comprehensive understanding of the plant. One way to achieve this is to investigate the presence of other classes of secondary metabolites, such as terpenoids or phenolics. These may have potential bioactive properties not detected in the current study. Another promising area of research is investigating the role of alkaloids in *Eumachia* species, which could provide insights into their defensive functions or signalling effects. Further research on *Eumachia* species could provide valuable information for discovering new natural products with potential therapeutic applications.

5. ABSTRACT

The genus *Eumachia* is classified within the *Psychotria* alliance, well known to accumulate indole alkaloids. Due to the complex taxonomic classification, chemodiversity studies have primarily focused on the genera *Psycotria* and *Palicourea* (Taylor et al., 2017). This study was intended to check the presence of alkaloid compounds in crude extracts of the leaves and stems bark of *Eumachia microdon* (D.C.) Delprete & J.H.Kirkbr. The extracts were analysed using chromatographic techniques such as MPLC, CC, TLC, and ion-exchange chromatography. The samples were analysed by HPCL, and by comparing the peaks of the HPLC profiles with the compounds' UV spectra database. Most of the secondary metabolites were found in the leaf extract, but none of the compounds corresponded to the known alkaloids normally present in this alliance. Among the phenolic compounds, two derivatives of quercetin and kaempferol were observed in the crude extract and two phenylpropanoids with UV spectra, similar to caffeic acid. In the same extract, an unknown peak could be identified as a catechin derivative compared to the compounds' database. Flavone C-glycosides, probably apigenin derived, were tentatively identified after acidic hydrolysis extraction was performed for the leaf extract, both in the lipophilic and hydrophilic phases. These glycosides were non-hydrolysable as typical for C-glycosides, as they undergo a Wessely-Moser rearrangement leading to isomeric structures as were found here. The presence of alkaloids could not be confirmed, although three spots reacted to the Dragendorff reagent as apparently false positives. Furthermore, they showed no migration on the TLC plate. Also, no corresponding compounds could be detected by comparative HPLC analysis. The lack of evidence for the presence of tryptamine-derived alkaloids (such as strictosidine) or chimonanthine may be linked to regional chemotypes or to different responses to biotic and abiotic stresses (Nascimento et al., 2015; Taylor et al., 2017).

6. ZUSAMMENFASSUNG

Die Gattung *Eumachia* wird der *Psychotria*-Allianz zugeordnet, die dafür bekannt ist, Indolalkaloide zu akkumulieren. Aufgrund der komplexen taxonomischen Einordnung haben sich Chemodiversitätsstudien hauptsächlich auf die Gattungen *Psychotria* and *Palycourea* fokussiert (Taylor et al., 2017).

In dieser Arbeit werden die Alkaloidverbindungen in Rohextrakten von Blättern und Stängelrinde von *Eumachia microdon* analysiert. Die Extrakte wurden mit chromatografischen Techniken wie MPLC, CC, TLC und Ionenaustauschchromatografie gemessen. Die Proben wurden mittels HPCL analysiert, wobei die HPLC-Profile mit der Datenbank der UV-Spektren verglichen wurden.

Viele der sekundären Metaboliten wurden im Blattextrakt gefunden. Unter den phenolischen Verbindungen wurden im Rohextrakt zwei Derivate von Quercetin und Kämpferol sowie zwei Phenylpropanoide mit UV-Spektren, die der Kaffeesäure ähneln, festgestellt. Im selben Extrakt konnte ein unbekannter Peak im Vergleich zur Datenbank als Catechin-Derivat identifiziert werden.

Bei der sauren Hydrolyse-Extraktion des Blattextrakts wurden Flavonglykoside sowohl in der lipophilen als auch in der hydrophilen Phase gefunden. Es wird vermutet, dass das Vorhandensein von Flavonglykosiden auf Wessely-Moser-Umlagerungen zurückzuführen ist, bei denen nach der Hydrolyse ein Zuckermolekül abgespalten wird und ein anderes an das Flavonoid gebunden bleibt (Dou et al., 2015).

Was die Isolierung von Alkaloiden betrifft, so war es nicht möglich, sie zu isolieren; drei falsch positive Substanzen reagierten auf das Dragendorff-Reagenz und wanderten nicht durch die TLC-Platte. Die vergleichenden Analysen der Blatt- und Stammextrakte ergaben, dass das Vorhandensein von Tryptamin-Alkaloiden (wie Strictosidin) oder Chimonanthin-Derivaten nicht bestätigt werden konnte. Das Vorhandensein dieser Verbindungen könnte mit regionalen Chemotypen zusammenhängen (Brand et al., 2012; Nascimento et al., 2015).

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8. BIBLIOGRAPHY

Abdallah, I. I., & Quax, W. J. (2017). A Glimpse into the Biosynthesis of Terpenoids. *KnE Life Sciences*, *3*(5), 81–98. https://doi.org/10.18502/kls.v3i5.981

Adedokun, K. A., Imodoye, S. O., Bello, I. O., Lanihun, A.-A., & Bello, I. O. (2023). Therapeutic potentials of medicinal plants and significance of computational tools in anti-cancer drug discovery. In *Phytochemistry, Computational Tools and Databases in Drug Discovery* (pp. 393–455). Elsevier. https://doi.org/10.1016/B978-0-323-90593-0.00017-4

Aldred, E. M. (2008). Terpenes. In Aldred E. M., Buck C., & Vall K. (Eds.), *Pharmacology* (pp. 167–174). Churchill Livingstone. https://doi.org/10.1016/B978-0-443-06896-0.00022-0

Anadón, A., Martínez-Larrañaga, M. R., Ares, I., & Martínez, M. A. (2016). Interactions between Nutraceuticals/Nutrients and Therapeutic Drugs. In *Nutraceuticals: Efficacy, Safety and Toxicity* (pp. 855–874). Academic Press. https://doi.org/10.1016/B978-0-12-802147-7.00060-7

Aniszewski, T. (2007). Alkaloids - Secrets of life. Alkaloid chemistry, biological significance, applications and ecological role. In *Alkaloids: Chemistry, biology, ecology and applications. Elsevier* (1st ed.). Elsevier.

Badshah, S. L., Faisal, S., Muhammad, A., Poulson, B. G., Emwas, A. H., & Jaremko, M. (2021). Antiviral activities of flavonoids. *Biomed. Pharmacother.*, *140*, 1–30. https://doi.org/10.1016/j.biopha.2021.111596

Ballard, C. R., & Maróstica, M. R. (2019). Health Benefits of Flavonoids. In M. R. Segura Campos (Ed.), *Bioactive Compounds* (pp. 185–201). Elsevier. https://doi.org/10.1016/B978-0-12-814774-0.00010-4

Barrabé, L. (2013). *Systématique et Evolution du genre Psychotria (Rubiaceae) en Nouvelle-Calédonie* [Doctoral thesis]. Université de la Nouvelle-Calédonie.

Berger, A. (2012). *Distribution and systematic significance of selected secondary metabolites within Psychotrieae/Palicoureeae (Rubiaceae)* [Bachelor thesis]. University of Vienna.

Berger, A., Kostyan, M. K., Klose, S. I., Gastegger, M., Lorbeer, E., Brecker, L., & Schinnerl, J. (2015). Loganin and secologanin derived tryptamine-iridoid alkaloids from *Palicourea crocea* and *Palicourea padifolia* (Rubiaceae). *Phytochemistry*, *116*(1), 162–169. https://doi.org/10.1016/j.phytochem.2015.05.013

Berger, A., Preinfalk, A., Robien, W., Brecker, L., Valant-Vetschera, K., & Schinnerl, J. (2016). New reports on flavonoids, benzoic- and chlorogenic acids as rare features in the *Psychotria* alliance (Rubiaceae). *Biochem. Syst. Ecol.*, *66*, 145–153. https://doi.org/10.1016/j.bse.2016.02.027

Berger, A., & Schinnerl, J. (2019). Taxonomical and phytochemical diversity of Costa Rica *Palicoureeae* and *Psychotrieae* (Rubiaceae). *Acta ZooBot Austria*, *156*, 231–248.

Berger, A., Valant-Vetschera, K., Schinnerl, J., & Brecker, L. (2022a). A revised classification of the sister tribes *Palicoureeae* and *Psychotrieae* (Rubiaceae) indicates genus-specific alkaloid accumulation. In *Phytochemistry Reviews* (Vol. 21, Issue 3, pp. 941–986). Springer Science and Business Media B.V. https://doi.org/10.1007/s11101-021-09769-x

Bergman, M. E., Davis, B., & Phillips, M. A. (2019). Medically Useful Plant Terpenoids: Biosynthesis, Occurrence, and Mechanism of Action. *Molecules* , *24*(21), 3961. https://doi.org/10.3390/MOLECULES24213961

Bhattacharya, A., Sood, P., & Citovsky, V. (2010). The roles of plant phenolics in defence and communication during *Agrobacterium* and *Rhizobium* infection. *Mol. Plant Pathol.*, *11*(5), 705–719. https://doi.org/10.1111/J.1364-3703.2010.00625.X

Bourgeois, K., & Eggleston, W. (2023). Yohimbine. *Reference Module in Biomedical Sciences*. https://doi.org/10.1016/B978-0-12-824315-2.00564-9

Bremei, B. (2009). A review of molecular phylogenetic studies of Rubiaceae. *Annals of the Missouri Botanical Garden*, *96*(1), 4–26. https://doi.org/10.3417/2006197

Bremer, B., & Eriksson, T. (2009). Time tree of Rubiaceae: Phylogeny and dating the family, subfamilies, and tribes. *International Journal of Plant Sciences*, *170*(6), 766–793. https://doi.org/10.1086/599077

Bremer, B., & Manen, J. F. (2000). Phylogeny and classification of the subfamily Rubioideae (Rubiaceae). *Plant Systematics and Evolution*, *225*(1–4), 43–72. https://doi.org/10.1007/BF00985458

Calixto, N. O., Pinto, M. E. F., Ramalho, S. D., Burger, M. C. M., Bobey, A. F., Young, M. C. M., Bolzani, V. S., & Pinto, A. C. (2016). The genus *Psychotria*: Phytochemistry, chemotaxonomy, ethnopharmacology and biological properties. In *Journal of the Brazilian*

Chemical Society (Vol. 27, Issue 8, pp. 1355–1378). Sociedade Brasileira de Quimica. https://doi.org/10.5935/0103-5053.20160149

Carbajal-Valenzuela, I. A., Apolonio–Hernandez, N. M., Gutierrez-Chavez, D. V., González-Arias, B., Jimenez-Hernandez, A., torres-Pacheco, I., Rico-García, E., Feregrino-Pérez, A. A., & Guevara-González, R. G. (2022). Biological macromolecules as nutraceuticals. In *Biological Macromolecules* (pp. 97–138). Elsevier. https://doi.org/10.1016/B978-0-323-85759-8.00001-4

Céspedes, C. L., Marín, J. C., Domínguez, M., Avila, J. G., & Serrato, B. (2006). Plant growth inhibitory activities by secondary metabolites isolated from Latin American flora. In *Advances in Phytomedicine* (Vol. 2, Issue C, pp. 373–410). Elsevier. https://doi.org/10.1016/S1572-557X(05)02021-0

Ciorîță, A., Zăgrean-tuza, C., Moț, A. C., Carpa, R., & Pârvu, M. (2021). The phytochemical analysis of *Vinca* l. Species leaf extracts is correlated with the antioxidant, antibacterial, and antitumor effects. *Molecules*, *26*(10). https://doi.org/10.3390/molecules26103040

Courdavault, V., Papon, N., Clastre, M., Giglioli-Guivarc'h, N., St-Pierre, B., & Burlat, V. (2014). A look inside an alkaloid multisite plant: the *Catharanthus* logistics. *Curr. Opin. Plant Biol.*, *19*, 43–50. https://doi.org/10.1016/j.pbi.2014.03.010

Davis, A. P., Govaerts, R., Bridson, D. M., Ruhsam, M., Moat, J., & Brummitt, N. A. (2009). A Global assessment of distribution, diversity, endemism, and taxonomic effort in the Rubiaceae. *Annals of the Missouri Botanical Garden*, *96*(1), 68–78. https://doi.org/10.3417/2006205

de Carvalho, A. R., de Carvalho, M. G., Braz-Filho, R., & Vieira, I. J. C. (2016). *Psychotria* Genus: Chemical Constituents, Biological Activities, and Synthetic Studies. In *Studies in Natural Products Chemistry* (Vol. 48, pp. 231–261). Elsevier B.V. https://doi.org/10.1016/B978-0-444-63602-7.00007-2

De Luca, V., Salim, V., Thamm, A., Masada, S. A., & Yu, F. (2014). Making iridoids/secoiridoids and monoterpenoid indole alkaloids: progress on pathway elucidation. *Curr. Opin. Plant Biol.*, *19*, 35–42. https://doi.org/10.1016/j.pbi.2014.03.006

Delprete, P. G., & Kirkbride, J. H. (2015). New combinations in *Eumachia* (Rubiaceae) for species occurring on the guiana shield. *J. Bot. Res. Inst. Tex.*, *9*(1), 75–79. https://www.jstor.org/stable/24621246

Dudley, Q. M., Jo, S., Guerrero, D. A. S., Chhetry, M., Smedley, M. A., Harwood, W. A., Sherden, N. H., O'Connor, S. E., Caputi, L., & Patron, N. J. (2022). Reconstitution of monoterpene indole alkaloid biosynthesis in genome engineered *Nicotiana benthamiana*. *Commun. Biol.*, *5*(1), 1–12. https://doi.org/10.1038/s42003-022-03904-w

Elshafie, H. S., Camele, I., & Mohamed, A. A. (2023). A Comprehensive Review on the Biological, Agricultural and Pharmaceutical Properties of Secondary Metabolites Based-Plant Origin. *Int. J. Mol. Sci.*, *24*(4), 3266. https://doi.org/10.3390/IJMS24043266

Falcone Ferreyra, M. L., Rius, S. P., & Casati, P. (2012). Flavonoids: biosynthesis, biological functions, and biotechnological applications. *Front. Plant Sci.*, *3*. https://doi.org/10.3389/fpls.2012.00222

Fu, Y., Huang, L., Wang, X., Li, X., Li, K., Wu, S., & Liu, Y. (2015). Studies on chemical constituents from *Psychotria straminea*. *Chin. Med. J.*, *40*(11), 2138–2143.

Gimeno Creus, E. (2004). Compuestos fenólicos. Un análisis de sus beneficios para la salud. *Offarm*, *23*(6), 80–84. https://www.elsevier.es/es-revista-offarm-4-articulo-compuestos-fenolicos-un-analisis-sus-13063508

Graf, B. A., Milbury, P. E., & Blumberg, J. B. (2005). Flavonols, Flavones, Flavanones, and Human Health: Epidemiological Evidence. *J. Med. Food*, *8*(3), 281–290. https://doi.org/10.1089/jmf.2005.8.281

Grujic, S., Radojevic, I., Vasic, S., Comic, L., & Topuzovic, M. (2014). Antimicrobial activity and some phytochemical analysis of two extracts *Vinca minor* L. *Kragujevac Journal of Science*, *36*, 145–154. https://doi.org/10.5937/kgjsci1436145g

Gutiérrez-Grijalva, E. P., López-Martínez, L. X., Contreras-Angulo, L. A., Elizalde-Romero, C. A., & Heredia, J. B. (2020). Plant alkaloids: Structures and bioactive properties. In *Plant-derived Bioactives: Chemistry and Mode of Action* (pp. 85–117). Springer Singapore. https://doi.org/10.1007/978-981-15-2361-8_5

Hamerski, L., Bomm, M. D., Silva, D. H. S., Young, M. C. M., Furlan, M., Eberlin, M. N., Castro-Gamboa, I., Cavalheiro, A. J., & Da Silva Bolzani, V. (2005). Phenylpropanoid glucosides from leaves of *Coussarea hydrangeifolia* (Rubiaceae). *Phytochem.*, *66*(16), 1927–1932. https://doi.org/10.1016/j.phytochem.2005.06.019

Heinrich, M., Mah, J., & Amirkia, V. (2021). Alkaloids used as medicines: Structural phytochemistry meets biodiversity—An update and forward look. In *Molecules* (Vol. 26, Issue 7). MDPI AG. https://doi.org/10.3390/molecules26071836

Hostetler, G. L., Ralston, R. A., & Schwartz, S. J. (2017). Flavones: Food Sources, Bioavailability, Metabolism, and Bioactivity. *Advances in Nutrition*, *8*(3), 423–435. https://doi.org/10.3945/an.116.012948

Hussein, R. A., & El-Anssary, A. A. (2019). Plants Secondary Metabolites: The Key Drivers of the Pharmacological Actions of Medicinal Plants. In *Herbal Medicine*. IntechOpen. https://doi.org/10.5772/intechopen.76139

Imran, M., Rauf, A., Abu-Izneid, T., Nadeem, M., Shariati, M. A., Khan, I. A., Imran, A., Orhan, I. E., Rizwan, M., Atif, M., Gondal, T. A., & Mubarak, M. S. (2019). Luteolin, a flavonoid, as an anticancer agent: A review. *Biomedicine & Pharmacotherapy*, *112*, 108612. https://doi.org/10.1016/j.biopha.2019.108612

Jamison, C. R., Badillo, J. J., Lipshultz, J. M., Comito, R. J., & MacMillan, D. W. C. (2017). Catalyst-controlled oligomerization for the collective synthesis of polypyrroloindoline natural products. *Nat. Chem.*, *9*(12), 1165–1169. https://doi.org/10.1038/nchem.2825

Jan, R., Asaf, S., Numan, M., Lubna, & Kim, K. M. (2021a). Plant secondary metabolite biosynthesis and transcriptional regulation in response to biotic and abiotic stress conditions. In *Agronomy* (Vol. 11, Issue 5). MDPI AG. https://doi.org/10.3390/agronomy11050968

Jannic, V., Guéritte, F., Laprévote, O., Serani, L., Martin, M. T., Sévenet, T., & Potier, P. (1999). Pyrrolidinoindoline alkaloids from *Psychotria oleoides* and *Psychotria lyciiflora*. *J. Nat. Prod.*, *62*(6), 838–843. https://doi.org/10.1021/np9805387

Katerova, Z. (2012). *Influence of ultraviolet radiation on plant secondary metabolite production Cytokinin oxidase/dehydrogenase-impact of some growth regulators and abiotic stress factors on the enzyme activity View project*. http://www.ifrg-bg.com

Keglevich, P., Hazai, L., Kalaus, G., & Szántay, C. (2012). Modifications on the Basic Skeletons of Vinblastine and Vincristine. *Molecules*, *17*(5), 5893. https://doi.org/10.3390/MOLECULES17055893

Kulagina, N., Méteignier, L.-V., Papon, N., O'Connor, S. E., & Courdavault, V. (2022). More than a *Catharanthus* plant: A multicellular and pluri-organelle alkaloid-producing factory. *Curr. Opin. Plant Biol.*, *67*, 102200. https://doi.org/10.1016/j.pbi.2022.102200

Kumar, N., & Goel, N. (2019). Phenolic acids: Natural versatile molecules with promising therapeutic applications. In *Biotechnology Reports* (Vol. 24). Elsevier B.V. https://doi.org/10.1016/j.btre.2019.e00370

Lattanzio, V. (2013). Phenolic compounds: Introduction. In *Natural Products: Phytochemistry, Botany and Metabolism of Alkaloids, Phenolics and Terpenes* (pp. 1543–1580). Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-642-22144-6_57

Li, S. S., Wu, J., Chen, L. G., Du, H., Xu, Y. J., Wang, L. J., Zhang, H. J., Zheng, X. C., & Wang, L. S. (2014). Biogenesis of C-Glycosyl Flavones and Profiling of Flavonoid Glycosides in Lotus (*Nelumbo nucifera*). *PLoS ONE*, *9*(10). https://doi.org/10.1371/JOURNAL.PONE.0108860

Lin, C.-Z., Wu, A.-Z., Zhong, Y., Wang, Y.-M., Peng, G.-T., Su, X.-J., Liu, B.-X., Deng, Y., Zhu, C.-C., & Zhang, C.-X. (2015). Flavonoids from *Psychotria serpens* L., a Herbal Medicine with Anti-Cancer Activity. *J. Can. Res. Updates*, *4*, 60–64.

Liu, R. H. (2013). Dietary Bioactive Compounds and Their Health Implications. *J. Food Sci.*, *78*(s1), A18–A25. https://doi.org/10.1111/1750-3841.12101

Liu, W., Feng, Y., Yu, S., Fan, Z., Li, X., Li, J., & Yin, H. (2021). The Flavonoid Biosynthesis Network in Plants. *Plants. Int. J. Mol. Sci*, *22*(23), 12824. https://doi.org/10.3390/ijms222312824

Liu, X. Y., & Qin, Y. (2022). Recent advances in the total synthesis of monoterpenoid indole alkaloids enabled by asymmetric catalysis. *GSC*, *3*(1), 25–39. https://doi.org/10.1016/J.GRESC.2021.10.009

Lopes, S., von Poser, G. L., Kerber, V. A., Farias, F. M., Konrath, E. L., Moreno, P., Sobral, M. E., Zuanazzi, J. A. S., & Henriques, A. T. (2004). Taxonomic significance of alkaloids and iridoid glucosides in the tribe *Psychotrieae* (Rubiaceae). *Biochemical Systematics and Ecology*, *32*(12), 1187–1195. https://doi.org/10.1016/j.bse.2004.04.015

Lou, H., Hu, L., Lu, H., Wei, T., & Chen, Q. (2021). Metabolic Engineering of Microbial Cell Factories for Biosynthesis of Flavonoids: A Review. *Molecules*, *26*(15), 4522. https://doi.org/10.3390/molecules26154522

Lu, H. X., Liu, L. Y., Li, D. P., Li, J. Z., & Xu, L. C. (2014). A new iridoid glycoside from the root of *Psychotria rubra*. *Biochem. Syst. Ecol.*, *57*, 133–136. https://doi.org/10.1016/J.BSE.2014.07.024

Mabry, T. J., Markham, K. R., & Thomas, M. B. (1970). The Aglycone and Sugar Analysis of Flavonoid Glycosides. In *The Systematic Identification of Flavonoids* (pp. 23–32). Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-88458-0_3

Mačiulaitis, R., Kontrimavičiute, V., Bressolle, F. M. M., & Briedis, V. (2008). Ibogaine, an anti-addictive drug: Pharmacology and time to go further in development. A narrative review. In *Human and Experimental Toxicology* (Vol. 27, Issue 3, pp. 181–194). https://doi.org/10.1177/0960327107087802

Maeda, H., & Dudareva, N. (2012). The shikimate pathway and aromatic amino acid biosynthesis in plants. In *Annual Review of Plant Biology* (Vol. 63, pp. 73–105). https://doi.org/10.1146/annurev-arplant-042811-105439

Magnani, C., Isaac, V. L. B., Correa, M. A., & Salgado, H. R. N. (2014). Caffeic acid: A review of its potential use in medications and cosmetics. In *Analytical Methods* (Vol. 6, Issue 10, pp. 3203–3210). Royal Society of Chemistry. https://doi.org/10.1039/c3ay41807c

Maltese, F., Van Der Kooy, F., & Verpoorte, R. (2009). Solvent Derived Artifacts in Natural Products Chemistry. *NPC*, *4*(3), 447–454. https://doi.org/10.1177/1934578X0900400326

Martins, D., & Nunez, C. V. (2015). Secondary metabolites from Rubiaceae species. *Molecules*, *20*(7), 13422–13495. https://doi.org/10.3390/molecules200713422

Masyita, A., Mustika Sari, R., Dwi Astuti, A., Yasir, B., Rahma Rumata, N., Emran, T. Bin, Nainu, F., & Simal-Gandara, J. (2022). Terpenes and terpenoids as main bioactive compounds of essential oils, their roles in human health and potential application as natural food preservatives. *Food Chemistry: X*, *13*. https://doi.org/10.1016/j.fochx.2022.100217

Matsuura, H. N., Porto, D. D., & Fett-Neto, A. G. (2013). Bioactive alkaloids from South American *Psychotria* and related Rubiaceae. In *Natural Products: Phytochemistry, Botany*

and Metabolism of Alkaloids, Phenolics and Terpenes (pp. 119–147). Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-642-22144-6_101

Miao, Z., Zhao, Y., Chen, M., & He, C. (2022). Using flavonoids as a therapeutic intervention against rheumatoid arthritis: The known and unknown. *Pharmacological Research - Modern Chinese Medicine*, *3*, 100014. https://doi.org/10.1016/j.prmcm.2021.100014

Moha, R. (2017). Chemotherapy medication of Vincristine and Vinblastine. *JCRCT*, *1*(1), 01–02. https://doi.org/10.31579/2640-1053/007

Mohammed, A. E., Abdul-Hameed, Z. H., Alotaibi, M. O., Bawakid, N. O., Sobahi, T. R., Abdel-Lateff, A., & Alarif, W. M. (2021). Chemical Diversity and Bioactivities of Monoterpene Indole Alkaloids (MIAs) from Six Apocynaceae Genera. *Molecules*, *26*(2). https://doi.org/10.3390/MOLECULES26020488

Nascimento, R., Pimenta, A., De Lima Neto, P., Junior, J. R. C., Costa-Lotufo, L. V., Ferreira, E. G., Tinoco, L. W., Braz-Filho, R., Silveira, E. R., & Lima, M. A. S. (2015). New alkaloids from *Margaritopsis carrascoana* (Rubiaceae). *J. Braz. Chem. Soc.*, *26*(6), 1152–1159. https://doi.org/10.5935/0103-5053.20150079

Ngnokam Jouogo, D. C., Tamokou, J.-D.-D., Teponno, R. B., Matsuete-Takongmo, G., Voutquenne-Nazabadioko, L., Tapondjou, L. A., & Ngnokam, D. (2022). Chemotaxonomy and Antibacterial Activity of the Extracts and Chemical Constituents of *Psychotria succulenta* Hiern. (Rubiaceae). *BioMed Research International*, *2022*, 1–10. https://doi.org/10.1155/2022/7856305

Ninkuu, V., Zhang, L., Yan, J., Fu, Z., Yang, T., & Zeng, H. (2021). Biochemistry of terpenes and recent advances in plant protection. *Int. J. Mol. Sci.*, *22*(11). https://doi.org/10.3390/ijms22115710

O'Connor, S. E., & Maresh, J. J. (2006). Chemistry and biology of monoterpene indole alkaloid biosynthesis. In *Natural Product Reports* (Vol. 23, Issue 4, pp. 532–547). https://doi.org/10.1039/b512615k

Oldfield, E., & Lin, F. Y. (2012). Terpene biosynthesis: Modularity rules. In *Angewandte Chemie - International Edition* (Vol. 51, Issue 5, pp. 1124–1137). https://doi.org/10.1002/anie.201103110

Panche, A. N., Diwan, A. D., & Chandra, S. R. (2016). Flavonoids: An overview. In *Journal of Nutritional Science* (Vol. 5). Cambridge University Press. https://doi.org/10.1017/jns.2016.41

Peebles, C. A. M., Hong, S. B., Gibson, S. I., Shanks, J. V., & San, K. Y. (2006). Effects of terpenoid precursor feeding on *Catharanthus roseus* hairy roots over-expressing the alpha or the alpha and beta subunits of anthranilate synthase. *Biotechnol. Bioeng.*, *93*(3), 534–540. https://doi.org/10.1002/BIT.20739

Petrussa, E., Braidot, E., Zancani, M., Peresson, C., Bertolini, A., Patui, S., & Vianello, A. (2013). Plant Flavonoids—Biosynthesis, Transport and Involvement in Stress Responses. *Int. J. Mol. Sci.*, *14*(7), 14950–14973. https://doi.org/10.3390/ijms140714950

Pietta, P.-G. (2000). Flavonoids as Antioxidants. *J. Nat. Prod.*, *63*(7), 1035–1042. https://doi.org/10.1021/np9904509

Porto, D. D., Henriques, A. T., & Fett-Neto, A. G. (2009). Bioactive Alkaloids from South American *Psychotria* and Related Species. *The Open Bioactive Compounds Journal*, *2*(1), 29–36. https://doi.org/10.2174/1874847300902010029

Procházková, D., Boušová, I., & Wilhelmová, N. (2011). Antioxidant and prooxidant properties of flavonoids. *Fitoterapia*, *82*(4), 513–523. https://doi.org/10.1016/j.fitote.2011.01.018

Rauter, A. P., Lopes, R. G., & Martins, A. (2007). C-Glycosylflavonoids: Identification, bioactivity and synthesis. *NPC*, *2*(11), 1175–1196.

Razafimandimbison, S. G., Rydin, C., & Bremer, B. (2008). Evolution and trends in the *Psychotrieae* alliance (Rubiaceae)-A rarely reported evolutionary change of many-seeded carpels from one-seeded carpels. *Molecular Phylogenetics and Evolution*, *48*(1), 207–223. https://doi.org/10.1016/j.ympev.2008.03.034

Salehi, B., Venditti, A., Sharifi-Rad, M., Kręgiel, D., Sharifi-Rad, J., Durazzo, A., Lucarini, M., Santini, A., Souto, E., Novellino, E., Antolak, H., Azzini, E., Setzer, W., & Martins, N. (2019). The Therapeutic Potential of Apigenin. *International Journal of Molecular Sciences*, *20*(6), 1305. https://doi.org/10.3390/ijms20061305

Seigler, D. S. (1998). Flavonoids. In *Plant Secondary Metabolism* (pp. 151–192). Springer Science+Business Media. https://doi.org/10.1007/978-1-4615-4913-0_11

Shen, N., Wang, T., Gan, Q., Liu, S., Wang, L., & Jin, B. (2022). Plant flavonoids: Classification, distribution, biosynthesis, and antioxidant activity. *Food Chemistry*, *383*, 132531. https://doi.org/10.1016/j.foodchem.2022.132531

Shukla, S., & Gupta, S. (2010). Apigenin and cancer chemoprevention. *Bioactive Foods in Promoting Health*, 663–689. https://doi.org/10.1016/B978-0-12-374628-3.00041-4

Singh, B., & Sharma, R. A. (2015). Plant terpenes: defense responses, phylogenetic analysis, regulation and clinical applications. *3 Biotech*, *5*(2), 129–151. https://doi.org/10.1007/s13205-014-0220-2

Soto, M. L., Falqué, E., & Domínguez, H. (2015). Relevance of natural phenolics from grape and derivative products in the formulation of cosmetics. *Cosmetics*, *2*(3), 259–276. https://doi.org/10.3390/cosmetics2030259

Srivastav, V. K., Egbuna, C., & Tiwari, M. (2019). Plant secondary metabolites as lead compounds for the production of potent drugs. In *Phytochemicals as Lead Compounds for New Drug Discovery* (pp. 3–14). Elsevier. https://doi.org/10.1016/B978-0-12-817890-4.00001-9

Szostak, H., & Kowalewski, Z. (1975). The flavonoids in the leaves of *Vinca minor* L. (Apocynaceae). *Pol J Pharmacol Pharm*, *27*, 657–663.

Taylor, C. M. (2005). Botanic Garden Meise *Margaritopsis* (Rubiaceae, Psychotrieae) in the Neotropics. In *Geography of Plants* (Vol. 75, Issue 2).

Taylor, C. M., Razafimandimbison, S. G., Barrabé, L., Jardim, J. G., & Barbosa, M. R. V. (2017). Eumachia expanded, a pantropical genus distinct from Psychotria (Rubiaceae, Palicoureeae). *Candollea*, *72*(2), 289–318. https://doi.org/10.15553/c2017v722a6

Teoh, E. S. (2016). Secondary Metabolites of Plants. In *Medicinal Orchids of Asia* (pp. 59–73). Springer International Publishing. https://doi.org/10.1007/978-3-319-24274-3_5

Thippeswamy, M., Rajasreelatha, V., Chalannavar, R. K., & Jogaiah, S. (2021). Terpenoid indole alkaloids, a secondary metabolite in plant defense response. In *Biocontrol Agents and Secondary Metabolites: Applications and Immunization for Plant Growth and Protection* (pp. 443–457). Woodhead Publishing. https://doi.org/10.1016/B978-0-12-822919-4.00018-1

Tomassini, L., Cometa, M. F., Serafini, M., & Nicoletti, M. (1995). Isolation of Secoiridoid Artifacts from *Lonicera japonica. J. Nat. Prod.*, *58*(11), 1756–1758.

https://www.academia.edu/19031157/Isolation_of_Secoiridoid_Artifacts_from_Lonicera_japonica

Tu, Y. (2016). Artemisinin—A Gift from Traditional Chinese Medicine to the World (Nobel Lecture). *Angew. Chem. Int.*, *55*(35), 10210–10226. https://doi.org/10.1002/ANIE.201601967

Veitch, N. C., & Grayer, R. J. (2011). Flavonoids and their glycosides, including anthocyanins. In *Natural Product Reports* (Vol. 28, Issue 10, pp. 1626–1695). https://doi.org/10.1039/c1np00044f

Vlase, L., Sabin, A., Leti, A., & Arsene, I. (2017). Phytochemical investigations on four Galium species (Rubiaceae) from Romania. *Ser J Exp Clin Res*, *64*, 95–99.

Wani, M. C., Taylor, H. L., Wall, M. E., Coggon, P., & Mcphail, A. T. (1971). Plant antitumor agents. VI. The isolation and structure of taxol, a novel antileukemic and antitumor agent from Taxus brevifolia. *J Am Chem Soc.*, *93*(9), 2325–2327. https://doi.org/10.1021/JA00738A045

Wink, M. (2003). Evolution of secondary metabolites from an ecological and molecular phylogenetic perspective. In *Phytochemistry* (Vol. 64, Issue 1, pp. 3–19). Elsevier Ltd. https://doi.org/10.1016/S0031-9422(03)00300-5

Yang, H., Zhang, H., Yang, C., & Chen, Y. (2016). Chemical Constituents of Plants from the Genus *Psychotria*. In *Chemistry and Biodiversity* (pp. 807–820). Wiley-VCH Verlag. https://doi.org/10.1002/cbdv.201500259

Zappi, D. C., Miguel, L. M., Sobrado, S. V., & Salas, R. M. (2017). Flora das cangas da Serra dos Carajás, Pará, Brasil: Rubiaceae. *Rodriguésia*, *68*(3), 1091–1137. https://doi.org/10.1590/2175-7860201768347