MASTERARBEIT | MASTER'S THESIS

Titel | Title

Chemodiversity studies of selected Notopleura (Rubiaceae) species from Costa Rican rain forests.

verfasst von | submitted by

Stefanie Schmid BSc

angestrebter akademischer Grad | in partial fulfilment of the requirements for the degree of

Master of Science (MSc)

Wien | Vienna, 2025

Studienkennzahl lt. Studienblatt | Degree programme code as it appears on the student record sheet:	UA 066 832
Studienrichtung lt. Studienblatt | Degree programme as it appears on the student record sheet:	Masterstudium Botany
Betreut von | Supervisor:	ao. Univ.-Prof. i.R. Dr. Karin Vetschera
Mitbetreut von | Co-Supervisor:	Mag. Andreas Berger PhD

"The most beautiful thing we can experience is the mysterious. It is the source of all true art and science."

Albert Einstein

Photograph of *Notopleura uliginosa* (Sw.) Bremek. by Andreas Berger.

Acknowledgment

First and foremost, I would like to express my sincere gratitude to my supervisor, ao. Univ.-Prof. i.R. Dr. Karin Vetschera at the University of Vienna, for her guidance and the constructive feedback on this thesis.

I am also deeply grateful to my co-supervisor, Mag. Andreas Berger, PhD, for introducing me to essential laboratory techniques, for his invaluable contribution in collecting the plant material in Costa Rica, and for generously providing me with illustrative photographs.

My sincere appreciation goes to Mag. Dr. Johann Schinnerl for his extensive support in the laboratory work, his insightful assistance during the writing process, and for his thorough review of this thesis. His mentorship has been invaluable throughout this project.

I would like to extend my thanks to Univ.-Prof. Dipl.-Chem. Dr. Lothar Brecker and his team at the Department of Organic Chemistry of the University of Vienna for their expertise and for providing the crucial NMR and mass spectrometry data.

On a personal note, I would like to thank my partner for his unwavering patience and emotional support throughout the demanding process of completing this Master's thesis. Finally, I am deeply indebted to my family for their continuous encouragement and for providing me with the opportunity to pursue my academic endeavors at the University of Vienna.

Table of Contents

Abstract

The neotropical genus *Notopleura* (Benth. & Hook. f.) Bremek (Rubiaceae), comprising approximately 100 species of leathery to succulent herbs and shrubs, represents an interesting subject for phytochemical investigation. Numerous species in the Rubiaceae family, including species from the genus *Notopleura*, have an important ethnomedical relevance in a variety of cultures due to their complex biochemical pathways and wide range of specialized metabolites. This rich metabolite diversity has long fueled phytochemical research within the family. But even though Rubiaceae has shown promise as a source of bioactive compounds, the genus *Notopleura* has not yet been thoroughly studied from a wide-ranging, comparative phytochemical point of view. By examining the specialized metabolites found in *Notopleura* species and assessing their possible importance, this study seeks to close this gap. Based on chromatographic analyses using high-performance liquid chromatography (HPLC) and the determination of the total phenolic content, the study characterizes the chemical profiles of 16 different *Notopleura* species, comparing them with analytical data of five closely related genera. This comparative analysis revealed a chemical distinction between the two subgenera of *Notopleura*. Furthermore, the four substances harounoside (**1**), 2-aza-anthraquinone (**2**), its derivative 6-hydroxy-2-aza-anthraquinone (**3**) and the neolignane derivative glochidiobioside (**4**) were successfully identified from *N. panamensis*. Glochidiobioside (**4**) represents a novel discovery within the genus, and 6-hydroxy-2-aza-anthraquinone (**3**) is currently an undescribed compound. These findings highlight the potential of *Notopleura* as a source of unique specialized metabolites. Additionally, antifeedant assays employing polyphagous caterpillars were conducted to assess the ecological relevance of the two previously identified compounds harounoside (**1**) and 2-aza-anthraquinone (**2**), providing insights into their potential role in plant defense. These results highlight the need for more studies to clarify the pharmacological and ecological significance of *Notopleura's* specialized metabolites and significantly broaden our knowledge of the chemical diversity within Rubiaceae.

Keywords: *Notopleura*, Rubiaceae, specialized metabolites, Costa Rica, chemosystematics

Zusammenfassung

Die neotropische Gattung *Notopleura* (Benth. & Hook. f.) Bremek (Rubiaceae), die etwa 100 Arten ledrige bis sukkulente Kräuter und Sträucher umfasst, ist ein interessanter Gegenstand für phytochemische Untersuchungen. Zahlreiche Arten der Rubiaceae-Familie, darunter auch Arten der Gattung *Notopleura*, haben aufgrund ihrer komplexen biochemischen Stoffwechselwege und einer breiten Palette an speziellen Pflanzenmetaboliten in einer Vielzahl von Kulturen eine wichtige ethnomedizinische Bedeutung. Diese reiche Vielfalt an Metaboliten hat die phytochemische Forschung innerhalb der Familie seit langem vorangetrieben. Doch obwohl die Rubiaceae sich als vielversprechende Quelle für bioaktive Verbindungen erwiesen haben, wurde die Gattung *Notopleura* bisher noch nicht umfassend aus einem breit gefächerten, vergleichenden phytochemischen Blickwinkel untersucht. Die vorliegende Studie versucht, diese Lücke zu schließen, indem sie die in *Notopleura*-Arten gefundenen speziellen Pflanzenmetaboliten untersucht und ihre mögliche Bedeutung bewertet. Anhand von chromatographischen Analysen unter Anwendung von Hochleistungsflüssigkeits-chromatographie (HPLC) und des Gesamtphenolgehalts, charakterisiert die Studie die chemischen Profile von 16 verschiedenen *Notopleura*-Arten und vergleicht diese weiters mit analytischen Daten von Arten aus fünf nahe verwandten Gattungen. Diese vergleichende Analyse ergab eine chemische Unterscheidung zwischen den beiden Untergattungen von *Notopleura*. Darüber hinaus wurden die vier Substanzen Harounosid (**1**), 2-Azaanthrachinon (**2**), das Anthrachinonderivat 6-Hydroxy-2-aza-anthrachinon (**3**) und das Neolignanderivat Glochidiobioside (**4**) erfolgreich in *N. panamensis* identifiziert. Glochidiobioside (**4**) wurde erstmals in einer *Notopleura* Art nachgewiesen und das 6-Hydroxy-2-aza-anthrachinon (**3**), ist bisher noch nicht als Naturstoff beschrieben. Dieses Ergebnis unterstreicht das Potenzial von *Notopleura* als Quelle einzigartiger spezieller Pflanzenstoffe. Darüber hinaus wurden Fraßhemmungs-Tests mit polyphagen Raupen durchgeführt, um die ökologische Relevanz der beiden zuvor identifizierten Verbindungen Harounoside (**1**) und 2-Aza-anthrachinon (**2**) zu bewerten und Einblicke in ihre mögliche Rolle bei der Pflanzenabwehr zu gewinnen. Diese Ergebnisse machen deutlich, dass weitere Studien erforderlich sind, um die pharmakologische und ökologische Bedeutung der speziellen Metaboliten von *Notopleura* umfassend zu klären und unser Wissen über die chemische Vielfalt innerhalb der Rubiaceae erheblich zu erweitern.

1 Introduction

Many of the plant species found in Costa Rica's tropical rainforests are still poorly understood in terms of their phytochemical composition. With its wide variety of species, the family Rubiaceae is one of them and has many uses and is of substantial economic worth. The family offers many resources, ranging from the widely grown *Coffea arabica*, to decorative species like *Ixora* and *Mussaenda*, as well as genera with edible fruits that belong to the Gardenieae tribe (Berger, 2012). In addition to these applications, species of Rubiaceae are frequently used for their specialized metabolites that have historically been applied as poisons, hallucinogens, and natural cures (Dobkin de Rios, 1972; Delprete, 2004; Shan et al., 2016; Gong et al., 2017). Because of these useful properties, these compounds may also be used as building blocks for developing novel medications. A potentially abundant source of new substances is represented by this remarkable but till now mostly unexplored diversity in tropical areas. Among these plants is the genus *Notopleura* (Benth. & Hook. f.) Bremek., which provides an interesting topic for research. This genus comprises about 200 species of which only a few have been studied more in detail. It was shown that the so far studied species of *Notopleura* synthesize a diverse range of specialized metabolites, whereat alkaloids otherwise typical for Rubiaceae could so far not be detected in this genus (Solis et al., 1995; Jacobs et al., 2008; Berger, 2012; Berger et al., 2016; Kostyan, 2017). The diverse chemistry observed so far is thought to be related to a variety of functions in biotic interactions, but there are hardly any data available on this subject. The species of *Notopleura* and other understudied species in this family require further phytochemical research in order to advance scientific understanding and possibly find significant natural resources. These kinds of studies are crucial for understanding the chemical diversity of plants as well as for encouraging their conservation and sustainable use.

Until now the genus *Notopleura* has not been subject to broad comparative phytochemical analyses, including both distribution of specialized metabolites and related functional aspects. Thus, the major aims of this study will be:

a) Phytochemical profiling by HPLC of new and neglected species, and comparison with those that have already been studied as well as those that have received little attention so far.
b) Elaboration of species-specific accumulation tendencies and structural determination of relevant compounds, alongside determining the total phenolic content to understand unspecific defense chemicals.
c) Bioactivity studies, such as insect deterrence activity, to address functional aspects.

2 Systematics & Morphology

2.1 Rubiaceae Juss.

The Rubiaceae Juss., also known as the "coffee family," is a family of at least 614 genera and 13,465 species in the order Gentianales and belongs to one of the largest families of angiosperms (Davis et al., 2009; Govaerts et al., 2011). Although they are found on every continent in the world (except Antarctica), members of the Rubiaceae are most prevalent in tropical areas. The neotropics are home to almost half of the species and roughly one-third of the genera, which have adapted to a wide variety of environments (Stevens, 2001; Delprete, 2004; Davis et al., 2009; Govaerts et al., 2011). The majority of Rubiaceae species are small trees or shrubs that make up a significant portion of the understory of rainforests at low and mid-altitudes, while annuals and perennial herbs dominate in the temperate zone. High trees, lianas, vines, and herbs are less common and are primarily terrestrial (Robbrecht, 1988). A combination of vegetative and generative characteristics, such as opposite or whorled leaves with complete margins, entire or divided interpetiolar stipules, bisexual, actinomorphic, tetracyclic 4-5-merous flowers, and an inferior ovary with two carpels, are characteristics that largely identify the family (Robbrecht, 1988; Delprete, 2004; Robbrecht & Manen, 2006).

Within the Rubiaceae, the split into subfamilies has varied significantly over time and remains unclear to this day. The current division into the three subfamilies Cinchonoideae, Ixoroideae and Rubioideae follows Delprete (2004) and Bremer (2009), while Cinchonoideae and Rubioideae are the only two major clades described by Robbrecht & Manen (2006). However, the description of the Rubioideae subfamily, which includes the species group under study, has stayed consistent over the last years.

2.2 *Psychotria* alliance

The hyperdiverse *Psychotria* alliance is a prominent supertribe of the subfamily Rubioideae. It is a well-supported monophyletic clade which currently consists of nine distinct tribes: Cratispermeae, Gaertnereae, Mitchelleae, Morindeae, Palicoureeae, Prismatomerideae, Psychotrieae, Schizocoleeae, and Schradereae (Figure 1) (Robbrecht & Manen, 2006; Razafimandimbison et al., 2008; Bremer & Eriksson, 2009; Thureborn et al., 2022). Even though this classification offers a strong framework, research is still being done to better understand the interactions within this intricate group. Palicoureeae and Psychotrieae are two of the alliance's most notable tribes, which comprises 91% of the species within the *Psychotria* alliance and 24% of all Rubiaceae species (Razafimandimbison et al., 2014) and are therefore

the subject of this current study. The distinguishing features between these two tribes are: Psychotrieae possess deciduous stipules and reticulate, striate, columellate pollen, and lack pyrenes containing distinct proanthocyanidins, while Palicoureeae exhibit persistent stipules and such pyrenes containing distinct proanthocyanidins, and lack the reticulate, striate, columellate pollen (Andersson, 2002b; Robbrecht & Manen, 2006; Razafimandimbison et al., 2008). A fascinating variety of genera may be found in these two tribes, which are renowned for their exceptional ecological diversity and species richness. *Psychotria* L.*, Eumachia* D.C., *Geophila* D. Don*, Notopleura* (Benth. & Hook. F.) Bremek*, Palicourea* Aubl., and *Rudgea* Salisb. are genera that best represent the remarkable diversity and evolutionary success of the *Psychotria* alliance.

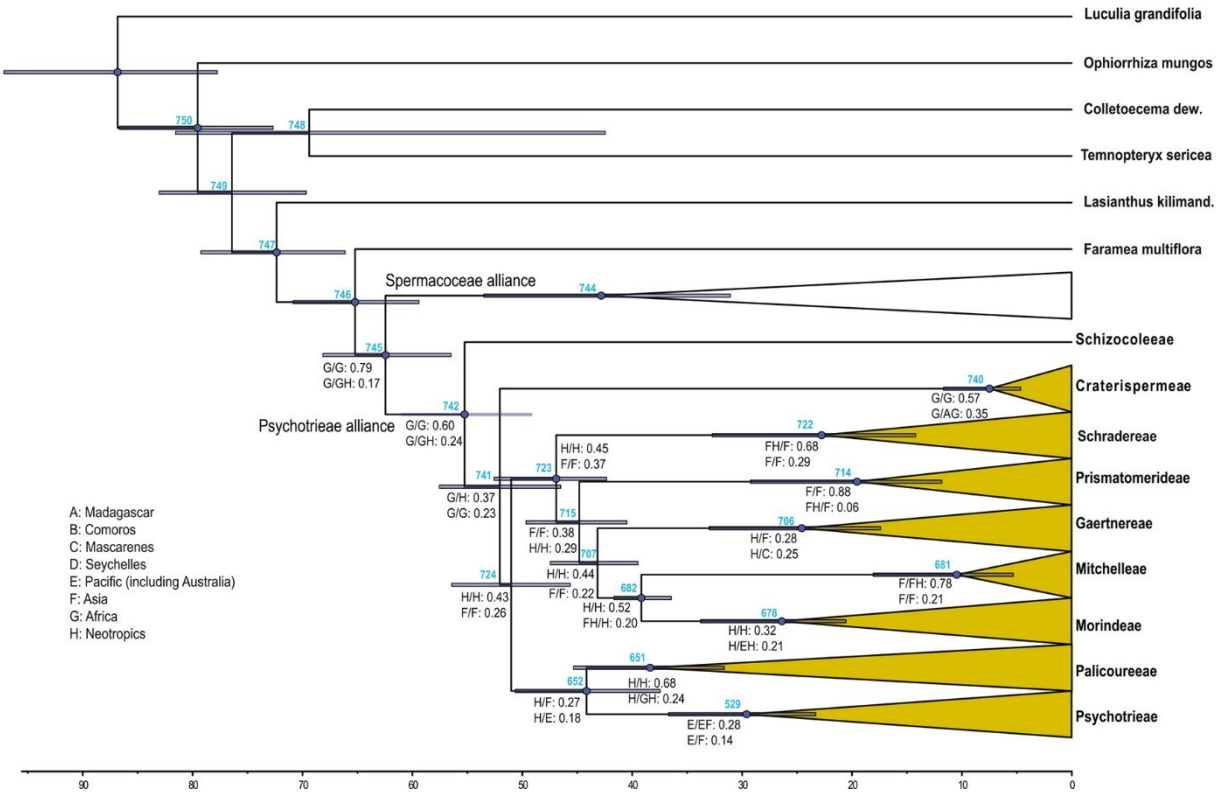


Figure 1 Extract of the Maximum clade credibility (MCC) phylogeny tree from the BEAST analyses of the combined *Psychotria* alliance datasets including the nine tribes: Cratispermeae, Gaertnereae, Mitchelleae, Morindeae, Palicoureeae, Prismatomerideae, Psychotrieae, Schizocoleeae, and Schradereae. (Razafimandimbison et al., 2017; modified by Stefanie Schmid)

2.3 Psychotrieae Cham. & Schltdl.

2.3.1 *Psychotria* L.

With at least 1,645 species, the pantropical genus *Psychotria* L. is one of the largest genera of flowering plants (Stevens, 2001; Berger et al., 2021). It is mainly found as small trees in the understory of tropical forests (Taylor, 1996) and is primarily defined by its reddish-brown, or

grayish dried color; interpetiolar, triangular, and caducous stipules; small, insect-pollinated flowers with straight tubes and white, cream, or greenish corollas (Lachenaud, 2019; Taylor, 1996; 2020). The flowers are possibly melittophilous and psychophilous, though detailed pollination studies are lacking (Csekits, 2008) and the red drupaceous fruits are bird dispersed (Snow, 1981; Poulin et al., 1999).

2.4 Palicoureeae Robbr. & Manen

2.4.1 *Eumachia* D.C.

The pantropical genus *Eumachia* D.C. comprises 83 species spread across the Neotropics, Africa, Asia, and the Pacific (Stevens, 2001; Taylor, 2005). *Eumachia* species are distinguished by their shrubby habit, frequently flattened and longitudinally ridged internodes, persistent stipules, leaves that dry greyish or pale yellowish-green, terminal inflorescences with green to whitish axes, actinomorphic, white, creamy, or yellow-green corollas with straight bases, and orange to red fruits (Taylor, 2005; Barrabé et al., 2012; Razafimandimbison et al., 2014; Delprete & Kirkbride, 2015; Taylor et al., 2017; Berger et al., 2021).

2.4.2 *Geophila* D. Don

With roughly 20–25 species of creeping plants or shrubs, the pantropical genus *Geophila* D. Don is most varied in the Neotropics and Africa, while some species are also found in Asia. Orange, red, or black fruits; cordate, long-petiolate leaves with mostly bifid stipules; white corollas; creeping, stoloniferous, and herbaceous habits; and frequently twisted pyrenes with one to several ribs but lack clear preformed germination slits are characteristics that easily identify this genus (Taylor, 1996; Piesschaert et al., 1999; Razafimandimbison, et al. 2014; Berger et al., 2021).

2.4.3 *Palicourea* Aubl.

With more than 600 species of shrubs or small trees, the Neotropical genus *Palicourea* Aubl. is the sixth largest genus of Rubiaceae. It is found from Mexico, the Bahamas, and the Greater Antilles south to northern Argentina especially as understory or subcanopy trees and shrubs of wet rainforests (Taylor, 1996, 1997; Davis et al., 2009; Taylor et al., 2010; Borhidi, 2011; Berger et al., 2021). *Palicourea* is distinguished by its rather greenish dried appearance, persistent stipules, metallic blue or purple-black mature fruits and pyrenes with preformed germination slits. The wide range of pollination syndromes is reflected in the features of flowers. Small, white, greenish, or yellow corollas with short tubes (pollinated by bees) or white

corollas with long tubes (pollinated by hawk moths) are characteristic of insect-pollinated species (Taylor, 1996, 1997). *Palicourea* species with long, tube-shaped, vibrant flowers are suited for hummingbird pollination. According to Taylor (1997) and Ornelas et al. (2004a, 2004b), birds spread the drupes that make up the fruits.

2.4.4 *Rudgea* Salisb.

The neotropical genus *Rudgea* Salisb. can be found from Mexico and the Lesser Antilles to northern Argentina and has more than 150 species of shrubs and small to occasionally big trees. The species has prolonged or fragmenting, whole, round, truncate to acute stipules with marginal glands, typically early caducous glandular appendages; terminal, frequently whitish inflorescences with corollas up to 7 cm long which are suited for either psychophily or melittophily (Zappi, 2003; Taylor & Zappi, 2006; Csekits, 2008). Usually white, spongy drupes with a persistent calyx on top, the fruits are thought to be disseminated by birds, though specific evidence on this is missing (Zappi, 2003; Csekits, 2008).

2.5 *Notopleura* (Benth. & Hook. f.) Bremek.

The neotropical genus *Notopleura* (Benth. & Hook. f.) Bremek, a member of the Palicoureeae tribe within the *Psychotria* alliance, constitutes the main focus of this work. With over 200 species of succulent herbs and shrubs, this genus thrives in wet forests, including swamps and stream borders, and is found across a wide range, from central Mexico and the Antilles to Brazil and Bolivia (Taylor, 2001; 2003; Stevens, 2001). Before being acknowledged as a distinct genus (Bremekamp, 1934; Nepokroeff et al., 1999; Taylor, 2001), this neotropical group was long categorized as *Psychotria* sect. *Notopleura* Benth. & Hook. f. (Bentham & Hooker, 1873). Furthermore, the genus *Notopleura* can be divided into two subgenera: the terrestrial and little branched subg. *Notopleura* and the epiphytic branched subg. *Viscagoga* (Baill.) C.M. Taylor (Taylor, 2001; Kostyan, 2017). Besides that, species of the genus *Notopleura* are easily identified by their characteristic fleshy stipules forming a sheath usually baring a succulent appendage; and by their inflorescences, which are typically pseudoaxillary containing small white flowers forming spongy drupaceous, white to red (in subg. *Viscagoga*) or black fruits (Figure 2) (Taylor, 2001; 2003; Taylor & Zappi, 2006; Razafimandimbison et al., 2014). Although no pollination studies have been conducted, the little flowers may be melittophilous and psychophilous in their pollination mechanism (Csekits, 2008) and birds are said to spread the drupes (Taylor, 2001).

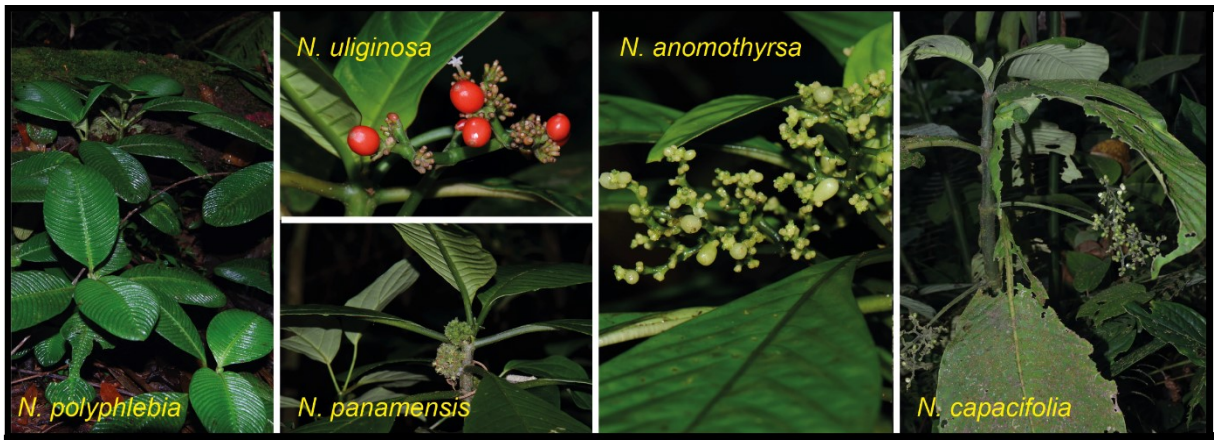


Figure 2 Photos from selected *Notopleura* species by Andreas Berger

2.5.1 *Notopleura panamensis* (Dwyer) C.M. Taylor

The succulent herb *Notopleura panamensis* (Dwyer) C.M. Taylor can be found from Costa Rica to Panama (Taylor, 2001) and reaches a height of around 1.25 meters. It features oblong leaves up to 35 cm long and jointed, dark green stems. The leaves are thin, slightly succulent with visible veins. The plant can be smooth or slightly hairy. It has small, persistent stipules (leaf-like appendages). The flowers are small, yellowish, clustered in heads up to 4.5 cm wide, and have pale greenish to whitish petals. The fruit is a white, round drupe about 6 mm in diameter, containing two flat, ridged 'pyrenes' (Dwyer, 1980).

Figure 3 Morphological details of *Notopleura panamensis*: A) whole plant, B) inflorescence, and C) single flower taken by Andreas Berger

3 Phytochemistry - Specialized metabolites (SPM)

Plant specialized (secondary) metabolites (SPM) are a diverse group of organic compounds that, unlike primary metabolites essential for basic survival, serve special ecological roles. These compounds are presumably not directly involved in growth or reproduction but are crucial for a plant's interaction with its environment (Chen et al., 2022; Jeyasri et al., 2023; Reshi et al., 2023; Tariq et al., 2023). While the purpose of specialized metabolites was historically debated (Bourgard et al., 2001; Hartmann, 2007; Wink, 2010), with some considering them waste products, the defense theory, pioneered by Stahl (1888) who studied snails feeding on plants and strongly supported by subsequent research, now highlights their crucial role in plant protection against herbivores (Swain, 1977; Harborne, 1993; Bernays & Chapman, 1994; Wink, 1988; 1992, 1993a; 2010). They provide a huge variety of complex chemical compositions and possible applications e.g. chemical defenses against herbivores and pathogens, attracting pollinators and seed dispersers, and mediate symbiosis or competition with other plants (Bourgaud et al., 2001; Ncube & Van Staden, 2015; Guerriero et al., 2018; Kadereit et al., 2021). Beyond defense, they play a role in creating the vibrant colors of flowers, the unique fragrances of leaves and the distinct flavors of fruits (Reshi et al., 2023).

Plant specialized metabolite biosynthesis is highly reactive and precisely controlled, both in location and timing, to maximize the output e.g. for defense purposes. Young, vital tissues like seeds and seedlings, as well as reproductive organs like flowers and fruits, are prioritized for SPM accumulation, adhering to the "optimal defense theory." The strategic allocation of defenses directly impacts plant morphology, ensuring vulnerable and critical parts are well-protected while simultaneously influencing leaf number, area, and plant height (Paul-Victor et al., 2010; Wink, 2010; Züst et al., 2015; Jeyasri et al., 2023; Reshi et al., 2023). Surface features like glandular trichomes often contain SPM which act as chemical deterrents upon contact or wounding, while high concentrations of SPMs stored in vacuoles provide a defense against initial attacks (Wink, 1992; 1997; 2010; Taiz et al., 2018; Holzbach et al., 2021; Kadereit et al., 2021). The specific placement of SPMs within plant tissues further reinforces their role as defense compounds. The strategic deployment of pre-existing specialized metabolites lays the groundwork for understanding the diverse mechanisms of plant defense, which can be broadly categorized as either constitutive or induced. Constitutive defenses, like alkaloids, are always-present traits providing continuous protection, while induced defenses, such as phytoanticipins and phytoalexins, are activated or amplified in response to attack, through chemical modifications or microbial encounters (Karban & Baldwin, 1997, Taiz et al., 2018; Kadereit et al., 2021). Plants may also leverage exogenous help through mutualistic relationships,

employing indirect defenses or hosting endophytes/epibionts to enhance their overall protective capabilities (Heil, 2008; Rodriguez et al., 2009). However, certain insects have been observed to sever leaf veins, effectively disrupting the flow of defense compounds to the feeding site (Dussourd & Eisner, 1987). Others have developed specialized detoxification mechanisms, allowing them to metabolize and tolerate otherwise harmful substances (Jeckel et al., 2022). This intricate interplay exemplifies the ongoing evolutionary arms race between plants and their attackers, where adaptations and counter-adaptations continuously shape each other's evolutionary development (Wink, 2010; Karinho-Betancourt, 2018). The significance of plant specialized metabolites extends beyond their ecological roles, as they have become valuable resources for humans. They are the source of numerous pharmaceuticals derived from plant-based substances, including aspirin, morphine, and taxol (Raskin et al., 2002; Dewick, 2009; Wink, 2010; Cragg & Newman, 2013; Atanasov et al., 2015), and contribute to the flavors and fragrances of foods and spices (Herrmann, 1997; Burdock, 2010). In agriculture, they offer potential for natural pesticides (Isman, 2008; Dayan et al., 2009), and in industry, they find applications in areas such as leather tanning and the production of resins (Zinkel & Russel, 1989; Covington, 2009). This multifaceted value highlights the significant impact of plant specialized metabolites on natural ecosystems and human society.

Despite their distinct roles, primary and specialized metabolic pathways are interconnected, with primary metabolites providing the necessary precursors for SPM synthesis (War et al., 2020; Singh et al., 2022). The major classes of specialized metabolites include terpenoids, phenolic compounds, and alkaloids, each with distinct biosynthetic pathways and functions (Reshi et al., 2023), which are described in chapter 3.2 Terpenoids and 3.3 Phenolics. Due to their chemical complexity, diverse distribution, and functional specificity, these metabolites can serve as chemotaxonomic markers, reflecting distinct chemical signatures across plant lineages (Bourgaud et al., 2001).

3.1 Specialized metabolites in Rubiaceae with focus on the *Psychotria* alliance

The Rubiaceae family, particularly the tribes Psychotrieae and Palicoureeae, has been of high interest for phytochemical studies, due to its ethnomedical uses and its remarkable diversity of SPMs and biosynthetic pathways (Porto et al., 2009; Sanz-Biset et al., 2009; Gong et al., 2017; Shan et al., 2016; Berger et al., 2021). Recent phylogenetic revisions have significantly reshaped the classification of the Psychotrieae and Palicoureeae tribes, leading to a more nuanced understanding of their chemical profiles (Taylor et al., 2017; Berger et al., 2021;

Razafimandimbison et al., 2014; 2024). Traditional phytochemical studies, focused on individual species and their isolated components, missed the opportunity to reveal the ecological and evolutionary significance of specialized metabolites. This restricted focus has resulted in a critical lack of insight into diversification within related plant lineages, their accumulation trends, and, crucially, their impact on ecological interactions and survival (Berger, 2012).

Historically, *Psychotria* encompassed a broad range of species, but contemporary studies have delineated distinct genera within the Palicoureeae tribe, notably *Palicourea*. Psychotrieae, and specifically the genus *Psychotria*, are characterized by a predominance of polyphenols and tannins, with only sporadic reports of polypyrroloindoline alkaloids (Berger et al., 2021). Crucially, monoterpene indole alkaloids (MIA), previously attributed to *Psychotria* (Martins & Nunez, 2015; Calixto et al., 2016; Yang et al., 2016; de Carvalho Junior et al., 2017), are now established to be absent, challenging earlier assumptions (Berger et al., 2021).

The Palicoureeae tribe stands in contrast to Psychotrieae, displaying a much higher chemical diversity. Although indole alkaloid and/or monoterpene-indole alkaloid biosynthesis is common across the tribe, the specific compounds produced vary significantly. *Palicourea* is known for accumulation of indole alkaloids like strictosidine and its derivatives, while *Geophila* and *Rudgea* accumulate alstrostine derivatives, polyphenols and triterpenoids (Luo et al., 2011; Berger et al., 2021), and *Eumachia* polypyrroloindoline alkaloids (Jamison et al., 2017). The genus *Notopleura* in particular exhibits a phytochemically unique pattern within the Palicoureeae tribe, exhibiting a complete absence of alkaloids and instead accumulating quinones, flavonoids, and megastigmanes, demonstrating a distinct chemodiversity (Martins & Nunez, 2015; Kostyan, 2017; Berger et al., 2012; 2016; 2021). This highlights that the genera within the *Psychotria* alliance can be separated not only by morphology, but also by their production of different specialized metabolites.

Phytochemical analyses carried out so far of *Notopleura* species have revealed distinct compound profiles. Studies to date showed that *Notopleura polyphlebia* yields in the 1,4-naphthoquinones harounoside and 2-aza-anthraquinone, the flavonoid dihydroflavanol (2R,3R)-7,4′-*O*-dimethyl-aromadendrin5-*O*-β-D-apiofuranosyl-(1⟶6)-β-D-glucopyranoside, and the megastigmane glucoside roseoside (Berger, 2012; Kolar, 2012; Berger et al., 2016). *Notopleura camponutans* instead contains the 1,4-naphthoquinones psychorubrin and 2-aza-anthraquinone, the megastigmanes 3-oxo-α-ionol, blumenol A and loliolide, and the flavonoids dihydroflavanol and quercetin derivatives (Solis et al., 1995; Jabocs et al., 2008; Kostyan,

2017). The following discourse will provide an in-depth examination of the major specialized metabolite classes characterized within the genus *Notopleura*.

3.2 Terpenoids

Terpenoids, representing the largest group of SPMs with approximately 60,000 identified compounds, are ubiquitous across all life forms. Their fundamental building block is the universal five-carbon isoprene unit. Terpenoids are classified by the number of these incorporated isoprene units, ranging from hemiterpenoids (C5) to polyterpenoids (C>40) (Figure 4), with each class exhibiting distinct properties, significance, and functional roles in biological and industrial contexts (Tholl, 2015; Abdallah & Quax, 2017).

The biosynthesis of terpenoids is fundamentally dependent upon two key starter molecules: Isopentenyl diphosphate (IPP) (Figure 5A) and its allylic isomer, dimethylallyl diphosphate (DMAPP) (Figure 5B) (McGarvey & Croteau, 1995). These essential precursors are produced by two separate and distinct biochemical pathways, with each pathway playing a critical role in providing the foundational building blocks for the big variety of terpenoid molecules (Eisenreich et al, 1998; Lange et al., 2000; Dewick 2001; Dudareva et al., 2013; Abdallah & Quax, 2017).

The mevalonic acid (MVA) pathway, operates within the cellular cytoplasm. This pathway is a six-step enzymatic process and converts acetyl-CoA into IPP and DMAPP. It begins with three acetyl-CoA molecules condensing to form 3-hydroxy-3-methylglutaryl-CoA, which is then reduced to mevalonate (MVA), followed by phosphorylations and a decarboxylation/elimination to yield IPP and DMAPP (Figure 6) (Eisenreich et al, 1998; Lange et al., 2000; Dewick, 2001; Dudareva et al., 2013; Abdallah & Quax, 2017). This cytosolic (including endoplasmic reticulum and peroxisomes) terpenoid production pathway is present in eukaryotes (including humans and the cytosol/mitochondria of plants), fungi, archaea, and certain eubacteria (Simkin et al., 2011; Pulido et al., 2012) and leads to sesqui- and triterpenoids.

The methylerythritol phosphate (MEP) pathway, is confined to the plastids, the specialized organelles of plant cells. The MEP pathway is an eight-step process and converts glyceraldehyde 3-phosphate (GAP) and pyruvate (Pyr) into isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP). It begins with the condensation of GAP and Pyr to 1-deoxy-D-xylulose 5-phosphate, which is then reduced to MEP. Five further steps lead to IPP and DMAPP (Figure 6). (Andriotis et al., 2010; Joyard et al., 2010; Bayer et al., 2011; Dudareva et al., 2013; Abdallah & Quax, 2017). This pathway, which functions inside plastids,

is present in eubacteria, algae, cyanobacteria, and plant chloroplasts (Hsieh et al., 2008) and leads to monoterpenes (including iridoids), diterpenoids as well as tetraterpenoids.

The remarkable structural diversity of terpenoids arises from the activity of terpene synthases and cyclases, which often exhibit broad substrate specificity (Tholl, 2006; Degenhardt et al., 2009b; Bleeker et al., 2011). These enzymes facilitate various modifications, including rearrangements, cyclization reactions, and combinations with other biosynthetic pathways, exemplified by monoterpene indole alkaloids (Dudareva et al., 2004; Wang et al., 2005; Tholl, 2006). The function of a specific terpenoid is intrinsically linked to its structure and the number of isoprene units it contains (Dudareva et al., 2013; Abdallah & Quax, 2017).

Their diverse bioactivities, including antimicrobial, antifungal, antiviral, antiparasitic, antihyperglycemic, antiallergenic, anti-inflammatory, antispasmodic, immunomodulatory, and chemotherapeutic properties, underscore their significant medicinal and commercial potential. Furthermore, terpenoids play crucial roles in agriculture as natural insecticides and storage protectants, and are emerging as vital components in the biofuel industry. Notable examples of medicinally relevant terpenoids include artemisinin (antimalarial acitivities) and taxol (anticancer activities) (Wang et al., 2005; Ajikumar et al., 2008; Thoppil & Bishayee, 2011; Guan et al., 2015; Abdallah & Quax, 2017).

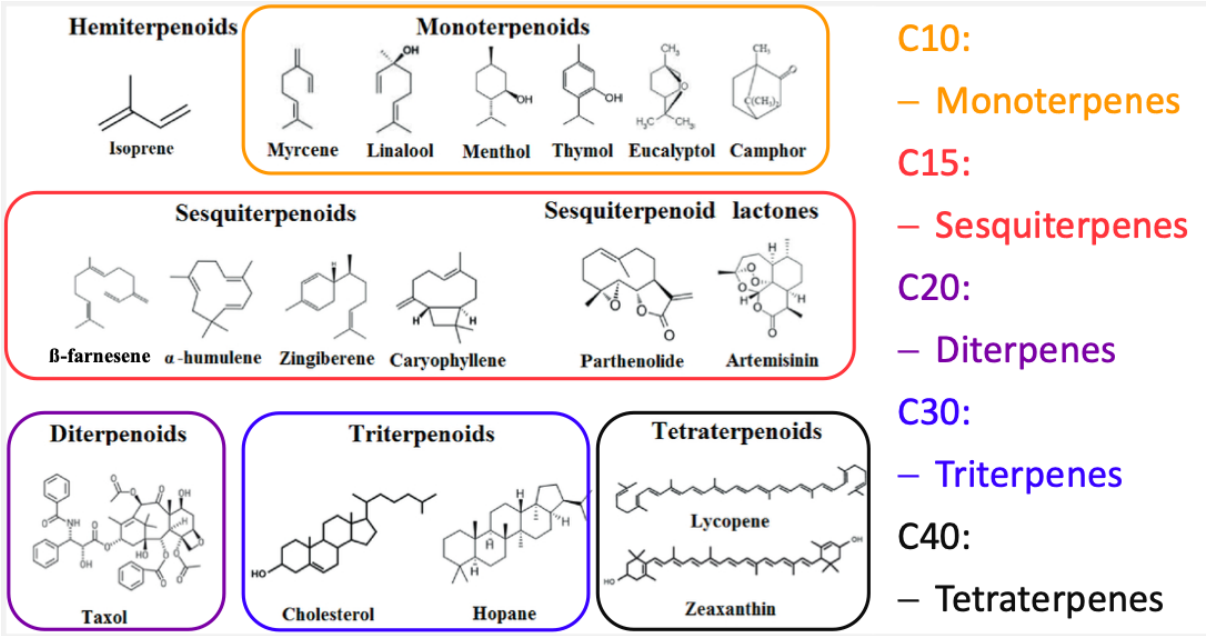


Figure 4 Terpenoid classification by the number of incorporated isoprene units (Holden, 2019; modified by Karin Vetschera and Stefanie Schmid).

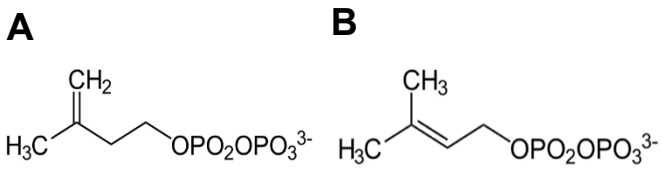


Figure 5 Starter molecules for terpenoid biosynthesis: A) Isopentenyl diphosphate (IPP) and B) dimethylallyl diphosphate (DMAPP)

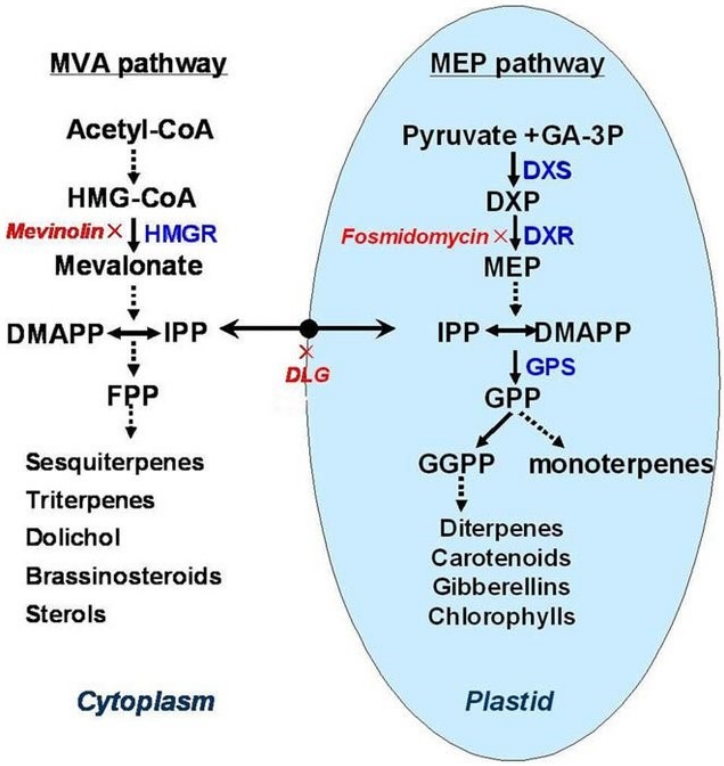


Figure 6 Comparative overview of the mevalonate (MVA) and methylerythritol phosphate (MEP) pathways for terpenoid synthesis. HMGR, 3hydroxy-3-methylglutaryl coenzyme A reductase; DMAPP, dimethylallyl pyrophosphate; IPP, isopentenyl diphosphate; FPP, farnesyl diphosphate; DLG, D, L -glyceraldehyde; GA-3P, glyceraldehyde 3-phosphate; DXP, 1-deoxy-D-xylulose 5-phosphate; DXS, 1-deoxy-D-xylulose 5phosphate synthase; DXR, 1-deoxy-D-xylulose 5-phosphate reductoisomerase; MEP, 2-C-methyl-d-erythritol-4-phosphate; GGPP, geranylgeranyl diphosphate; GPP, geranyl diphosphate; GGPPS, geranylgeranyl diphosphate synthase (Dongfeng et al., 2012; modified by Stefanie Schmid).

3.2.1 Iridoids

Iridoids are a large family of biologically active monoterpene lactones found in both, plants and insects. While predominantly found in plants, the significance of iridoids extends to the insect world, as exemplified by iridomyrmecin, a defensive secretion compound of ants from the genus *Iridomyrmex,* which also gave its name to this class of compounds (Cavill et al., 1956). They are structurally characterized by their fundamental cyclopentan-[c]pyran core structure, typically featuring a δ-lactone ring, which contributes significantly to their biological activity. They occur as iridoid aglycones, featuring a hydroxyl group at the C-1 position of the pyran-ring, as iridoid glucosides, where a glucosyl group is present, or as secoiridoids with a cleaved cyclopentane ring (Figure 7). Plants exhibit a remarkable diversity of iridoid derivatives,

classified based on substituents, linkages to other molecules, and ring modifications and can therefore serve as important precursor for monoterpene indole alkaloids (Jensen, 1991; El-Sayed & Verpoorte, 2007; Dinda et al., 2009; 2011; De Luca et al., 2014; Boland, 2017).

The biosynthesis of iridoids in plants primarily follows the 2-methyl-D-erythritol 4-phosphate (MEP) pathway, initiating with geraniol diphosphate and proceeding through a series of oxidation, reduction, glycosylation, and methylation reactions (De Luca et al., 2014; Dinda & Debnath, 2013; Huang et al., 2016; Boland, 2017).

Iridoids play a multifaceted role in plant defense and exhibit a range of biological activities. Their bitter taste serves as a deterrent to vertebrates (Rodriguez et al., 1998), while their ability to crosslink dietary and tissue proteins deters generalist herbivores (Konno et al., 1999; Kim et al., 2000; Dobler et al., 2011). The toxic effects of iridoids arise from the release of the highly reactive aglycones iridodial or iridotrial, which are generated from non-toxic iridoid glucosides and are stored in plant organelles (Konno et al., 1999; Kim et al., 2000). Specialist insects, however, have evolved mechanisms to sequester iridoid glycosides, converting them into aglycones for their own defense. This sequestration, often age-dependent, suggests a dual role in defense against predation and immune suppression (e.g. Stermitz et al., 1994; Bowers & Stamp, 1997; Willinger & Dobler, 2001). Beyond their ecological roles, specific iridoids, such as nepetalactones, display a range of biological activities, including insecticidal, insect repellent, antimicrobial, anti-inflammatory, cytostatic, and phytotoxic effects (Biere et al., 2004; Baden & Dobler, 2009; Dobler et al., 2011; Whitehead et al., 2015; Uenoyama et al., 2021). These diverse functions highlight the significance of iridoids in both plant defense and potential pharmaceutical applications (Ghisalberti, 1998; Tundis et al., 2008; Dinda & Debnath, 2013).

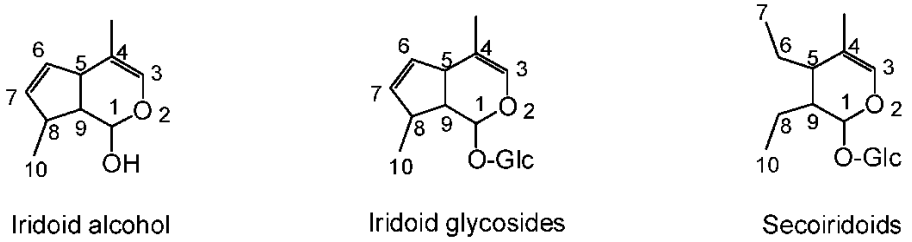


Figure 7 Basic structural representations of the cyclopentan-[c]pyran core structure of iridoids, including aglycone iridoid (iridoid alcohol), iridoid glycoside (showing the added sugar component), and secoiridoid glucoside (demonstrating the characteristic ring cleavage) (Wang et al., 2020; modified by Stefanie Schmid).

3.2.2 C13 Norisoprenoids (Megastigmanes)

Megastigmanes are a subclass of nor-sesquiterpenes characterized by a C13 skeleton and stereogenic centers at C-6 and C-9 (Figure 8). The biosynthetic plasticity of this class of compounds is demonstrated by the large range of megastigmane derivatives with different

oxidation states and glycosylation patterns, despite the relatively simple C13 framework (Ayinampudi, 2017).

The biosynthesis of megastigmanes initiates with the MVA pathway, generating precursors for geranylgeranyl pyrophosphate and carotenoids. Subsequently, carotenoid cleavage dioxygenases oxidatively cleave these carotenoids, yielding C13 fragments that undergo further modifications to produce these diverse megastigmane structures (Sefton et al., 1989; Baumesa et al., 2002; Ayinampudi, 2017).

Megastigmanes exhibit diverse bioactivities, including anti-inflammatory, antibacterial and antioxidant properties, as evidenced by studies demonstrating their ability to modulate cytokine expression and combat oxidative stress. (Nomoto et al., 2013; Lv et al., 2018; Pan et al., 2019; Elshamy et al., 2021). However, Kornpointer et al. (2022) only partially agreed with these findings, reporting weak or negligible antibacterial activity and failing to detect antioxidant effects in the studied compounds. This suggests that the previously reported bioactivities are not consistently observed across all studies. Megastigmanes seem to demonstrate potential in other areas as well, including neuroprotection and anti-tumor activity, highlighting their therapeutic potential and the need for further investigation into their diverse pharmacological effects (Cho et al., 2014; Lee et al., 2015). Additionally, C13 norisoprenoids, such as β-damascenone, are significant aroma compounds found in various plants and products like roses, wine, honey, starfruits, and tea (Ohloff & Demole, 1987; Winterhalter et al., 1990; Herderich et al., 1992; Winterhalter & Rouseff, 2001; Jerkovic & Kus, 2014).

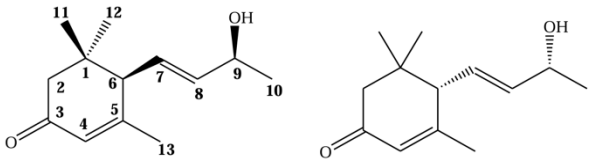


Figure 8 C13 skeleton and stereogenic centers at C-6 and C-9 of megastigmanes (Ayinampudi, 2017; modified by Stefanie Schmid)

3.3 Phenolics

Phenolic compounds are characterized by their aromatic ring substituted by one or more hydroxyl (OH) groups (Figure 9). They are broadly classified into distinct groups based on their structural features, encompassing phenolic acids, flavonoids, stilbenoids, lignans, tannins (incl. catechins), coumarins, and others (Hahlbrock & Scheel, 1989; Kadereit et al., 2021). Phenolics can be biosynthesized through different metabolic pathways, including the shikimate pathway, acetate-malonate pathway, terpenoid synthesis pathway (Kadereit et al., 2021). However, the

majority of phenolic compounds, particularly phenylpropanoids, are derived from shikimic acid, progressing through the phenylpropanoid pathway, where hydroxycinnamic acids (HCAs) emerge as key intermediates, serving either as direct precursors or ester components for a wide array of phenolic-based metabolites (Hahlbrock & Scheel, 1989).

The shikimate pathway is a crucial metabolic route in plants and microorganisms located within the plastids, leading to the biosynthesis of aromatic amino acids, which serve as precursors for a wide array of phenolic compounds, particularly phenylpropanoids (Schmid & Amrhein, 1995; Rippert et al, 2009; Vogt, 2010). The shikimate pathway (Figure 10) initiates with the condensation of phosphoenolpyruvate (PEP) and erythrose-4-phosphate (E4P), generating shikimic acid, which is subsequently transformed into phenylalanine through a series of enzymatic reactions. This phenylalanine is then converted to cinnamic acid by the enzyme phenylalanine ammonia-lyase (PAL), initiating the diversification into a wide array of phenylpropanoid phenolics. Importantly, a key product derived from this pathway, specifically through the action of PAL, is *para*-coumaroyl-CoA. This compound serves as a central intermediate in the generation of a vast array of phenylpropanoid compounds, acting as a crucial branch point in the biosynthesis of these diverse metabolites (Hahlbrock & Grisebach, 1979; Hahlbrock & Scheel, 1989; Vogt, 2010).

Widely distributed throughout the plant kingdom, phenolic compounds, known for their multiple biological effects such as antioxidant capacity and antimicrobial activity (Dapkevicious et al., 1998; Rauha et al., 2000; Proestos et al., 2005), play a crucial role in plant responses to environmental stress such as drought, UV radiation or wounding (Smirnoff, 1993; Grantz et al., 1995; Landry et al., 1995). Phenolic compounds effectively neutralize free radicals which are increasing during this stress, by donating a hydrogen atom from their hydroxyl groups, resulting in unreactive stable free radicals (Figure 9). This stability results from the delocalization of the unpaired electron on the oxygen atom into the aromatic ring structure, thereby distributing the electron density and minimizing reactivity (Scott, 1997; Duthie, 1999).

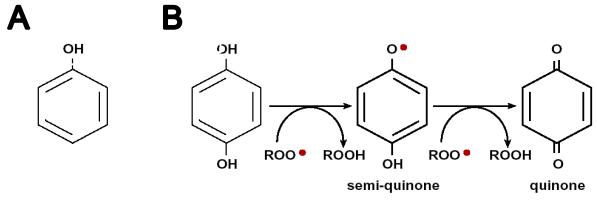


Figure 9 Phenolic structure and antioxidant activity. A) core structure with key functional groups, aromatic ring and hydroxyl group. B) simplified mechanism of hydrogen donation forming a stable free radical (Duthie, 1999; modified by Stefanie Schmid).

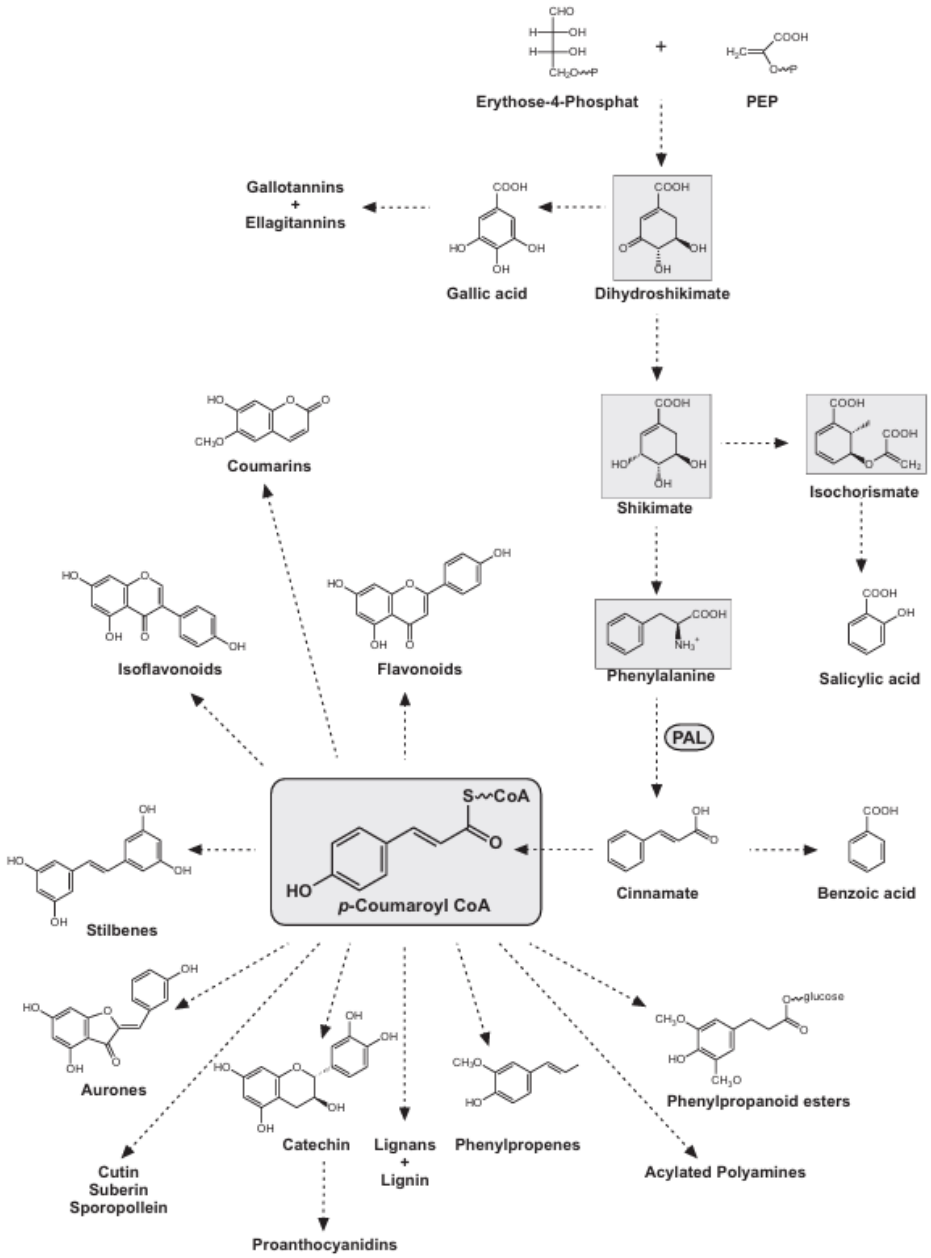
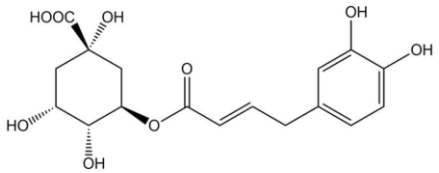


Figure 10 Overview of the shikimate pathway, showing the series of enzymatic reactions that convert erythrose-4-phosphate and phosphoenolpyruvate to the precursor *p*-coumaroyl-CoA (Vogt, 2010; modified by Stefanie Schmid)

3.3.1 Chlorogenic acids

Chlorogenic acids, ester formed from caffeic acid and L-quinic acid, is a functional polyphenol characterized by its axial hydroxyls on carbons 1 and 3 and equatorial hydroxyls on carbons 4 and 5 (Figure 11). This compound is widely distributed throughout the plant kingdom, notably abundant in coffee (Clifford, 2000; American Chemical Society, 2013). The biosynthesis of chlorogenic acids relies on the three key enzymes: Phenylalanine ammonia-lyase (PAL), shikimic acid/quinic acid hydroxyl cinnamyl transferase (HCT), and quinic acid cinnamate hydroxyltransferase (HQT). The enzyme PAL starts the process by converting L-phenylalanine

to cinnamic acid. Further, HCT and HQT subsequently condense caffeic acid to quinic acid, forming chlorogenic acids (Wang et al., 2022). Chlorogenic acids exhibits a diverse range of bioactivities, including antibacterial properties and the ability to reduce tumor growth (Su et al., 2019; Gupta et al., 2022; Herath et al., 2024). Furthermore, chlorogenic acids plays a significant role in plant defense. They defend plants by deterring herbivores through growth inhibition and reduced palatability (Nicholson & Hammerschmidt, 1992), exhibiting antimicrobial and antifungal properties against pathogens (Sung & Lee, 2010; Santana-Gálvez et al., 2017), accumulating upon injury or attack (Mandal et al., 2010), and strengthening cell walls (Treutter, 2006), making them crucial for plant survival.



5-CQA

Figure 11 Chemical structure of 5-caffeoylquinic acid, a common phenolic conjugate, often referred to as chlorogenic acid (CGA) (Clifford, 2000; Wang et al., 2022; modified by Stefanie Schmid)

3.3.2 Flavonoids

Flavonoids are a remarkably diverse group of plant specialized metabolites which are ubiquitous throughout the plant kingdom. With over 10,000 distinct compounds already identified (Ferrer et al., 2008; Ferreyra et al., 2012; Kozlowska & Szostak-Wegierek, 2014), this biologically significant class of molecules showcases a wide range of chemical structures. Fundamentally, flavonoids possess a 15-carbon skeleton, arranged as a C6-C3-C6 structure, comprising two phenyl rings (A and B) connected by a heterocyclic ring (C) containing oxygen (Figure 12) (D'Amelia et al., 2018; Dias et al., 2021). This basic structure allows for extensive variations through hydroxylation, glycosylation, and other substitutions, leading to six subgroups: flavan-3-ols, flavones, flavonols, flavanones, isoflavones, and anthocyanins (Figure 13) (Winkel-Shirley, 2001; 2006; Šamec et al., 2021). These structural variations directly influence their biological activities. Flavonoids occur in various compartments throughout plants, accumulating as e.g. glycosides within vacuoles and aglycones in exudates, and are also localizing within organelles like chloroplasts and nuclei (Winkel-Shirley, 2001).

The biosynthesis of flavonoids represents a highly conserved metabolic process, integrating contributions from both the malonic acid pathway (for the A-ring) and the shikimate pathway, specifically through 4-coumaroyl CoA (for the B-ring). Chalcone synthase catalyzes the initial committed step, generating chalcone scaffolds that serve as the fundamental precursors for all

flavonoid subgroups. Although the core biosynthetic pathway exhibits remarkable conservation across plant taxa, the diversification of flavonoid structures is achieved through the action of a suite of modifying enzymes. These include isomerases, reductases, hydroxylases, and $Fe^{2+/2-}$-oxoglutarate-dependent dioxygenases, which introduce structural variations to the basic flavonoid skeleton, yielding the extensive array of flavonoid compounds observed in nature (Martens et al., 2010; Ferreyra et al., 2012)

Flavonoids have diverse biological roles, impacting human health (Parr & Bolwell, 2000) with medicinal and pharmacological effects (De Bruyne et al., 1999; Kong et al., 2003; Marles et al., 2003; Yilmaz & Toledo, 2004). In plants, they act as stress protectors, signaling molecules, and contribute to resistance (Barcelò & Poschenrieder, 2002; Ryan et al., 2002; Tattini et al., 2004). The pigments that color most flowers, fruits, and seeds are flavonoids, contributing to the attraction of pollinators (Ferreyra et al., 2012), thereby facilitating pollination (Zerback et al., 1989; Van Der Meer et al., 1992). Flavonoids also mediate plant-microbe interactions (Hungria & Stacey, 1997; Broughton et al., 2003; Mathesius, 2003; Cooper, 2004; Kobayashi et al., 2004) and plant-plant interactions via antimicrobial effects (Inderjit & Gross, 2000; Treutter, 2006). These multifaceted roles underscore the significance of flavonoids in plant biology, ecology, and human applications.

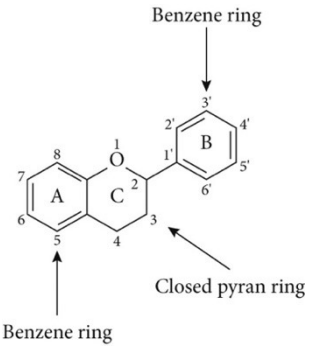


Figure 12 Basic chemical structure of flavonoids, depicting the A and B phenyl rings connected by a heterocyclic C ring (Arpita et al., 2022; modified by Stefanie Schmid).

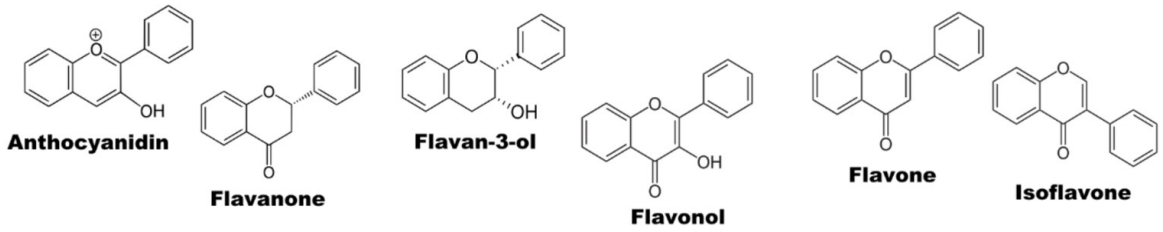


Figure 13 Overview of the six major subclasses of flavonoids and their core structures. (Lemos et al., 2019; modified by Stefanie Schmid)

3.3.3 Quinones (Benzoquinones, Naphthoquinones and Anthraquinones)

Quinones are ubiquitously distributed throughout nature, found extensively across plant families, including both higher and lower plants like algae and lichens, and are localized within various plant tissues such as roots, stems, leaves, seeds, fruits, and flowers (Dave & Ledwani, 2012). Beyond the plant kingdom, quinones are prevalent in bacteria, fungi, marine animals like sponges, sea urchins, and insects, highlighting their broad ecological significance and potential roles in diverse biological processes (Shikov et al., 2018; Christiansen et al., 2021; Soleimani et al., 2022; Dong et al., 2024).

The distinction between the three subgroups benzoquinones, naphthoquinones, and anthraquinones fundamentally resides in their polycyclic aromatic hydrocarbon backbones, reflecting a progressive increase in structural complexity. Benzoquinones, characterized by a single benzene ring bearing two carbonyl functionalities (Figure 14a), represent the simplest structural element, followed by the naphthoquinones with the fusion of two benzene rings (Figure 14b). Anthraquinones, the most complex of the series, are defined by three benzene rings which are linearly annulated (Figure 14c), again incorporating the characteristic quinone functionalities. The physicochemical characteristics and biological activities of these compounds are determined by the gradual development of the aromatic framework, which also affects their lipophilicity, redox potentials, and interactions with biological targets (Abaham et al., 2011; Kumagai et al., 2012; Dulo et al., 2021; Patel et al., 2021; Zhang et al., 2025).

Quinone biosynthesis in plants is remarkably diverse, multiple biosynthetic pathways lead to this structurally diverse class of compounds. Widhalm and Rhodes (2016) highlighted at least four distinct metabolic routes for 1,4-naphthoquinones, each utilizing different starting molecules, as confirmed by numerous biochemical studies (Bentley and Campbell, 1974; Bentley, 1975; Leistner, 1980; Meyer et al., 2021). These pathways include the acetate-polymalonate pathway, used for both, naphthoquinone and anthraquinone biosynthesis, in various plant species (Leistner & Zenk, 1969; Leistner, 1971; Fairbain & Muhtadi, 1972; Durand & Zenk, 1974), the aromatic amino acid pathway where phenylalanine is converted into precursors for benzoquinones (Bolkart & Zenk, 1968a), and also the shikimic acid-*o*-succinoylbenzoic acid, and the MVA (mevalonic acid) pathways (Leistner, 1981; Meyer et al., 2021).

Quinones exhibit versatile biological activities, primarily functioning as potent antioxidants by scavenging free radicals and inhibiting oxidative reactions (Figure 9), which protect cellular components from damage (Gutteridge & Halliwell, 2006; Vasileva et al., 2016; Maimon et al., 2018; Jeremić et al., 2018; Halliwell et al., 2021). Additionally, they display antimicrobial

properties by disrupting bacterial DNA replication, protein synthesis, and inducing oxidative stress, leading to cell death (Stasevych et al., 2018; Kim & Ma, 2019; Dighe & Collet, 2020; Sabnis, 2020; Quiping et al., 2023). Furthermore, quinones demonstrate significant antitumor potential through various mechanisms, including cell cycle arrest, apoptosis induction, DNA damage, and synergistic effects with other therapeutic agents, showcasing their broad applicability in combating oxidative stress, infections, and cancer (Oliveira et al., 2020; Atteia et al., 2021; Mahmud et al., 2022; Li, 2023; Dong et al., 2024). Some naphthoquinones, like juglone, function as phytotoxins, exhibiting allelopathic effects by inhibiting the germination and the growth of other plants (Stoessl, 1981; Rietveld, 1983; Powell & Spencer, 1988). Quinones exist in plants as highly oxidized forms contributing to pigmentation (Velisek et al., 2008) making anthraquinones a large group of natural occurring pigments, including approximately 700 compounds, which were used as dyes due to their characteristic blue color throughout history (Seigler, 1998; Velisek et al., 2008; Duval et al., 2016).

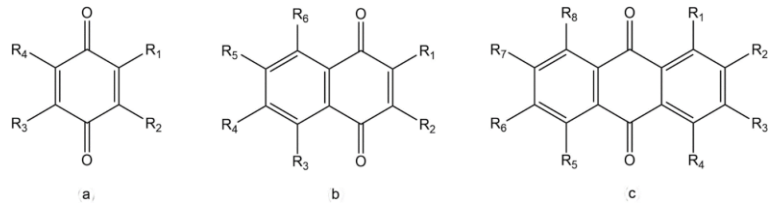


Figure 14 Chemical structure of **a)** benzoquinone, **b**) naphthoquinone and **c**) anthraquinone (Dulo et al., 2021; modified by Stefanie Schmid)

4. Material and Methods

4.1 Plant material

A total of 93 samples from 42 individuals from 16 *Notopleura* species (Table 1) were collected in Costa Rica for this study. These samples primarily consisted of leaves (77), with additional stems (12), roots (3) and fruits (1). For comparative purposes, additional samples from the five related genera *Palicourea*, *Psychotria*, *Eumachia, Rudgea* and *Geophila* and 42 different species were collected in Costa Rica in 2022 to compare to *Notopleura* for this study. To identify the distinguishing characteristics a total of 291 samples from 97 individuals (3 leaves per individual) were selected (Table A8).

From the investigated air-dried plant parts 40 mg (± 1 mg) were weighed into 2.0 mL micro centrifuge tubes, as far as this was possible with the amount of material available. Occasionally, only 20 mg (± 1 mg) per leaf could be weighed in when not more material was available. The dried material was then mechanically crushed using three glass beads in a swing mill (Qiagen–Tissue Lyser II) at a frequency of 30 Hz for 5 min.

Table 1 *Notopleura* species used in this study

Taxon	samples	Leaves	Stems	Roots	fruits	Region	Conservation Area
N. aggregata	9	9				Monteverde	ACAT
N. anomothyrsa	3	1	1		1		
N. capacifolia	1	1					
N. costaricensis	2	1	1				
N. maxonii	9	9				Juan Castro Blanco	ACAHN
N. nepokroeffiae	2	2					
N. panamensis	2	1	1				
N. aff. *panamensis*[1]	9	9				Monteverde	ACAT
N. peperomiae	9	9				Catarata Toro, Juan Castro Blanco	ACAHN
N. pithecobia	4	4				Tapanti	ACC
N. polyphlebia	14	11	1	2		Chilamate	ACAHN
N. siggersiana	3	1	2				
N. tolimensis	7	3	4				
N. tonduzii	1	1					
N. uliginosa	16	14	1	1		Chilamate, Boca Tapada–Camino de San Juan	ACAHN
N. subg. *Viscagoga*[1]	2	1	1				

Leaves (L), stem (S), roots (R); [1] species not exactly determined

4.2 Analytical methods

4.2.1 Thin layer chromatography (TLC)

Analytical thin layer chromatography (TLC) is a technique used to analyze non-volatile mixtures and their containing compounds. In TLC, the sample is spotted onto an aluminum- or glass-backed silica gel plate and developed using a suitable solvent system. The compounds separate based on their polarity, with more polar compounds interacting more strongly with the polar silica gel and migrating slower up the plate through capillary action.

Small volumes of the samples of interest were spotted on the bottom of a TLC plate and it was then developed in either a versatile solvent mixture (petrol ether/ethyl acetate) or a polar mixture consisting of ethyl acetate/methanol (MeOH) or $CHCl_3$/MeOH in different ratios depending on the compound examined. The developed plates were then analyzed under UV light at 254 nm and 366 nm to detect compounds with chromophores. To visualize additional compounds, the plates were sprayed with the staining reagent anisaldehyde (4-methoxybenzaldehyde; 0.48% (v/v)) solution made up of 85 mL MeOH, 10 mL acetic acid, 8 mL concentrated sulfuric acid, 0.5 mL anisaldehyde (Stahl, 1967). This reagent reacts with various classes of compounds producing colored products after heating, noticeable under visible light, thus aiding in the identification of potential compound classes. For compounds identified during this work this detection reagent was suitable.

4.2.2 High-performance liquid chromatography (HPLC)

High-performance liquid chromatography (HPLC) was employed to analyze the crude extracts and to identify potential target compounds. HPLC separates compounds based on their differential interactions with a stationary phase consisting in many cases of particles with diameters of 10–3 µm. As the sample is eluted through the column, compounds with different polarities and affinities for the stationary phase are separated and eluted at distinct retention times. HPLC relies on the detection of compounds based on their absorbance of light, which is related to their chromophoric group present in the chemical structures. If a compound is present at a very low concentration or lacks a strong chromophore, it may not be detected.

The measurements were carried out with an Agilent 1100 Series HPLC System. The used components as well as the parameters (Table 2) for the measurements are shown below:

- G1322A Degasser
- G1312A Binary Pump
- G1367A Well plate sampler

- G1316A Column oven
- G1315C Diode array detector

Separation of the crude extracts for the genera and species comparison was achieved on a Hypersil BDS C18 100 × 4.0 mm 3 µm column, while a Kinetex 5 µm PFP 100 A, 250 × 4.6 mm LC Column was used for monitoring the isolation procedure of *N. panamensis*. The mobile phase consisted of 10 mM ammonium acetate dissolved in HPLC grade water as solvent A and MeOH as solvent B. The gradients used are given in Table 2. The analytical evaluations were carried out using Agilent ChemStation B 04.02 SP1.

Prior HPLC analysis, the prepared samples of the crude extracts were centrifuged for 15 min at 14,000 rpm to avoid insoluble particles. After centrifugation 20 µL of the samples were transferred into a 96-microwell plate and diluted with 80 µL dimethyl sulfoxide (DMSO). This led to a final concentration of 10 mg/mL. The monitoring wavelength was 230 nm and the identification of each compound was based on a combination of retention time and UV spectra matching with an in-house UV database constructed during previous research (Berger et al., 2021 and references cited therein).

Table 2 Timetable of the eluent gradient used in this work, A: 10 mM ammonium acetate, B: MeOH. 1) gradient for *Notopleura* species comparison; 2) gradient for genera comparison; 3) gradient for extraction of *N. panamensis*

Time [min]	A [%]	B [%]	Flow [mL/min]
0.00	95	5	0.5
2.00	95	5	0.5
2.01	80	20	0.5
15.00	20	80	0.5
15.01	5	95	0.5
20.00	5	95	0.5

Time [min]	A [%]	B [%]	Flow [mL/min]
0.00	95	5	0.5
2.00	95	5	0.5
2.01	60	40	0.5
11.00	5	95	0.5
15.00	5	95	0.5

Time [min]	A [%]	B [%]	Flow [mL/min]
0.00	80	20	0.5
0.00	80	20	1.0
15.00	10	90	1.0
15.10	1	99	1.0
22.00	1	99	1.0

4.2.3 Nuclear magnetic resonance spectroscopy (NMR)

Nuclear magnetic resonance spectroscopy (NMR) is a method that uses a strong magnetic field to elucidate the structure of molecules, and it was employed to identify the purified compounds of interest. The NMR spectra were recorded at the Department of Organic Chemistry, University of Vienna and further analyzed by Univ.-Prof. Dipl.-Chem. Dr. Lothar Brecker and his team.

4.2.4 Total phenolic content

Subsequently the extracted samples were used to determine the concentration of the total phenolic content using the Folin-Ciocalteu method (Folin & Ciocalteu, 1927). This colorimetric assay involves the oxidation of phenolic compounds by a mixture of phospho-molybdic acid and phospho-tungstic acid, resulting in a blue-colored complex. The intensity of the blue color, measured spectrophotometrically, is directly proportional to the concentration of phenolic compounds.

In this work, the calibration parameters using gallic acid as standard were assessed in the conc. range from 0 to 100 mg/mL. A mixture consisting of 798 µL water, 2 µL of the sample at a conc. of 10 mg/mL, 50 µL diluted Folin-Ciocalteu reagent (1:1 with H_2O) and 150 µL 20% aqueous Na_2CO_3 solution was incubated at 40°C for 30 min. Subsequently, 200 µL of each sample was transferred to a 96-well plate and measured spectrophotometrically at 765 nm using a Tecan - Infinite M Nano plate reader.

4.2.5 Molar extinction coefficient ε of 6-hydroxy-2-aza-anthraquinone (**3**)

From this sample an amount of 2.2 mg was dissolved in 1 mL of MeOH, then diluted with MeOH to achieve a final concentration of 0.022 mg/mL (0.0977 mol/L). This solution was subsequently measured using a UV/Vis-Spectrophotometer (Analytik Jena – Specord PC 205) at wavelenghts of 230 nm (log ε 4.26) and 256 nm (log ε 3.95) which correspond with the observed UV maxima in the UV spectrum. The calculation was performed according to the Lambert-Beer law (Otto, 2011):

$$E = \varepsilon * c * d$$

E= extinction (Absorbance of the material for light of the wavelength λ)
ε= decadic extinction coefficient
c= mass concentration of the absorbing substance in the medium [mol/L]
d= pathlengths of the cuvette (1 cm)

4.3 Preparative methods

4.3.1 Liquid-liquid extraction

Liquid-liquid extraction is a technique that separates compounds into two distinct layers: A lipophilic (organic) layer and a hydrophilic (aqueous) layer. This separation is based on the differing solubilities of compounds in two immiscible solvents and was used as first separation step. Therefore, the crude extract was suspended in deionized water and subsequently transferred to a separation funnel. Petrolether (PE) was added to create a 1:1 water-to-petrolether ratio, and the funnel was shaken with caution and allowed to rest for a few minutes for phase separation. The two layers were then drained and collected in different vessels. Subsequently, the same extraction procedure was repeated subsequently with chloroform ($CHCl_3$) and ethyl acetate. Each step was repeated three times.

4.3.2 Column chromatography (CC)

Column chromatography using silica gel 60 was employed to separate components based on their polarity. More polar compounds interact more strongly with the polar silica gel stationary phase, leading to slower elution rates. To facilitate this separation, silica gel with a particle size of 0.04-0.063 mm was mixed with the starting solvent PE prior to packing the column. The sample was adsorbed onto silica gel with a larger particle size (0.2–0.5 mm) and carefully added to the top of the column. A gradient elution was performed, starting with PE and progressively increasing the polarity of the solvent system by first adding ethyl acetate followed by MeOH with a 10% increase.

Medium pressure liquid chromatography (reversed-phase MPLC)

Medium pressure liquid chromatography is a method that uses a tightly packed column to assure a better separation of pre-purified samples using elevated pressure. Therefore, a prepacked column (size 310 × 25 mm, Lichroprep RP-18 40–63µm) with a gradient mixture of HPLC-grade water and MeOH (90:10) with an increase of 10% MeOH per step was used. The different fractions were collected according to the absorbances given by the UV/VIS detector at 230 nm.

Gel permeation chromatography (GPC)

Gel permeation chromatography using Sephadex LH-20 (GE Healthcare), a dextran-based polymer with isopropyl side chains, separates compounds based on their hydrodynamic volume. Smaller molecules are retained within the pores of the Sephadex LH20 beads, while larger molecules pass through quicker (Janson, 1987). In this work the selected sample was dissolved

in MeOH and loaded onto a pre-packed Sephadex-LH20 column and eluted isocratically with MeOH. The volume of the collected fractions was approx. 3–5 mL depending of the absorbances given be the employed UV/VIS detector at 230 nm.

4.4 Extraction for HPLC-profiling and TPC

The ground and homogenized samples were extracted with MeOH. For this purpose, 500 µL MeOH was added to the micro centrifuge tubes and the samples were shortly vortexed, ultrasonicated for 15 min (VWR Ultrasonic cleaner) and centrifuged for a further 15 min at 14,000 rpm (Eppendorf Centrifuge 5417 R). This formed a pellet at the bottom of the tubes and the supernatant remaining on top was accurately transferred to new pre-weighed 2 mL micro centrifuge tubes. This extraction procedure was performed three times and the supernatants of each extraction step were pooled. The MeOH was evaporated and the samples were subsequently placed in a vacuum rotary evaporator to remove any remaining solvent (Savant–Integrated SpeedVac System ISS 100) for a further 15 min and then weighed again to determine the mass of the obtained extract. DMSO was then added to the dried samples to achieve a concentration of 10 mg/mL.

4.5 Extraction and Isolation of constituents of *Notopleura panamensis*

4.5.1 Leaves

Air dried material of the leaves, weighing 46.3 g, was subjected to MeOH extraction. Therefore, the leaves of *N. panamensis* were ground in a commercial kitchen mill and then covered in a slight excess of MeOH. The mixture was then sonicated for 15 minutes and filtered using filter paper (Carl Roth GmbH + Co. KG, ROTILABO® Typ 600 Cellulose, 500 × 500 mm). This process was repeated 3 times in total yielding 5.4 g crude extract. This extract was then processed through liquid-liquid extraction resulting in 400 mg of $CHCl_3$ phase. This extract was then separated using a CC resulting in 16.7 mg of fraction 6–9 which was further purified using GPC, yielding 1.8 mg of pure sample labeled ST-PN03 (**3**) in fractions 18–20. This purified compound was further analyzed by NMR spectroscopy and mass spectrometry.

4.5.2 Stem

Air dried material of the stem, weighing 49.5 grams, was subjected to MeOH extraction, yielding 3.5 g of crude extract. This filtrate was further purified through liquid-liquid extraction resulting in 100 mg of the ethyl acetate phase. The ethyl acetate phase was then separated using GPC, yielding 59 mg in the fractions 16–19. These fractions were further purified using MPLC

resulting in the desired fraction 27, weighing 3.1 mg of the sample labeled ST-PN02 (**4**). This purified compound was further analyzed by NMR spectroscopy and mass spectrometry.

4.6 Calibration for harounoside (**1**) and 2-aza-anthraquinone (**2**)

Linear calibration curves were established for the two substances harounoside (**1**) and 2-aza-anthraquinone (**2)** as external standards to be able to determine the concentration of the compounds in the investigated samples. Pre-existing stock solutions of harounoside (**1**) and 2-aza-anthraquinone (**2**), isolated from *N. polyphlebia*, were purified by gel permeation chromatography (GPC). The purified fractions were subsequently used to prepare dilution series. The dilution series consisted of five different concentrations for each measured substance (Table 3). Measurements were then carried out using HPLC and the results plotted and determined using a linear regression whereby the following equation: y= ax+b and the coefficient of determination (R^2) was determined.

Table 3. Calibration data for harounoside (**1**) and 2-aza-anthraquinone (**2**).

Compound	conc. [mg/mL]	area	Reg. parameters	R^2
harounoside (**1**)	0.9890	9144.8	y= 13.032x + 163.24	0.993
	0.4945	5109.9		
	0.2473	2455.4		
	0.1236	1243.5		
	0.0618	639.4		
2-aza-anthraquinon (**2**)	0.7752	10163.3	y=15.862x + 44.886	0.9992
	0.3876	5033.3		
	0.1938	2401.0		
	0.0969	1250.7		
	0.0485	611.3		

4.7 Antifeedant assay with *Spodoptera littoralis*

Spodoptera littoralis (Boisduval) also known as Egyptian cotton leafworm belonging to the family Noctuidae, is a moth native to Africa, Madagascar, Europe, and the Middle East. Its distribution in Europe is limited by low temperatures during winter, as this species lacks a diapause stage (Miller, 1976, Sidibe & Lauge, 1977, CABI, 2022). The European and Mediterranean Plant Protection Organization (EPPO) classifies *S. littoralis* as an A2 quarantine

pest (OEPP/EPPO, 2015), with the primary phytosanitary concern being its introduction into greenhouses where it can damage a wide range of crops. While insecticide control is available, widespread resistance and a lack of effective biological control methods pose significant challenges (CABI, 2022).

For this study the larvae of *S. littoralis* were used, due to their highly polyphagous feeding habits (Brown & Dewhurst, 1975; Prasad & Bhattacharya, 1975; Holloway, 1989; EPPO, 2024). The Egyptian cotton leafworm has been reported to attack more than 40 plant families, including at least 87 economically important species and is therefore a major pest worldwide (Salama et al., 1970; EPPO, 2024). For this reason, *S. littoralis* and its relatives *S. exigua* and *S. frugiperda* has already been used in a large number of studies and thus provides good comparative data (Sneh et al., 1981; Martinez & van Emden, 2001; Pavela, 2005; Korrat et al., 2012; Shishehbor & Hemmati, 2022)

The *Spodoptera littoralis* caterpillars used for these experiments were reared from eggs on white bean and yeast-based artificial diet (prepared according to in-house recipe: 150 g beans, 710 mL water, 1.5 mL vitamin solution, 3 g ascorbic acid, 3 g *para*-hydroxybenzoic acid ethylester (nipagin), 30 g yeast, 7 g agar-agar) and maintained in an environmental chamber with a 12h day-night light rhythm at room temperature (22 ± 2 °C). All experiments were carried out with 3rd instar caterpillars with an approximate size of 1 cm. The caterpillars were starving for 16 h to create the best possible interest for the food provided.

In this assay petri dishes with 5.5 cm diameter were used with moistened filter paper to minimize water loss and wilting of the used food source. The food source was a leaf disc of 1.3 cm diameter of a commercially available lettuce (*Lactuca sativa* L.), which was carefully washed in advance to remove any residues from the leaf surface. Afterwards, 40 µL of each of the prepared substances (Table 4) diluted in water:acetone (80:20) were dripped onto this disc with three replicates each. Whereby caffeine, D-salicin and aristolochic acid were used as positive controls based on the results of Glendinning et al. (2002). The caterpillars were then each placed in the center of the already prepared petri dish exposing them to the lettuce disc with the added substances at room temperature. Additionally, a wilt control with just the leaf discs without caterpillars was performed to monitor the shrinkage caused by water loss.

The petri dishes were then photographed at the beginning of the experiment and after 15, 30, 45 and 60 minutes, as well as after 2 and 3 h, to record the feeding progress. After 3 hours the experiment was aborted and caterpillars undergoing ecdysis (shedding) were excluded from the experiment's evaluation, as feeding is suspended during this process. To minimize disturbance of the caterpillars during the experiment, the petri dishes were not moved during the experiment.

The pictures were then processed using the ImageJ software (Wayne Rasband (NIH)) by adapting the threshold and analyzed using the pixel counting function to measure the ratio between petri dish and leaf disc size. The determined areas are given in Table 4.

Table 4 Overview of *Spodoptera littoralis* (Noctuidae) antifeedant assay listing used controls, test substances and average of cosumed area after 3h.

Substance	**Concentration [mg/mL]**	**Consumed Areas [%] *****
control 1*	0.0	53.65
control 2**	2.5	51,02
caffeine	2.5	11,25
D-salicin	2.5	24,61
aristolochic acid	2.5	23,85
harounoside (**1**)	2.5	37,31
	1.25	27,68
	0.625	38,42
2-aza-anthraquinone (**2**)	2.5	18,56
	1.25	30,68
	0.625	24,35

* leaf disc + caterpillar without solvent; ** leaf disc + caterpillar with solvent water/acetone 80:20; *** average of consumed area at the end of the experiment

5. Results & Discussion

5.1. Comparative SPM Diversification of the *Psychotria* Alliance

5.1.1 HPLC-profiling

The analysis of chemical compound classes using HPLC-UV-diode array detection across the *Psychotria* alliance reveals a chemically diverse landscape. To illustrate the differences between the investigated genera, a representative HPLC profile was selected for each genus and the UV-spectra of the main peaks allowed the assignment of these compounds to the respective classes of compounds using the in-house UV database (Figure 15) (Berger et al., 2021 and references cited therein). While *Eumachia* and *Geophila* (Figure 15A, B) exhibited highly complex chromatograms with limited substance identification including potential flavonoid glycosides, chlorogenic acid, and various unknowns, other genera presented a clearer picture of their major metabolic trends. For instance, *Psychotria* species (Figure 15E) predominantly accumulated catechin derivatives and showed no hint of enrichment of alkaloids (Figure 16). This contrasts with *Palicourea (*Figure 15D), which mainly accumulated monoterpene indole alkaloids like strictosidine and derivatives.

The qualitative investigation is also mirrored by the assessed quantities of the classes of compounds. For this purpose, the peak areas of derivatives belonging to respective classes of compounds were quantified, summed up and compared using authentic standards (Table A5). Condensed tannins were further pooled with other phenolics. In *Palicourea* phenolics like flavonoids were also present beside alkaloids (Figure 16) but no condensed tannins could be identified (Figure 15D). This aligns with previous findings of Berger et al. (2021, and references cited therein) and Kornpointner et al. (2020), which highlighted the broad chemically distinct alkaloid diversification within *Palicourea*, where strictosidine and its derivatives are the main compounds, followed by phenolics except condensed tannins and a few iridoid derivatives. In contrast, *Eumachia* and *Geophila* contained only a minor amount of phenolics and of iridoids (Figure 16) that could be identified in this study, followed by *Rudgea (*Figure 15F), indicating the presence of unknown iridoid derivatives (Figure 16). The results from the investigated *Eumachia* samples are consistent with the work of Tarazona Montoya (2023) in which flavonoid derivatives were identified. The genus *Notopleura* (Figure 15C) exhibits primarily phenolics followed by quinones and a few iridoids (Figure 16). This suggests a degree of chemical specialization within the *Psychotria* alliance. This variation naturally leads to questions about the distribution of specialized metabolites. Berger (2012) also explored the

phytochemical diversity within the *Psychotria* alliance, noting the difficulties in systematic classification due to the group's morphological uniformity. Berger (2012) discovered that *Notopleura* species accumulate 2-aza-anthraquinone, a compound not found in other genera, and Kostyan (2017) further investigated the specialized metabolites present in *Notopleura*, revealing the presence of further quinones and megastigmanes. Furthermore, the undetectable monoterpene indole alkaloid content in *Notopleura* (Figure 16) indicates a distinct chemical profile for this genus within the *Psychotria* alliance, potentially characterized by a shift towards different metabolic pathways and the accumulation of unique compounds such as 2-aza-anthraquinone, which aligns with the findings of Kostyan (2017) and Berger (2012).

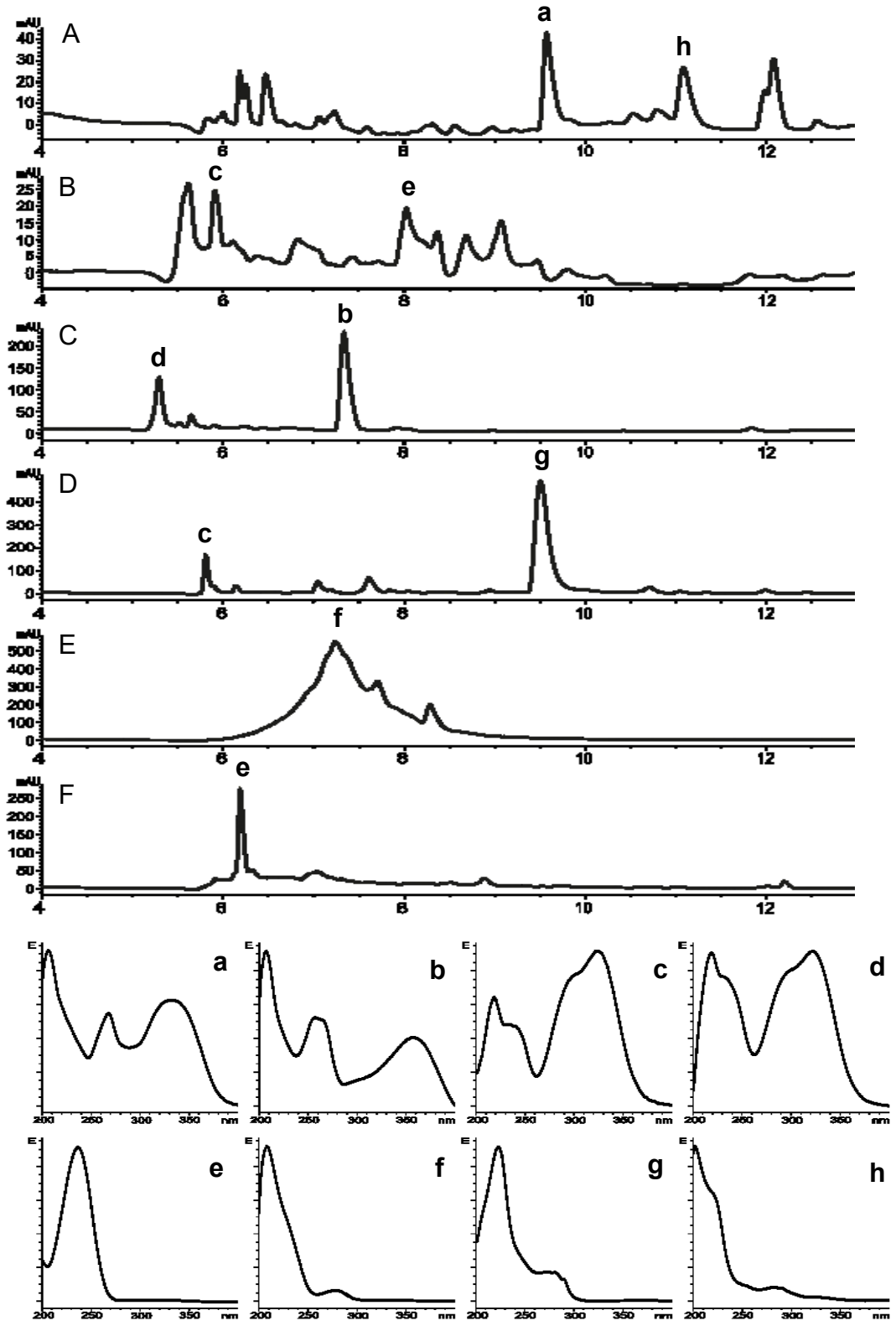


Figure 15 HPLC profiles of selected species belonging to various genera from the *Psychotria* alliance. A=*Eumachia haematocartpa*, B=*Geophila sp.,* C=*Notopleura pithecobia*, D=*Palicourea guianensis*, E=*Psychotria psychotriifolia*, F=*Rudgea reducticalyx*, depicted at 230 nm. Including UV-spectra of the most significant peaks present in the chromatograms. Comparison with UV-spectra of previously purified compounds indicated most likely **a=** as flavone glycoside (Holzbach et al., 2021), **b=** kaempferol or quercetin glycoside (Holzbach et al., 2021), **c** and **d=** chlorogenic acid derivatives; Berger et al., 2016), **e=** megastigmane *d*erivative (Kornpointner et al., 2022), **f=** catechine derivative (Berger, 2012), **g=** strictosidine (Berger et al., 2017). No match was obtained *for* **h**.

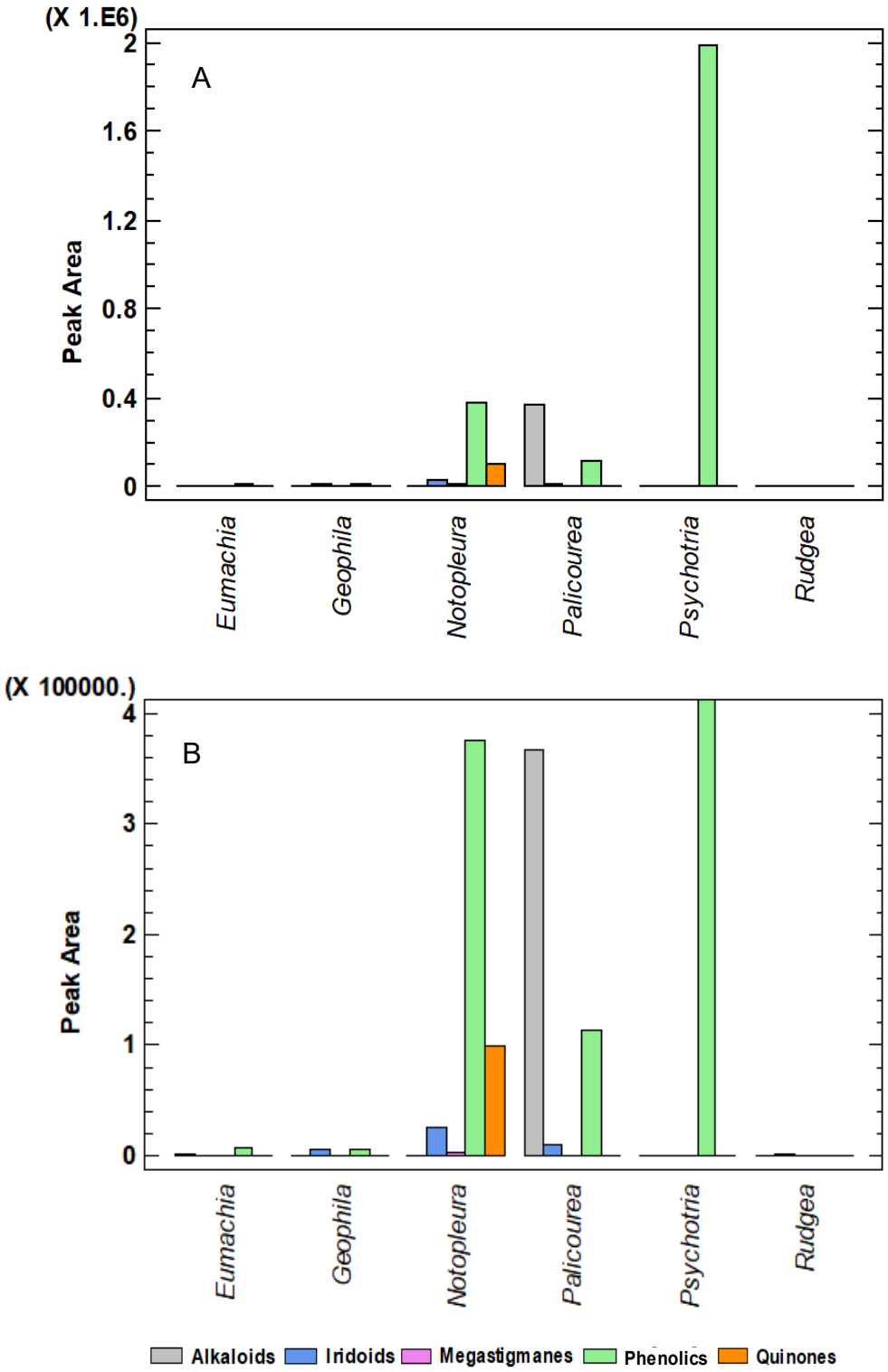


Figure 16 Quantitative assessment of peak areas of different compound groups obtained from HPLC profiles from the six different genera: *Eumachia* (18 samples/6 individuals), *Geophila* (10/4), *Notopleura* (66/39), *Palicourea* (191/64), *Psychotria* (114/38), *Rudgea* (12/4). A: original, B: magnified

5.1.2 Total phenolic content

Complementing the analysis of the generic comparison, a broader assessment of total phenolic content across these genera further supports the observed chemical divergence. As illustrated in Figure 17, the distribution of total phenolics, measured in gallic acid equivalents (GAE), confirms the trends seen in the previous analysis. The results obtained indicated a significantly higher phenolic content in *Psychotria* than in *Palicourea* and *Notopleura* (Figure 17), which reflects and supports the results in the previously mentioned findings of our HPLC analysis (Figure 16). *Palicourea* exhibits higher levels of total phenolics as compared to *Eumachia*, *Geophila*, *Notopleura* and *Rudgea*. These data suggest that the chemical distinction of *Palicourea* is not limited to alkaloids but extends to the accumulation of specialized metabolites belonging to various classes of compounds. However, it is important to note that the total phenolic content determined with the Folin-Ciocalteu method is not specific to phenols, but reflects the total reducing capacity of the samples, which can influence the interpretation of the results since the reagent used show also positive reactions with other substances e.g. sugars, proteins, organic acids (Cilliers et al., 1990; Prior et al., 2005; Everette et al., 2010; Pèrez et al., 2023).

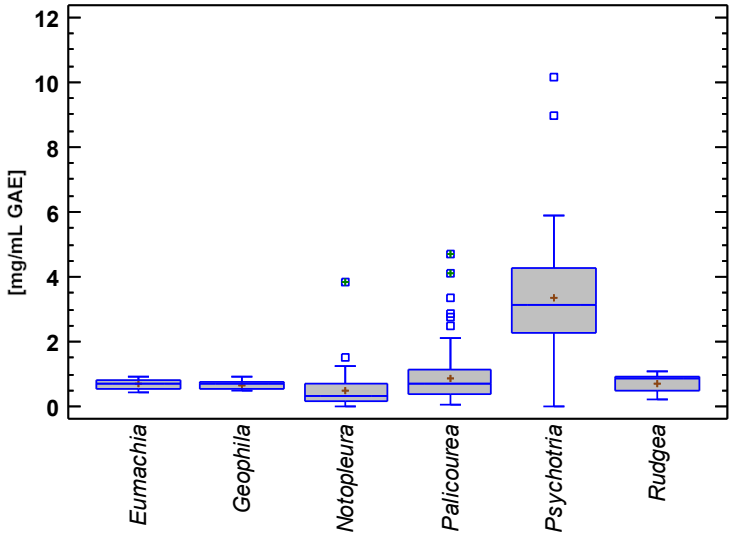


Figure 17 Total phenolic content given in mg/mL gallic acid equivalent (GAE) of the selected genera: *Eumachia* (17 samples/6 individuals), *Geophila* (10/4), *Notopleura* (92/41), *Palicourea* (190/64), *Psychotria* (114/38) and *Rudgea* (12/4).

5.2 *Notopleura*

5.2.1 HPLC-profiling

A representative HPLC profile was chosen for each species tested to visually illustrate the differences within the genus *Notopleura*. The primary identified compounds included quercetin and kaempferol derivatives, chlorogenic acid, harounoside (**1**), 2-aza-anthraquinone (**2**), iridoids, and methylated dihydroflavonol glycosides (Figure 18). Notably, the distribution of these compounds in the quantitative analysis was highly species-specific, as demonstrated by analysis of chemical profiles presented in Figure 19 (Table A6), which reveals distinct differences between epiphytic and terrestrial species. The epiphytic species *N. maxonii*, *N. peperomiae* and *N. pithecobia* exhibit a uniform pattern of substance distribution, characterized by a high content of chlorogenic acid and iridoids, while flavonoids, particularly kaempferol derivatives, are present only in small amounts. In contrast, the chemical profile of terrestrial species is significantly more complex. A common feature was the absence of detectable iridoids and an increased presence of quinones, especially 2-aza-anthraquinone derivatives. A notable exception is *N. siggersiana*, whose chemical profile differs drastically from all other terrestrial species. This unique metabolite profile might indicate a transitional evolutionary chemical position between terrestrial and epiphytic growth forms. The distribution of chlorogenic acid, kaempferol glycosides, and 2-aza-anthraquinones is highly diverse among the remaining terrestrial species and does not show a clearly common pattern. These results partially contradict the findings of Berger et al. (2021), who proposed quinones as a defining characteristic for *Notopleura*. The present study refutes this hypothesis, especially for the epiphytic species. However, the assumption by Berger et al. (2021) could still be relevant for the terrestrial species.

Interestingly, in the species *N. nepokroeffiae, N. anomothyrsa* and *N. costaricensis* no compounds were detectable by HPLC. This is primarily attributed to the fact that the concentrations of these compounds were below the detection limit of the applied HPLC method. To investigate the possibility that these species might contain terpenoids, a class of compounds that often lack chromophoric groups, thin-layer chromatography (TLC) analysis using anisaldehyde as staining reagent was performed. However, the TLC analysis also yielded no significant spots on the plates, suggesting that terpenoids or other classes of compounds without chromophoric groups were not present at detectable levels or were absent in the extracts of these species. Therefore, the absence of detectable compounds in the HPLC analysis is likely due to their low concentration or the absence of the specific compound classes targeted by the

methods used. Further investigation using more sensitive techniques or targeting different compound classes may be necessary to fully characterize the chemical profiles of these species.

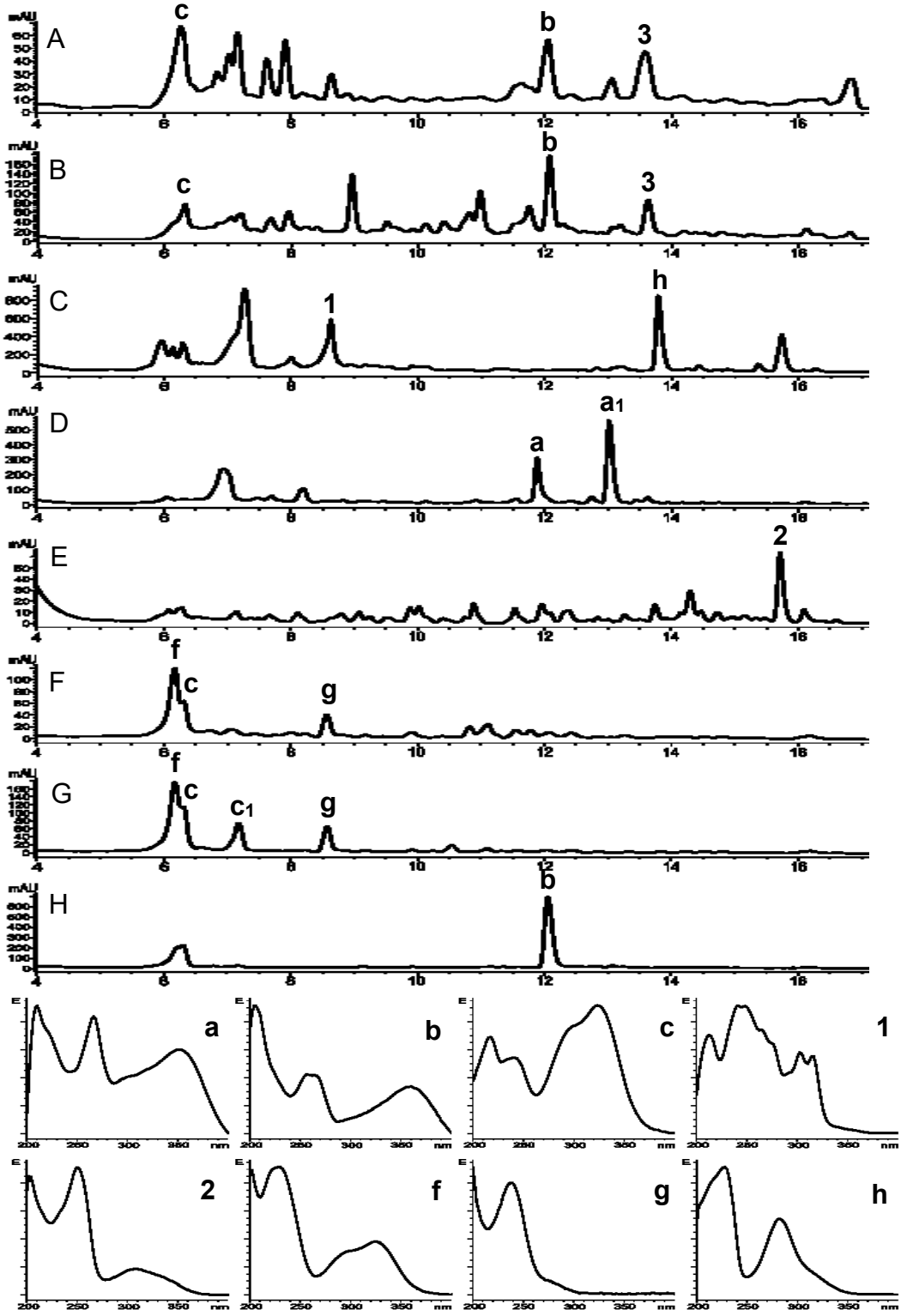


Figure 18 HPLC profiles of selected *Notopleura* species. Subg. *Notopleura:* A=*N.* aff. *panamensis,* B=*N. panamensis,* C=*N. polyphlebia,* D=*N. tolimensis,* E=*N. uliginosa.* Subg. *Viscagoga:* F=*N. maxonii,* G=*N. peperomiae,* H=*N. pithecobia,* depicted at 230nm. Including UV-spectra of the most significant peaks present in the chromatograms. Comparison with UV-spectra of previously purified compounds indicated most likely **a**= as flavone glycoside (Holzbach et al., 2021), **b**= kaempferol or quercetin glycoside (Holzbach et al., 2021), **c**= chlorogenic acid derivatives (Berger et al., 2016), **1**= harounoside, **2**= 2-aza-anthraquinone, **g**= iridoid, **h**= 7,4‘-Di-OMe-aromadendrin-5-*O*-ApiGlc; No match was obtained from **f**; x_1= almost identical UV spectrum.

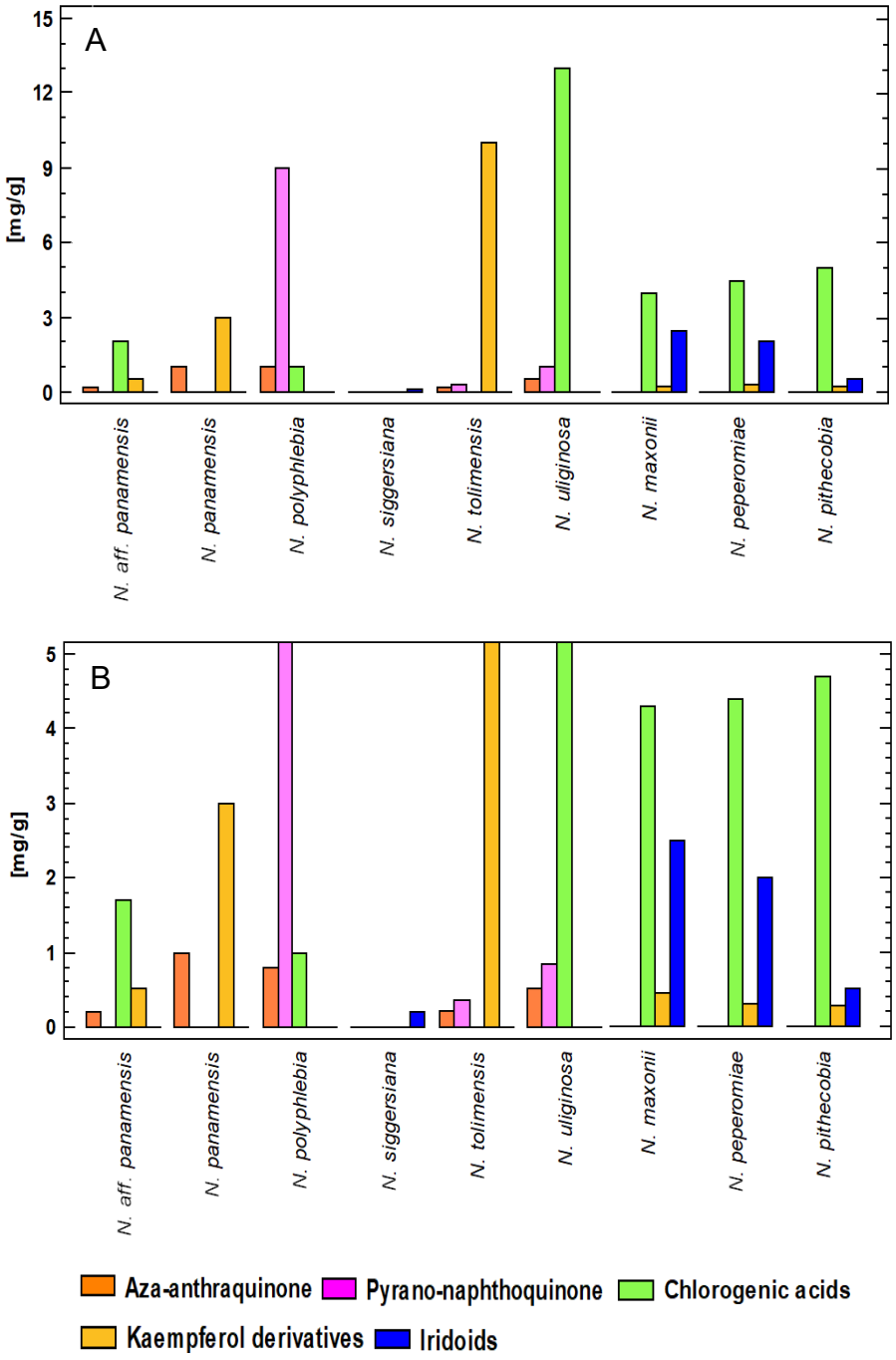


Figure 19 Quantitative comparison of peak areas of distribution of metabolites found among per *Notopleura* species given in mg g^{-1} dry weight. Subg. *Notopleura: N.* aff. *panamensis* (9 samples/3 individuals), *N. panamesis* (1/1), *N. polyphlebia* (10/6), *N. siggersiana* (1/1), *N. tolimensis* (4/4), *N. uliginosa* (15/11). Subg. *Viscagoga*: *N. maxonii* (9/3*), N. peperomiae* (9/3), *N. pithecobia* (4/2). A: original, B: magnified

5.2.2 Total phenolic content

In contrast to the distinct differences in the distribution of specific substances, the analysis of the total phenolic content (Figure 20) reveals no clear distinction between terrestrial and epiphytic species as indicated by the HPLC profiles (Figure 19). Within the epiphytic group, the total phenolic content varies considerably. For example, *N. maxonii* (mean 0.19 mg/mL) and *N. peperomiae* (0.25 mg/mL) exhibit a rather low content, whereas *N. pithecobia* (0.80 mg/mL) shows a higher content (Table A7). These differences suggest that the epiphytic habitat alone does not lead to a uniform phenolic composition. Similarly, among the terrestrial species, the amount of total phenolics varies strongly among the different species. No clear pattern is obvious for a distinct separation between the two habitat types and taxonomic entities. The results suggest that factors other than habitat, such as genetic differences or specific adaptations to local environmental conditions, may have a significant influence on the total phenolic content.

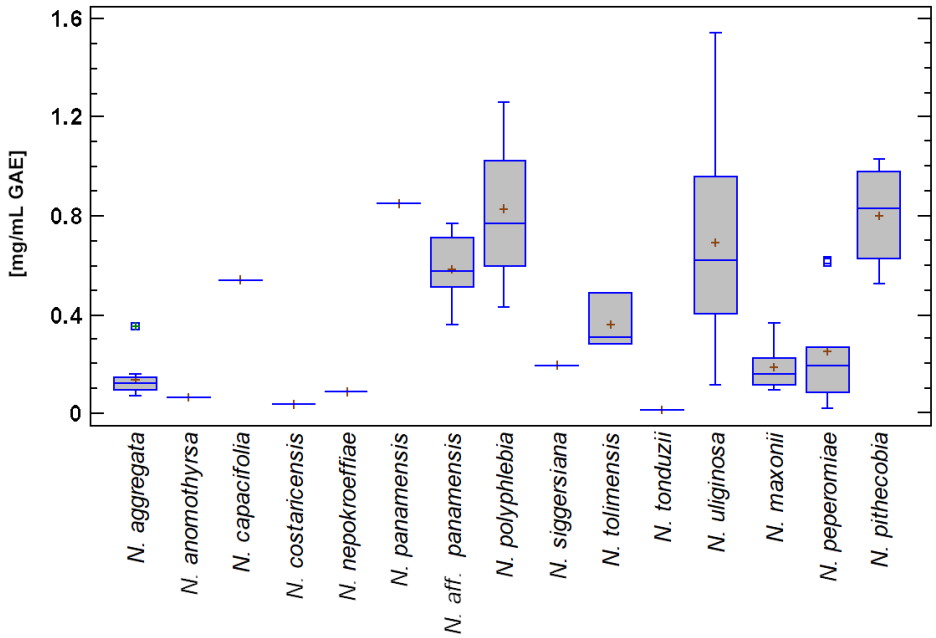


Figure 20 Total phenolic content of *Notopleura* species. The values are given in mg/mL gallic acid equivalents (GAE). Subg. *Notopleura: N. aggregata* (9 samples / 3 individuals), *N. anomothyrsa* (1/1), *N. capacifolia* (1/1), *N. costaricensis* (1/1), *N. nepokroeffiae* (1/1), *N. panamensis* (1/1), *N.* aff. *panamensis* (9/3), *N. polyphlebia* (11/7), *N. siggersiana* (1/1), *N. tolimensis* (3/3), *N. tonduzii* (1/1), *N. uliginosa* (14/8). Subg. *Viscagoga: N. maxonii* (9/3), *N. peperomiae* (9/3), *N. pithecobia* (4/2).

5.2.3 *Notopleura panamensis*

In the course of this research, the two compounds harounoside (**1**) and 2-aza-anthraquinone (**2**) could be identified by comparative HPLC analyses, and the two compounds 6-hydroxy-2-aza-anthraquinone (**3**) and the neolignan glochidioboside (**4**) (Figure 21) were isolated and structurally identified from *Notopleura panamensis* specimens collected in 2022. The compound 6-hydroxy-2-aza-anthraquinone (**3**) represents a so far undescribed compound, structurally related to 2-aza-anthraquinone (**2**). It was obtained as a white powder from the leaves of *N. panamensis* (Figure 21A) and exhibited characteristic UV absorption maxima in MeOH at 230 nm (log ε 4.14), 278 nm (4.07), 306 nm (3.78), and 350 nm (3.74). Structural features were further confirmed by detailed NMR spectroscopic analysis carried out by Univ.-Prof. Dipl.-Chem. Dr. Lothar Brecker and his team at the Department of Organic Chemistry of the University of Vienna. 6-Hydroxy-2-aza-anthraquinone (**3**) is a derivative of 2-aza-anthraquinone and both are characterized by a nitrogen atom incorporating at position 2 of the aromatic ring system, replacing a carbon atom. The found compound **3** is further characterized by the presence of an additional hydroxyl group (-OH) at C-6, which leads to increased polarity and therefore it may influence the compound's electronic properties, solubility, and reactivity. The hydroxyl group at the 6-position also has the potential to participate in hydrogen bonding, affecting the compound's biological activity and stability (Carey & Sundberg, 2007). This structural modification differentiates 6-hydroxy-2-aza-anthraquinone (**3**) from its most likely parent compound, **2** and potentially alters its chemical behavior, including its UV-VIS absorption and other spectroscopic characteristics (Zollinger, 1987).

The observed differential distribution of 6-hydroxy-2-aza-anthraquinone (**3**) in the leaves and of 2-aza-anthraquinone (**2**) in the stem material of *N. panamensis* may be attributed to several ecological, biochemical, and physiological factors. 6-Hydroxy-2-aza-anthraquinone (**3**), found predominantly in the leaves, could serve a protective role against herbivores, pathogens, or environmental stressors such as UV radiation. As the leaves are more exposed to these factors, the presence of this compound might be an adaptive defense mechanism. The tissue-specific production of these compounds could also be a result of distinct biosynthetic pathways regulated by tissue-specific enzymes or gene expression, with the hydroxylation step in 6-hydroxy-2-aza-anthraquinone (**3**) possibly requiring enzymatic activity more prevalent in leaves. Further investigation into the root tissues would be valuable to determine the presence and concentration of each compound, and to elucidate whether the biosynthetic pathway originates in the roots. Notably, while the study by Abouzeid et al. (2019) focused on stress-induced modifications of indole alkaloids, the same principle of enzymatic transformation of

existing secondary metabolites in result to stress, such as the hydroxylation of **2**, could also play a role in this differential distribution. Cytochrome P_{450} enzymes are known as key enzymes in alkaloid modifications, are versatile and might mediate such transformations in response to tissue-specific stress or developmental cues.

The neolignane glochidioboside (**4**) was identified in the stem of *N. panamensis* (Figure 21B) through HPLC and NMR. While glochidioboside (**4**) is a known compound first discovered by Takeda et al. (1998) in *Glochidion* (Phyllanthaceae), its detection in the genus *Notopleura* represents a novel finding, as it has not been previously reported in this plant group to the best of our knowledge. A previous report indicated antibacterial properties of glochidioboside, effectively combating both general and antibiotic-resistant pathogenic bacteria by disrupting bacterial membrane integrity (Lee et al., 2015). Furthermore, **4** exhibits antifungal activity against various pathogenic fungi, showing minimal harm to human red blood cells, which suggests a selective action. The mechanism behind its antifungal effect, similar to its antibacterial action, involves disrupting cell membranes, leading to depolarization, permeabilization, and shrinkage of fungal cells (Lee et al., 2014). The identification of these compounds highlights the potential of *N. panamensis* to biosynthesize 2-aza-anthraquinone and neolignans, providing a foundation for further exploration of their ecological roles and functional relevance.

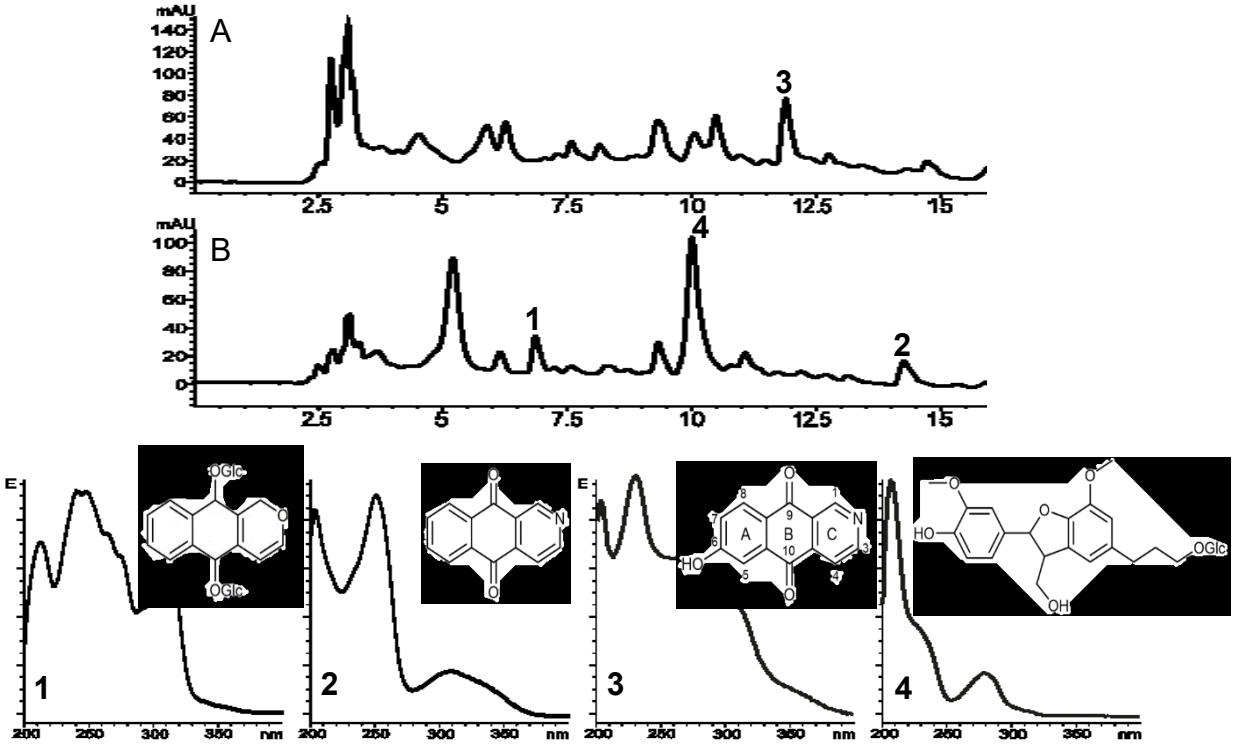


Figure 21 HPLC profiles of *Notopleura panamensis*. A=leaf, B=stem, depicted at 230nm. Including UV-spectra of the most significant peaks present in *N. panamensis* given in Figure 21. Comparison with UV-spectra of previously purified compounds indicated **1**= harounoside, **2**= 2-aza-anthraquinone, **3**= 6-hydroxy-2-aza-anthraquinone, **4**= glochidioboside.

5.3 Antifeedant assay with *Spodoptera littoralis*

The bioassay results have been grouped to assure a better readability. For the sake of simplicity, the controls (Figure A23), the comparison with the already known substances (Figure 22A), as well as the different concentrations of harounoside (**1**) (Figure 22B) and 2-aza-anthraquinone (**2**) (Figure 22C) were summarized in separate graphs. Initial controls of the wilt demonstrated minimal negligible wilting over the 180-minute experimental period, confirming that leaf disk consumption was primarily due to caterpillar feeding. Furthermore, the nearly identical feeding rates observed in the control 1 (leaf disc + caterpillar) and the control 2 (water:acetone, 80:20 + leaf disc + caterpillar) (Figure A23) showed no effect on the insect's behavior due to the used solvents, which allows us to conclude that any alternations in feeding patterns are primarily because of the compounds themselves. A Comparison to the known bitter compounds like aristolochic acid, a known feeding deterrent (Figure 22A) revealed a significant antifeedant effect of **2** indicating either a strong bitter or another deterrent effects which influences the uptake. In contrast, compound **1** showed minimal antifeedant activity, with leaf consumption patterns similar to the untreated control. Concentration-dependent studies (Figure 22C) showed that **2** exhibited a clear dose-response relationship. Increasing concentrations resulted in a progressive reduction in leaf disk consumption. Notably, at higher concentrations of **2**, the leaf disks tended to roll or curl, which complicated accurate measurement of the consumed area using the image analysis software. However, even with this technical challenge, a clear visual difference in leaf consumption was evident, confirming the potent antifeedant activity of **2**. Harounoside (**1**), however, did not show a comparable concentration-dependent effect (Figure 22B), further supporting its weak antifeedant properties. These findings suggest that **2** could potentially serve as a natural antifeedant in plant defense strategies. Further research is required to elucidate the specific mechanisms of action and to develop methods to quantify the effect of leaf curling on leaf area measurements.

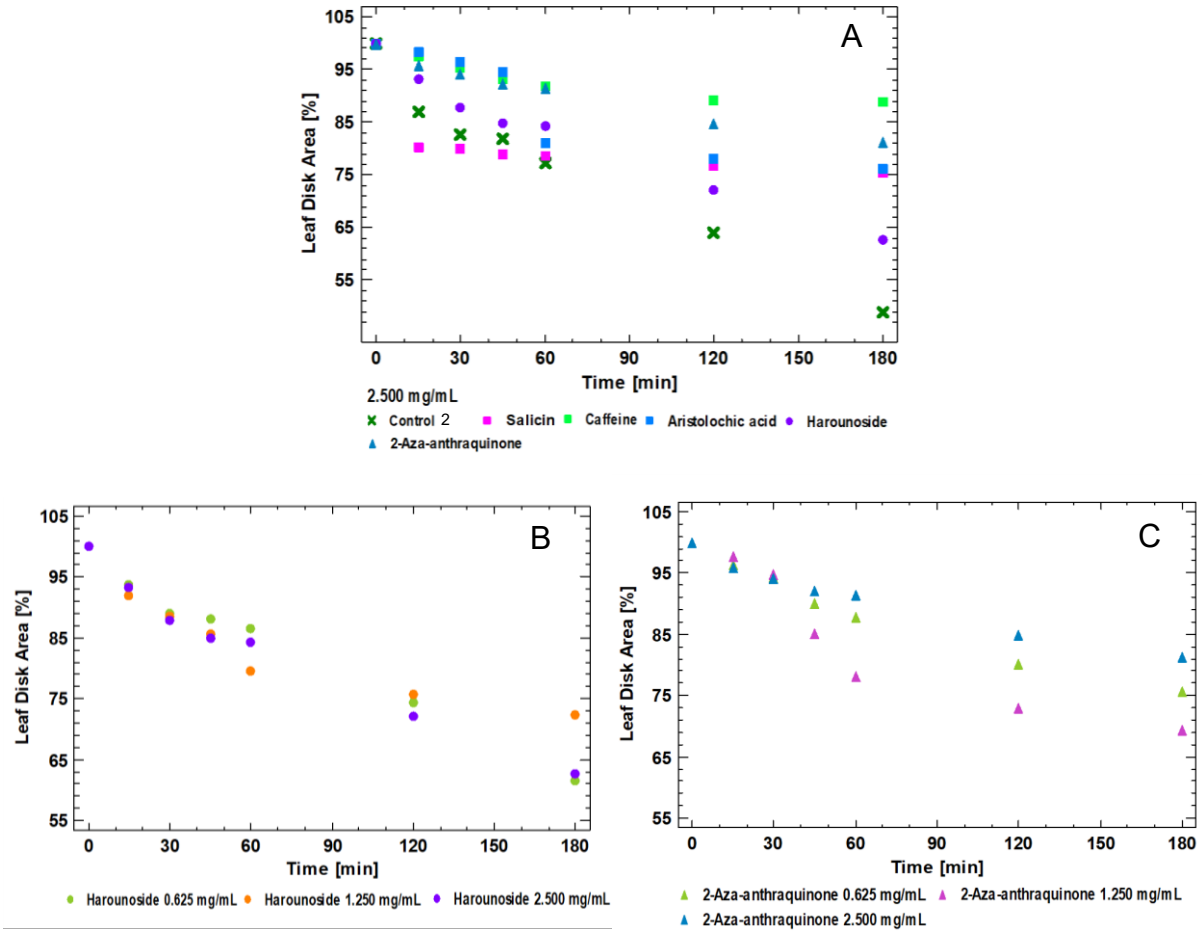


Figure 22: Antifeedant assay with *Spodoptera* caterpillars showing the remaining leaf disc area over time in %. A) comparison of the control 2 (caterpillar with solvent water/acetone 80:20) response and the already known "bitter" compounds (salicin, caffeine and aristolochic acid) with those of our two compounds of interest: harounoside (**1**) and 2-aza-anthraquinone (**2**) isolated from *Notopleura* species; B) results of the three different concentrations 0.625 mg/mL (green), (1.25 mg/mL (orange) and 2.5 mg/mL (violet) of harounoside (**1**); C) results of the three different concentrations 0.625 mg/mL (green), 1.250 mg/mL (pink) and 2.5 mg/mL (blue) of **2**.

6. Conclusion

Chemical analysis across Costa Rican species belonging to the *Psychotria* alliance reveals notable metabolic differences between genera. The tested species of *Eumachia* and *Geophila* exhibited complex chromatograms with numerous unidentified compounds. *Psychotria* primarily produced catechin derivatives, while *Palicourea* accumulated alkaloids, particularly strictosidine in this study. *Notopleura* stood out for its unique accumulation of quinones, highlighting a specialized metabolic pathway not seen in other genera. Overall, *Psychotria* is characterized by phenolics, *Palicourea* by alkaloids, and some *Notopleura* by quinones, suggesting distinct chemical strategies within the alliance.

The comparison of chemical profiles of different *Notopleura* species revealed a clear difference between terrestrial and epiphytic *Notopleura* species indicating metabolic differences. Terrestrial species, such as *N. polyphlebia*, exhibited a more complex metabolic profile with quinones like 2-aza-anthraquinone (**2**)**,** while epiphytic species showed more uniform patterns with higher concentrations of chlorogenic acid and iridoids. These differences suggest that the two groups of species have adapted to their respective environments through distinct metabolic strategies.

The detailed analysis of *Notopleura panamensis* further illustrated the genus's specialized metabolic profile. A novel compound, 6-hydroxy-2-aza-anthraquinone (**3**)**,** was isolated from the leaves, and its hydroxylated structure at position 6 suggests increased polarity, which could enhance its biological activity and contribute to the plant's defense mechanisms. In contrast, 2-aza-anthraquinone (**2**) was found in the stem alongside with the neolignane glochidioboside (**4**), likely playing a role in structural defense or protection against mechanical damage.

The conducted antifeedant assay revealed that 2-aza-anthraquinone (**2**) displayed significant antifeedant properties against *Spodoptera littoralis* caterpillars, supporting its role in plant defense. In contrast, harounoside (**1**) exhibited minimal antifeedant activity. These results imply that quinones may play a key role in plant defense in *Notopleura* species, suggesting that the plant utilizes a combination of specialized metabolites as part of its defensive strategy.

7. References

Abraham, I., Joshi, R., Pardasani, P., Pardasani, R.T. (2011). Recent advances in 1,4-benzoquinone chemistry. Journal of the Brazilian Chemical Society 22(3), 385–421.

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

Abouzeid, S., Beuling, U., Selmar, D. (2019). Stress-induced modification of indole alkaloids: Phytomodificines as new category of specialized metabolites. Phytochemistry 159, 102–107. Doi: 10.1016/J.phytochem.2018.12.015.

Ajikumar, P.K., Tyo, K., Carlsen, S., Mucha, O., Phon, T.H., Stephanopoulos, G. (2008). Terpenoids: Opportunities for biosynthesis of natural product drugs using engineered microorganisms. Molecular Pharmaceutics 5, 167–190.

American Chemical Society (2013). Molecule of the Week Archive–Chlorogenic acid retrieved 11.03.2025 Chlorogenic acid–American Chemical Society, retrieved March 2025.

Andersson, L. (2002b). Relationships and generic circumscriptions in the *Psychotria* complex (Rubiaceae, Psychotrieae). Systematics and Geography of Plants 72(1–2), 167–202.

Andriotis, V.M., Kruger, N.J., Pike, M.J., Smith, A.M. (2010). Plastidial glycolysis in developing *Arabidopsis* embryos. New Phytologist 185, 649–662.

Atanasov, A. G., Waltenberger, B., Pferschy-Wenzig, E. M., Linder, T., Wawrosch, C., Uhrin, P., ... & Malainer, C. (2015). Discovery and resupply of pharmacologically active plant natural products: A review. Biotechnology Advances 33(8), 1582–1614.

Atteia, H.H., Arafa, M. H., Mohammad, N. S., Amin, D. M. and Sakr, A. T. (2021). Thymoquinone upregulates miR-125a-5p, attenuates STAT3 activation, and potentiates doxorubicin antitumor activity in murine solid Ehrlich carcinoma, Journal of Biochemical and Molecular Toxicology 35, e22924.

Ayinampudi, S.R. (2017). Isolation, absolute configuration and bioactivities of megastigmanes or C13 isonorterpinoides. Chemistry International 3(1), 69–91.

Baden, C.U., Dobler, S. (2009). Potential benefits of iridoid glycoside sequestration in *Longitarsus melanocephalus* (Coleoptera, Chrysomelidae). Basic and Applied Ecology 10, 27–33.

Barceló, J., Poschenrieder, C. (2002). Fast root growth responses, root exudates, and internal detoxification as clues to the mechanism of *Aluminium* toxicity and resistance: a review. Environmental and Experimental Botany 48, 75–92.

Barrabé, L., Buerki, S., Mouly, A., Davis, A.P., Munzinger, J., Maggia, L. (2012). Delimitation of the genus *Margaritopsis* (Rubiaceae) in the Asian, Australasian and Pacific region, based on molecular phylogenetic inference and morphology. Taxon 61(6):1251–1268. Doi: 10.1002/tax.616007.

Baumesa. R., Wirtha, J., Bureaub, S., Gunatac, Y., Razunglesa, A. (2002). Biogeneration of C13-norisoprenoid compounds: Experiments supportive for an apo-carotenoid pathway in grapevines. Analytica Chimica Acta 458, 3–14.

Bayer, R.G., Stael, S., Csaszar, E., Teige, M. (2011). Mining the soluble chloroplast proteome by affinity chromatography. Proteomics 11, 1287–1299.

Bentham, G., Hooker, J. D. (1873). Rubiaceae. In: Genera plantarum, 2(1), 7–151, London.

Bentley, R. (1975). Biosynthesis of Quinones. In: T. A. Geissman and R. Bentley, in Biosynthesis, ed. T. A. Geissman, The Royal Society of Chemistry, 1975, vol. 3, pp. 181–246.

Bentley, R. and Campbell, I.M. (1974). In: The chemistry of Quinonoid Compounds (S. Patai, ed.), pp. 683–736, Wiley, New York.

Berger, A., Tanuhadi, E., Brecker, L., Schinnerl, J., Valant–Vetschera, K. (2017). Chemodiversity of tryptamine–derived alkaloids in some Costa Rican *Palicourea* species (Rubiaceae–Palicoureeae). Phytochemistry 143, 124–131.

Berger, A. (2012). Distribution and systematic significance of selected secondary metabolites within Psychotrieae/Palicoureeae (Rubiaceae). Diplomarbeit, University of Vienna.

Berger, A., Fasshuber, H., Schinnerl, J., Brecker, L., Greger, H. (2012). Various types of tryptamine-iridoid alkaloids from *Palicourea acuminata* (=*Psychotria acuminata* Rubiaceae). Phytochemistry Letters 5(3), 558–562. Doi: 10.1016/j.phytol.2012.05.013.

Berger, A., Preinfalk, A., Windberger, M., Fasshuber, H.K., Gastegger, M., Klose, I., Robien, W., Felsinger, S., Brecker, L., Valant-Vetschera, K., Schinnerl, J. (2016). New reports on flavonoids, benzoic- and chlorogenic acids as rare features in the *Psychotria* alliance (Rubiaceae). Biochemical Systematics and Ecology 66, 145–153. Doi: 10.1016/j.bse.2016.02.027.

Berger, A., Valant-Vetschera, K., Schinnerl, J., Brecker, L. (2021). A revised classification of the sister tribes Palicoureeae and Psychotrieae (Rubiaceae) indicates genus-specific alkaloid accumulation. Phytochemistry Reviews 21, 941–986. Doi: 10.1007/s11101-021-09769-x.

Bernays, E.A. and Chapman, R.F. (1994) Host-Plant Selection by Phytophagous Insects. Chapman &Hall, NewYork, p. 312.

Biere, A., Marak, H.B., van Damme, J.M. (2004). Plant chemical defense against herbivores and pathogens: generalized defense or trade-offs? Oecologia 140, 430–441.

Bleeker, P.M., Spyropoulou, E.A., Diergaarde, P.J., Volpin, H., De Both, M.T., Zerbe, P., Bohlmann, J., Falara, V., Matsuba, Y., Pichersky, E. (2011). RNA-seq discovery, functional characterization, and comparison of sesquiterpene synthases from *Solanum lycopersicum* and *Solanum habrochaites* trichomes. Plant Molecular Biology 77, 323–336.

Blois, M.S. (1958). Antioxidant determinations by the use of a stable free radical. Nature, 181, 1199–1200.

Boland, W. (2017). Deciphering the route to cyclic monoterpenes in *Chrysomelina* leaf beetles: source of new biocatalysts for industrial application? Zeitschrift Für Naturforschung C 72(9–10), 417–427. Doi: 10.1515/znc-2017-0015.

Bolkart, K.H, and Zenk, M.H. (1968). Tyrosine, a precursor of the quinone ring of 2,7-dimethyl-naphtoquinone (Chimaphilin), Naturwissenschaften 55, 444–445. Doi: 10.1007/BF00602666.

Bondet, V., Brand-Williams, W., Berset, C. (1997). Kinetics and mechanism of antioxidant activity using the DPPH free radical method. Lebensmittel-Wissenschaft & Technologie 30, 609–615.

Borhidi, A. (2011). Transfer of the Mexican species of *Psychotria* subgen. *Heteropsychotria* to *Palicourea* based on morphological and molecular evidences. Acta Botanica Hungarica 53(3-4), 241–250.

Bourgaud, F., Gravot, A., Milesi, S., Gontier, E. (2001). Production of plant secondary metabolites: a historical perspective. Plant Science 161, 839–851. Doi: 10.1016/S0168-9452(01)00490-3.

Bowers, M. D., & Stamp, N. E. (1997). Effect of hostplant genotype and predators on iridoid glycoside content of pupae of a specialist insect herbivore, *Junonia coenia* (Nymphalidae). Biochemical Systematics and Ecology 25(7), 571–580.

Bremekamp, C.E.B. (1934). Notes on the Rubiaceae of Surinam. Recueil des Travaux Botaniques Neerlandais 31, 248–308.

Bremer, B. (2009). A review of molecular phylogenetic studies of Rubiaceae. Annals of the Missouri Botanical Garden 96, 4–26. Doi: 10.3417/2006197.

Bremer, B., Eriksson, T. (2009). Timetree of Rubiaceae – Phylogeny and dating the family, subfamilies and tribes. International Journal of Plant Sciences 170(6), 766–793.

Broughton, W.J., Zhang, F., Perret, X., Staehelin, C. (2003) Signals exchanged between legumes and Rhizobium: agricultural uses and perspectives. Plant Soil 252, 129–137.

Brown, E.S., Dewhurst, C.F. (1975). The genus *Spodoptera* in Africa and the Near East. Bulletin of Entomological Research 65, 221-262.

Burdock, G.A. (2010). Fenaroli's handbook of flavor ingredients. CRC press. Boca Raton Doi: 10.1201/9781439847503.

CABI (2022). *Spodoptera littoralis* (cotton leafworm). CABI Compendium, CABI International, CABI Head Office, Wallingford, UK, Doi: 10.1079/cabicompendium.51070 (accessed 2024-12-17).

Calixto, N.O., Pinto, M.E.F., Ramalho, S.D., Burger, M., Bobey, A.F., Young, M.C.M., Bolzani, V.S., Pinto, A.C. (2016). The genus *Psychotria*: Phytochemistry, chemotaxonomy, ethnopharmacology and biological properties. Journal of the Brazilian Chemical Society 27(8), 1355–1378. Doi: 10.5935/0103-5053.20160149.

Carey, F.A., & Sundberg, R.J. (2007). Advanced Organic Chemistry (Parts A). Springer New York. Doi: 10.1007/978-0-387-44899-2.

Cavill, G.W., Ford, D.L., Locksley, H.D. (1956). The chemistry of ants. 1. Terpenoid constituents of some Australian *Iridomyrmex* species. Australian Journal of Chemistry 9, 288–293.

Chen, D., Mubeen, B., Hasnain, A., Rizwan, M., Adrees, M., Naqvi, S.A.H., Shahzad, S.M., Hussain, M., Alharby, H.F., Irshad, M.K., et al. (2022). Role of promising secondary metabolites to confer resistance against environmental stresses in crop plants: Current scenario and future perspectives. Front. Plant Sci. 2022; 13:881032. Doi: 10.3389/fpls.2022.881032.

Cho, H.K., Suh, W.S., Kim, K.H., Kim, S.Y., Lee, K.R. (2014). Phytochemical Constituents of *Salsola komarovii* and Their Effects on NGF Induction. Natural Product. Sciences, 20, 95–101.

Christiansen, J.V., Isbrandt, T., Petersen, C., Sondergaard, T.E., Nielsen, M.R., Pedersen, T.B., Sørensen, J.L., Larsen, T.O. and Frisvad, J.C. (2021). Fungal quinones: diversity, producers, and applications of quinones from *Aspergillus, Penicillium, Talaromyces, Fusarium*, and *Arthrinium*, Applied Microbiology and Biotechnology 105(21–22), 8157-8193.

Cilliers, J., Singleton, V., Lamuela-Raventos, R. (1990). Total polyphenols in apples and ciders; correlation with chlorogenic acid. Journal of Food Science 55, 1458–1459.

Clifford, M.N. (2000). Chlorogenic acids and other cinnamates – nature, occurrence, dietary burden, absorption and metabolism. Journal of the Science of Food and Agriculture 80, 1033-1043. Doi: 10.1002/(SICI)1097-0010(20000515)80:7<1033::AID-JSFA595>3.0.CO;2-T.

Cooper, J.E. (2004). Multiple Responses of rhizobia to flavonoids during legume root infection. Advances in Botanical Research 41(41), 1–62.

Covington, A.D. (2009). Tannin chemistry: the science of leather. Royal Society of Chemistry.

Cragg, G.M., & Newman, D.J. (2013). Natural products: a continuing source of novel drug leads. Biochimica et Biophysica Acta (BBA)-General Subjects 1830(6), 3670–3695.

Csekits, S. (2008). Blütenbiologische Beobachtungen an ausgewählten Rubiaceen des Esquinas-Regenwaldes (Costa Rica). Diploma thesis, University of Vienna.

D'Amelia V., Aversano R., Chiaiese P., Carputo D. (2018). The antioxidant properties of plant flavonoids: Their exploitation by molecular plant breeding. Phytochemistry Reviews. 2018; 17:611–625. Doi: 10.1007/s11101-018-9568-y.

Dapkevicious, A., Venskutonis, R., Van Beek, T.A., & Linssen, J.P.H. (1998). Antioxidant activity of extracts obtained by different isolation procedures from some aromatic herbs grown in Lithuania. Journal of the Science of Food and Agriculture 77, 140–146.

Dave, H., Ledwani, L. (2012). A review on anthraquinones isolated from *Cassia* species and their applications. Indian Journal of Natural Products and Resources. 3, 291–319.

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.

Dayan, F.E., Cantrell, C.L., & Duke, S.O. (2009). Natural products in crop protection. Bioorganic & Medicinal Chemistry 17(12), 4022–4034. Doi: 10.1016/j.bmc.2009.01.046.

de Carvalho Jr., A.R., Vieira, I.J.C., de Carvalho, M.G., Braz-Filho, R., Lima, M.A.S., Ferreira, R.O., Maria, J.E., de Oliveira, D.B. (2017). ^{13}C-NMR Spectral data of alkaloids isolated from *Psychotria* species (Rubiaceae). Molecules 22(1), 103. Doi: 10.3390/molecules22010103.

De Luca, V., Salim, V., Thamm, A., Masada, S.A., Yu, F. (2014). Making iridoids/ secoiridoids and monoterpenoid indole alkaloids: progress on pathway elucidation. Current Opinion in Plant Biology 19, 35–42.

DeBruyne, T., Pieters, L., Deelstra, H., Vlietinck, A. (1999), Condensed vegetable tannins: biodiversity in structure and biology activities. Biochemical Systematics and Ecology 27, 445–459.

Degenhardt, J., Köllner, T.G., Gershenzon, J. (2009b). Monoterpene and sesquiterpene synthases and the origin of terpene skeletal diversity in plants. Phytochemistry 70, 1621–1637.

Delprete, P.G. (2004). Rubiaceae. - In: Smith, N., Mori S. A., Henderson, A., Stevenson, D. W., Heald, S. V. (eds), Flowering Plants of the Neotropics. Princeton University Press. ISBN: 9780691116945, 328-333

Delprete, P.G., Kirkbride, Jr. J.H. (2015). New combinations in *Eumachia* (Rubiaceae) for species occurring on the Guiana Shield. Journal of the Botanical Research Institute Texas 9(1), 75–79.

Dewick, P.M. (2001). The mevalonate and deoxyxylulose phosphate pathways: terpenoids and steroids. In: Medicinal Natural Products. John Wiley & Sons, Ltd. p. 167 289.

Dewick, P. M. (2009). Medicinal natural products: a biosynthetic approach. John Wiley & Sons, Ltd. p. 187-310, ISBN:9780470741689.

Dias, M.C., Pinto, D.C.G.A., Silva, A.M.S. (2021). Plant Flavonoids: Chemical Characteristics and Biological Activity. Molecules 26(17), 5377. Doi: 10.3390/molecules26175377.

Dighe, S.N. and Collet, T. A. (2020). Recent advances in DNA gyrase-targeted antimicrobial agents, European Jorunal of Medicinal Chemistry 199, 112326.

Dinda, B., Chowdhury, D.R., Mohanta, B.C. (2009). Naturally occurring iridoids, secoiridoids and their bioactivity. An updated review, Part 3. Chemical and Pharmaceutical Bulletin 57, 765–96.

Dinda, B., Debnath, S., Banik, R. (2011). Naturally occurring iridoids and secoiridoids. An updated review, Part 4. Chemical and Pharmaceutical Bulletin 59, 803–33.

Dinda, B., Debnath, S. (2013). Monoterpenes: iridoids. In: Ramawat KG, Mérillon J-M, editors. Natural products: phytochemistry, botany and metabolism of alkaloids, phenolics and terpenes. Berlin, Heidelberg: Springer 3009–67.

Dobkin de Rios, M. (1972). Visionary vine: Psychedelic healing in the Peruvian Amazon. Chandler Publishing Company, San Francisco, CA, USA.

Dobler, S., Petschenka, G., Pankoke, H. (2011). Coping with toxic plant compounds – the insect's perspective on iridoid glycosides and cardenolides. Phytochemistry 72, 1593–604.

Dong, M., Xiaozhi, M., Tianyu, X., Nianjie, F., Mengyun, Z., Xurui, Y., Yi, H., Mengzhou, Z. and Qian, W. (2024). Recent research on the physicochemical properties and biological activities of quinones and their practical applications: a comprehensive review. Food & Function 18, 8973–8997. Doi: 10.1039/d4fo02600d.

Dongfeng, Y., Xuhong, D., Xiao L., Ruilian H., Zongsuo L., Yan, L., Fenghua L., Jianjun, Z. (2012). Different Roles of the Mevalonate and Methylerythritol Phosphate Pathways in Cell Growth and Tanshinone Production of *Salvia miltiorrhiza* Hairy Roots. PloS ONE. 7. e46797. Doi: 10.1371/journal.pone.0046797.

Dudareva, N., Pichersky, E., Gershenzon, J. (2004). Biochemistry of plant volatiles. Plant Physiology 135, 1893–1902.

Dudareva, N., Klempien, A., Muhlemann, J.K. and Kaplan, I. (2013). Biosynthesis, function and metabolic engineering of plant volatile organic compounds. New Phytologist, 198, 16–32. Doi: 10.1111/nph.12145.

Dulo, B., Phan, K., Githaiga, J. (2021). Natural Quinone Dyes: A Review on Structure, Extraction Techniques, Analysis and Application Potential. Waste and Biomass Valorization 12, 6339–6374. Doi: 10.1007/s12649-021-01443-9.

Durand, R., and Zenk, M.H. (1974). The homogentisate ring-cleavage pathway in the biosynthesis of acetate-derived naphthoquinones of the Droseraceae. Phytochemistry 13, 1483–1492.

Dussourd, D.E. and Eisner, T. (1987). Vein-cutting behavior: insect counterploy to latex defence of plants. Science, 237, 898–901.

Duthie, G. (1999). Determination of activity of antioxidants in human subjects. The Proceedings of the Nutrition Society 58, 1015–1024. Doi: 10.1017/S0029665199001330.

Duval, J., Pecher, V., Poujol, M., Lesellier, E. (2016). Research advances for the extraction, analysis and uses of anthraquinones: A review. Industrial Crops and Products 94, 812–833. Doi: 10.1016/j.indcrop.2016.09.056.

Dwyer, J.D. (1980). Part IX, Family 179. Rubiaceae – Part 1, In: Woodson, R.E.Jr., Schery, R.W., and Collaborators. Flora of Panama. Annals of the Missouri Botanical Garden 67, 286.

Eisenreich, W., Schwarz, M., Cartayrade, A., Arigoni, D., Zenk,M.H., Bacher, A. (1998). Thedeoxyxylulose phosphate pathway of terpenoid biosynthesis in plants and microorganisms. Chemistry and Biology 5, R221–R233.

El-Sayed, M., Verpoorte, R. (2007). *Catharanthus* terpenoid indole alkaloids: biosynthesis and regulation. Phytochemistry Reviews 6(2-3), 277–305.

Elshamy, A.I., Mohamend, T.A., Ibrahim, M.A.A., Atia, M.A.M, Yoneyama, T., Umeyama, A., Hegazy, M.-E.F. (2021). Two novel oxetane containing lignans and a new megastigmane from *Paronychia arabica* and in silico analysis of them as prospective SARS-CoV-2 inhibitors. RSC Advances 11, 20151–20163, Doi: 10.1039/d1ra02486h.

EPPO (2024). *Spodoptera littoralis*. EPPO datasheets on pests recommended for regulation. https://gd.eppo.int (accessed 2024-11-27).

Everette, J.D., Bryant, Q.M., Green, A.M., Abbey, Y.A., Wangila, G.W., Walker, R.B. (2010). Thorough study of reactivity of various compound classes toward the Folin−Ciocalteu reagent. Journal of Agricultural and Food Chemistry, 58, 8139–8144.

Fairbairn and Muhtadi, (1972). The Biosynthesis and metabolism of anthraquinones in *Rumex obtusifolius.* Phytochemistry 11, 215–219.

Ferreyra, F., Lorena, M., Rius, S., Casati, P. (2012). Flavonoids: biosynthesis, biological functions, and biotechnological applications. Frontiers in Plant Science 3, 222. Doi: 10.3389/fpls.2012.00222, ISSN=1664-462X.

Ferrer, J.L., Austin, M.B., Stewart Jr., C., Noel, J.P. (2008). Structure and function of enzymes involved in the biosynthesis of phenylpropanoids. Plant Physiology Biochemistry 46(3), 356–70. Doi: 10.1016/j.plaphy.2007.12.009.

Folin, O., Ciocalteu, V. (1927). On Tyrosine and Tryptophane determinations in proteins. Journal of Biological Chemistry, Volume 73, Issue 2, 1927, Pages 627–650, ISSN 0021-9258.

Ghisalberti, E.L. (1998). Biological and pharmacological activity of naturally occurring iridoids and secoiridoids. Phytomedicine 5, 147–63.

Glendinning, J.I., Davis, A., Ramaswamy, S. (2002). Contribution of Different Taste Cells and Signalling Pathways to the Discrimination of "Bitter" Taste Stimuli by an Insect. The Journal of Neuroscience 22(16), 7281–7287. Doi: 10.1523/JNEUROSCI.22-16-07281.2002.

Gong, X.-P., Sun, Y.-Y., Chen, W., Guo, X., Guan, J.K-., Li, D.-Y., Du, G. (2017). Antidiarrheal and anti-inflammatory activities of aqueous extract of the aerial part of *Rubia cordifolia*. BMC Complement, Alternative Medicine Review 17, 20. Doi: 10.1186/s12906-016-1527-9.

Govaerts, R., Ruhsam, M., Andersson, L., Robbrecht, E., Bridson, D., Davis, A., Schanzer, I., Sonké, B. (2011). World Checklist of Rubiaceae. The Board of Trustees of the Royal Botanic Gardens, Kew. Published on the Internet; http://www.kew.org/wcsp/rubiaceae/ accessed January 2025.

Grantz, A.A., Brummell, D.A., Bennett, A.B. (1995). Ascorbate free radical reductase mRNA levels are induced by wounding. Plant Physiologie 108, 411–418.

Guan, Z., Xue, D., Abdallah, I.I., Dijkshoorn, L., Setroikromo, R., Guiyuan, L. (2015). Metabolic engineering of *Bacillus subtilis* for terpenoid production. Applied Microbiology and Biotechnology 99, 9395–9406.

Guerriero, G., Berni, R., Muñoz-Sanchez, J.A., Apone, F., Abdel-Salam, E.M., Qahtan, A.A., Alatar, A.A., Cantini, C., Cai, G., Hausman, J.F., Siddiqui, K.S., Hernández-Sotomayor, S.M.T., Faisal, M. (2018). Production of Plant Secondary Metabolites: Examples, Tips and Suggestions for Biotechnologists. Genes (Basel) 9(6), 309. Doi: 10.3390/genes9060309.

Gupta A, Atanasov AG, Li Y, Kumar N, Bishayee A. (2022). Chlorogenic acid for cancer prevention and therapy: Current status on efficacy and mechanisms of action. Pharmacological Research 186, 106505. Doi: 10.1016/j.phrs.2022.106505.

Gutteridge, J.M.C., Halliwell, B. (2006). Free Radicals and Antioxidants in the Year 2000: A historical look to the future. Annals of the New York Academy of Science 899, 136–147. Doi: 10.1111/J.1749-6632.2000.tb06182.x.

Hahlbrock, K., Grisebach, H. (1979). Enzymic controls in the biosynthesis of lignin and flavonoids. Annual Review of Plant Physiology 30, 105–30.

Hahlbrock, K., Scheel, D. (1989). Physiology and molecular biology of phenylpropanoid metabolism. Annual Review of Plant Physiology and Plant Molecular Biology 40, 347–69.

Halliwell, B., Adhikary, A., Dingfelder, M., and Dizdaroglu, M. (2021). Hydroxyl radical is a significant player in oxidative DNA damage in vivo, Chemical Society Reviews 50, 8355–8360.

Harborne, J.B. (1993). Introduction to Ecological Biochemistry, 4th edn, Academic Press, London.

Hartmann, T. (2007). From waste products to ecochemicals: fifty years research of plant secondary metabolism. Phytochemistry 68, 2831–46.

Heil, M. (2008). Indirect defence via tritrophic interactions. New Phytologist 178, 41–61. Doi: 10.1111/j.1469-8137.2007.02330.x.

Herath, H.M.U.L., Piao, M.J., Kang, K.A., Fernando, P.D.S.M., Kang, H.K., Koh, Y.S., Hyun, J.W. (2024). The inhibitory effect of chlorogenic acid on oxidative stress and apoptosis induced by PM2.5 in HaCaT keratinocytes. Journal of Biochemical and Molecular Toxicology 38(9), e23806. Doi: 10.1002/jbt.23806.

Henderich, M., Neubert, C., Winterhalter, P., Schreier, P., Skouroumounis, G.K. (1992). Identification of C13-norisoprenoid flavour precursors in starfruit (*Averrhoa carambola* L.). Flavour and Fragrance Journal 7, 179–185. Doi: 10.1002/ffj.2730070403.

Herrmann, K. (1997). Occurrence and content of hydroxycinnamic and hydroxybenzoic acid compounds in foods. Critical reviews in food science and nutrition 37(5), 385–401.

Holden, Claire. (2019). Antioxidant Properties of Three *Salvia* Species and the Anti-proliferative Properties of *Salvia officinalis* on Triple Negative Breast Cancer Cells. Bachelor's thesis, Belmont University.

Holloway, J.D. (1989). The moths of Borneo: Family Noctuidae, trifine subfamilies: Noctuinae, Heliothinae, Hadeninae, Acronictinae, Amphipyrinae, Agaristinae. Malayan Nature Journal 42, 57–226.

Holzbach, B., Reuter, V., Bacher, M., Schinnerl, J., Brecker, L., Rosenau, T., Valant-Vetschera, K. (2021). Flavonoid diversification in different leaf compartments of *Primula auricula* (Primulaceae), Biochemical Systematics and Ecology 98, 104310, ISSN 0305-1978, Doi: 10.1016/j.bse.2021.104310.

Huang, Y.X., Tan, H.X., Guo, Z.Y., Wu, X.X., Zhang, Q.L., Zhang, L. (2016). The biosynthesis and genetic engineering of bioactive indole alkaloids in plants. Journal of Plant Biology 59, 203–14.

Hungria, M., Stacey, G. (1997). Molecular signals exchanged between host plants and rhizobia: basic aspects and potential application in agriculture. Soil Biology and Biochemistry 29, 819–830.

Inderjit, S., Gross E (2000) Plant phenolics: potential role in aquatic and terrestrial ecosystems. In: Martens S, Treutter D, Forkmann G (eds) Polypenols, pp 206–234.

Isman, M.B. (2008). Plant essential oils as natural insect repellents. Journal of pesticides science 33(3), 213–223.

Jacobs, J., Claessens, S., de Kimpe, N. (2008). First straightforward synthesis of 1-hydroxy-3,4-dihydro-1H-benz[g]isochromene- 5,10-dione and structure revision of a bioactive benz[g]isochromene-5,10-dione from *Psychotria camponutans*. Tetrahedron 64(2), 412–418. Doi: 10.1016/j.tet.2007.10.063.

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. Nature Chemistry 9(12), 1165–1169. Doi: 10.1038/nchem.2825.

Janson, J.C. (1987). On the history of the development of Sephadex. Chromatographia 23, 361–365. Doi: 10.1007/BF02316183.

Jeckel, A.M.., Beran, F., Züst, T., Younkin, G., Petschenka, G., Pokharel, P., Dreisbach, D., Ganal-Vonarburg, S.C., Robert, C.A.M. (2022). Metabolization and sequestration of plant specialized metabolites in insect herbivores: Current and emerging approaches. Frontiers in Physiology 13, 1001032. Doi: 10.3389/fphys.2022.1001032.

Jensen, S.R. (1991). Plant iridoids their biosynthesis and distribution in angiosperms. In: Harborne JB, Tomas-Barberan FA, editors. Eco logical chemistry and biochemistry of plant terpenoids. Oxford: Clarendon Press 1991, 133–58.

Jeremić, S., Amić, A., Stanojević-Pirković M., and Marković, Z. (2018). Selected anthraquinones as potential free radical scavengers and P-glycoprotein inhibitors. Organic & Biomolecular Chemistry 16, 1890–1902.

Jerkovic, I., Kus, P.M. (2014). Terpenes in honey: occurence, origin and their role as chemical biomarkers. RSC Advances 4, 31710–31728. Doi: 10.1039/C4RA04791E.

Jeyasri, R., Muthuramalingam, P., Karthick, K., Shin, H., Choi, S.H., Ramesh, M. (2023). Methyl Jasmonate and Salicylic Acid as Powerful Elicitors for Enhancing the Production of Secondary Metabolites in Medicinal Plants: An Updated Review. Plant Cell Tissue Organ Cult 53, 447–458. Doi: 10.1007/s11240-023-02485-8.

Joyard, J., Ferro, M., Masselon, C., Seigneurin-Berny, D., Salvi, D., Garin, J., Rolland, N. (2010). Chloroplast proteomics highlights the subcellular compartmentation of lipid metabolism. Progress in Lipid Research 49, 128–158.

Kadereit, J.W., Körner, C., Nick, P., Sonnewald, U. (2021). Strasburger – Lehrbuch der Pflanzenwissenschaften (38. Auflage). Springer Spektrum ISBN: 978-3-662-61942-1.

Karban, R., and Baldwin, I. (1997). Induced Responses to Herbivory. Chicago: University of Chicago Press. Doi: 10.7208/chicago/9780226424972.001.0001.

Karinho-Betancourt, E. (2018). Plant-herbivore interactions and secondary metabolites of plants: Ecological and ecolutionary perspectives. Botanical Sciences 96(1). Doi: 10.17129/botsci.1860.

Kim, A., and Ma, J. Y. (2019). Piceatannol-3-O-β-D-glucopyranoside (PG) exhibits in vitro anti-metastatic and anti-angiogenic activities in HT1080 malignant fibrosarcoma cells, Phytomedicine 57, 95–104.

Kim, D.H., Kim, B.R., Kim, J.Y., Jeong, Y.C. (2000). Mechanism of covalent adduct formation of aucubin to proteins. Toxicology Letters 114, 181–8.

Kobayashi, H., Naciri-Graven, Y., Broughton, W.J., Perret, X. (2004). Flavonoids induce temporal shifts in gene-expression of nod-box controlled loci in Rhizobium sp. NGR234. Molecular Microbiology 51, 335–347.

Kolar, P. (2012). Pflanzliche Chemodiversitätsforschung anhand von *Notopleura polyphlebia* und *Psychotria acuminata*. Bachelor's thesis, Universität Wien.

Kong, J.M., Chia, L.S., Goh,N.K., Chia, T.F., Brouillard, R. (2003). Analysis and biological activities of anthocyanins. Phytochemistry 64, 923–933.

Konno, K., Hirayama, C., Yasui, H., Nakamura, M. (1999). Enzymatic activation of oleuropein: a protein crosslinker used as a chemical defense in the privet tree. Proceedings of the National Academy of Science of the United States of America 96(16), 9159–9164. Doi: 10.1073/pnas.96.16.9159.

Kornpointner, C., Hochenegger, N.J., Shi, B.B., Berger, A., Theiner, J., Brecker, L., Schinnerl, J. (2022). Phytochemistry meets geochemistry – Blumenol C sulfate from *Palicourea luxurians* (Rubiaceae: Palicoureeae). Molecules 27, 7284. Doi: 10.3390/molecules27217284.

Kornpointner, C., Berger, A., Traxler, F., Habziadic, A., Massar, M., Matek, J., Brecker, L., Schinnerl, J. (2020). Alkaloid and iridoid glucosides from *Palicourea luxurians* (Rubiaceae: Palicoureeae) indicate tryptamine and tryptophan iridoid alkaloid formation apart the strictosidine pathway. Phytochemistry 173, 112296. Doi: 10.1016/j.phytochem.2020.112296.

Korrat, E.E.E., Abdelmonem, A.E., Helalia, A.A.R., Khalifa, H.M.S. (2012). Toxicological study of some conventional and nonconventional insecticides and their mixtures against cotton leaf worm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae). Annals of Agricultural Science 57, 145–152.

Kostyan, M.K. (2017). Comparative analysis of secondary metabolites in selected *Notopleura* species. Master thesis, University of Vienna. Doi: 10.25365/thesis.49735.

Kozlowska, A., Szostak-Wegierek, D. (2014). Flavonoids – Food sources and health benefits. Rocz Panstw Zakl Hig 65(2), 79–85.

Kumagai, Y., Shinkai, Y., Miura, T., Cho, A.K. (2012). The chemical biology of naphthoquinones and its environmental implications. Annual Review of Pharmacology and Toxicology 52, 221–247.

Lachenaud, O. (2019). Revision du genre *Psychotria* (Rubiaceae) en Afrique Occidentale et Centrale. Opera Botanica Belgica 17, 1–909.

Landry, L.G., Chapple, C., Last, R.L. (1995). *Arabidopsis* Mutants Lacking phenolic sunscreens exhibit enhanced ultraviolet-B injury and oxidative damage. Plant Physiology 109, 1159–1166. Doi: 10.1104/pp.109.4.1159.

Lange, B.M., Rujan, T., Martin, W., Croteau, R. (2000). Isoprenoid biosynthesis: the evolution of two ancient and distinct pathways across genomes. Proceedings of the National Academy of Sciences of USA 97, 13172–13177.

Lee, D.Y., Lee, S.C., Jeong, Y.T., Jeong, M.K., Lee, K.H., Seo, J.W., Lee, G.S., Kim, S.E., Lee, N.I., Baek, J., Hee, K. (2015). Antimelanogenic effects of picrionoside, an isolated from the leaves of Korean ginseng. Biological and Phramaceutical Bulletin 38, 1663–1667. Doi: 10.1248/bpb.b15-00410.

Lee, H., Choi, H., Ko, H.J., Woo, E.R., Lee, D.G., Lee, H. (2014). Antifungal effect and mode of action of glochidioboside against *Candida albicans* membranes. Biochemical and Biophysical Research Communications 444(1), 30–35. Doi: 10.1016/j.bbrc.2014.01.019.

Lee, H., Woo, E.R., Lee, D.G., Lee, H. (2015). Glochidioboside kills pathogenic bacteria by membrane perturbation. Current Microbiology 71(1), 1–7. Doi: 10.1007/s00284-015-0807-9.

Leistner, E., and Zenk, M.H. (1969). Chrysophanol (1,8-dihydroxy-3-methylanthraquinone) biosynthesis in higher plants. Journal of the Chemical Society D Chemical Communications 210–211.

Leistner, E. (1971). Second pathway leading to anthraquinones in higher plants. Phytochemistry 10, 3015–3020.

Leistner, E. (1980). In „pigments in Plants“ (I.-C. Czygan, ed.), pp. 352–369. G. Fischer Verlag, Stuttgart and New York.

Leistner, E. (1981). Biosynthesis of Plant Quinones. In: Conn EE (ed) The biochemistry of plants, vol VII. Academic Press, London New York, pp 403–423. Doi: 10.1016/B978-0-12-675407-0.50019-1.

Lemos, V.C., Reimer, J.J., Wormit, A. (2019). Color for Life: Biosynthesis and distribution of phenolic compounds in Pepper (*Capsicum annuum*). Agriculture 9(4), 81. Doi: 10.3390/agriculture9040081.

Li, W.W. (2023). Dietary phytochemicals against COVID–19: A focus on thymoquinone, eFood 4, e77.

Luo, Y., Li, G., Li, G., Yan, J., Yi, J., Zhang, G. (2011). Discovery and identification of 2-phenylethyl 2,6-dihydroxybenzoate as a natural lipid-lowering lead. Planta Medica 77(18), 2047–2049. Doi: 10.1055/s-0031-1280118.

Lv, X.J., Li, Y., Ma, S.G., Qu, J., Liu, Y.B., Li, Y.H., Zhang, D., Li L., and Yu, S.S. (2018). “Bioactive Megastigmane Glucosides and Monoterpenes from *Lyonia ovalifolia*.” Journal of Asian Natural Products Research 21 (6), 559–72. Doi: 10.1080/10286020.2018.1509313.

Mahmud, N.M., Paraoan, L., Khaliddin, N., and Kamalden, T.A. (2022). Thymoquinone in Ocular Neurodegeneration: Modulation of Pathological Mechanisms via Multiple Pathways, Frontiers in Cellular Neuroscience 16, 786926.

Maimon, E., Samuni, U., and Goldstein, S. (2018). Studying mechanism of radical reactions: From radiation to nitroxides as research tools, Radiation Physics and Chemistry 143, 14–19.

Mandal, S. M., Chakraborty, D., & Dey, S. (2010). Phenolic acids act as signaling molecules in plant-microbe symbioses. Plant Signaling & Behavior 5(4), 359–368.

Marles, M.A.S., Ray, H., Gruber, M.Y. (2003). New perspectives on pro anthocyanidin biochemistry and molecular regulation. Phytochemistry 64, 367–383.

Martens, S., Preuss, A., and Matern, U. (2010). Multifunctional flavonoid dioxygenases: flavonols and anthocyanin biosynthesis in *Arabidopsis thaliana* L. Phytochemistry 71, 1040–1049.

Martinez, S.S., van Emden, H.F. (2001). Growth disruption, abnormalities and mortality of *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) caused by Azadirachtin. Crop Protection. Neotropical Entomology. 30 (1). Doi: 10.1590/S1519-566X2001000100017.

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

Mathesius, U. (2003). Conservation and divergence of signaling pathways between roots and soil microbes—the Rhizobium-legume symbiosis compared to the development of lateral roots, mycorrhizal interactions and nematode-induced galls. Plant Soil 255, 105–119.

McGarvey, D.J., Croteau, R. (1995). Terpenoid metabolism. Plant Cell 7, 1015–1026.

Meyer, G.W., Bahamon Naranjo, M.A., Widhalm, J.R. (2021). Convergent evolution of plant specialized 1,4-napthoquinones: metabolism, trafficking, and resistance to their allelopathic effects. Journal of Experimental Botany 72(2), 167-176. Doi: 10.1093/jxb/eraa462.

Miller, G.W. (1976). Cold storage as a quarantine treatment to prevent the introduction of *Spodoptera littoralis* (Boisd.) into glasshouses in the UK. Plant Pathology 25(4), 193-196.

Ncube, B., Van Staden, J. (2015). Tilting plant metabolism for improved metabolite biosynthesis and enhanced human benefit. Molecules 20, 12698–12731. Doi: 10.3390/molecules200712698.

Nepokroeff, M., Bremer, B., Sytsma, K. J. (1999). Re-organization of the genus *Psychotria* (Rubiaceae) and tribe Psychotrieae inferred from ITS and rbcL sequence data. Systematic Botany 24(1), 5–27.

Nicholson, R. L., & Hammerschmidt, R. (1992). Phenolic compounds and their role in disease resistance. Annual Review of Phytopathology 30(1), 369–389.

Nomoto, Y., Sugimoto, S., Matsunami, K., Otsuka, H. (2013). Hirtionosides A-C, gallates of megastigmane glucosides, 3-hydroxyoctanoic acid glucosides and a phenylpropanoid glucoside from the whole plants of *Euphorbia hirta*. Journal of Natural Medicines 67, 350–358. Doi: 10.1007/S11418-012-0692-5.

OEPP/EPPO (2015). Diagnostic protocol for *Spodoptera littoralis, Spodoptera litura, Spodoptera frugiperda, Spodoptera eridania*. Bulletin OEPP/EPPO Bulletin, Standards PM 7/124(1) 45, 410–444.

Ohloff, G., Demole, E. (1987). Importance of the odoriferous principle of Bulgarian rose oil in flavor and fragrance chemistry. Journal of Chromatography A 406, 181–183. Doi:10.1016/S0021-9673(00)94029-9.

Oliveira, L.A., Nicolella, H.D., Furtado, R.A., Lima, N.M., Tavares, D.C., TCorrêa, T.A., and Almeida, M.V. (2020). Design, synthesis, and antitumor evaluation of novel anthraquinone derivatives. Bioorganic Chemistry 29, 1611–1620.

Ornelas, J.F., González, C., Jiménez, L., Lara, C., Martínez, A.J. (2004a). Reproductive ecology of distylous *Palicourea padifolia* (Rubiaceae) in a tropical montane cloud forest. II. Attracting and rewarding mutualistic and antagonistic visitors. American Journal of Botany 91(7), 1061–1069.

Ornelas, J.F., Jiménez, L., González, C., Hernández, A. (2004b). Reproductive ecology of distylous *Palicourea padifolia* (Rubiaceae) in a tropical montane cloud forest. I. Hummingbirds' effectiveness as pollen vectors. American Journal of Botany 91(7), 1052–1060.

Otto, M. (2011). *Analytische Chemie.* 4., überarbeitete und ergänzte Auflage. Wiley-VCH, Weinheim 2011, pp. 236–237. ISBN 978-3-527-32881-9.

Pan, S.P., Pirker, T., Kunert, O., Kretschmer, N., Hummelbrunner, S., Latkolik, S.L., Rappai, J., Dirsch, V.M., Zochkov, V., and Bauer, R. (2019). C13 Megastigmane Derivatives from *Epipremnum pinnatum*: β-Damascenone Inhibits the Expression of Pro-Inflammatory Cytokines and Leukocyte Adhesion Molecules as Well as NF-κB Signaling. Frontiers in Pharmacology 10, 1351. Doi: 10.3389/fphar.2019.01351.

Parr, A.J., Bolwell, G.P. (2000). Phenols in the plant and in man. The potential for possible nutritional enhancement of the diet by modifying the phenols content or profile. Journal of the Science of Food and Agriculture 80, 985–1012.

Patel, O.P.S., Beteck, R.M., Legoabe, L.J. (2021). Antimalarial application of quinones; A recent update. European Journal of Medicinal Chemistry 210, 113084.

Paul-Victor, C., Züst, T., Rees, M., Kliebenstein, D. J., and Turnbull, L. A. (2010). A new method for measuring relative growth rate can uncover the costs of defensive compounds in *Arabidopsis thaliana*. New Phytology 187, 1102–1111. Doi: 10.1111/j.1469-8137.2010.03325.x.

Pavela, R. (2005). Insecticidal activity of some essential oils against larvae of *Spodoptera littoralis*, Fitoterapia, 76(7–8), 691–696, ISSN 0367-326X.

Pérez, M., Dominguez-López, I., and Lamuela-Raventós, R.M. (2023). The Chemistry Behind the Folin–Ciocalteu Method for the Estimation of (Poly)phenol Content in Food: Total Phenolic Intake in a Mediterranean Dietary Pattern. Journal of Agricultural and Food Chemistry 71(46), 17543–17553. Doi: 10.1021/acs.jafc.3c04022.

Piesschaert, F., Robbrecht, E., Poulsen, A. D., Smets, E. (1999). Pyrene and pollen observations in the pantropical genus *Geophila* (Rubiaceae-Psychotrieae). Nordic Journal of Botany 19(1), 93–100.

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, 29–36. Doi: 10.2174/1874847300902010029.

Poulin, B., Wright, S.J., Lefebvre, G., Calderón, O. (1999). Interspecifc synchrony and asynchrony in the fruiting phenologies of congeneric bird-dispersed plants in Panama. Journal of Tropical Ecology 15, 213–227.

Powell, R.G., Spencer, G.F. (1988). Phytochemical inhibitors of Velvetleaf (*Abutilon theophrasti*) germination as model for new biorational herbicides, in: Biologically active natural products. 211–232. Doi: 10.1021/bk-1988-0380.ch014.

Prasad, J., Bhattacharya, A.K. (1975). Growth and development of *Spodoptera littoralis* (Boisd.) (Lepidoptera, Noctuidae) on several plants. Zeitschrift für Angewandte Entomologie 79, 34-48. Doi: 10.1111/j.1439-0418.1975.tb02313.x.

Prior, R.L., Wu, X., Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. Journal of Agricultural and Food Chemistry 53, 4290–4302.

Proestos, C., Chorianopoulos, N., Nychas, J.G.E., & Komaitis, M. (2005). RP-HPLC analysis of the phenolic compounds of plant extracts. Investigation of their antioxidant capacity and antimicrobial activity. Journal of Agricultural and Food Chemistry 53,1190–1195.

Pulido, P., Perello, C., Rodriguez-Concepcion, M. (2012). New insights into plant isoprenoid metabolism. Molecular Plant 5, 964–967.

Qiuping, Z., Jingni, W., Fu, L., Honglan, Z., Fengmin, L., Tuo, M., Danrong, L., and Huaxin, H. (2023). Synthesis and DNA interaction of aloe-emodin α-amino phosphate derivatives, Journal of Molecular Structure 1279, 134950.

Raskin, I., Ribnicky, D. M., Komarnytsky, S., Ilic, N., Poulev, A., Borisjuk, N., ... & Kennelly, E. J. (2002). Plants and human health in the twenty-first century. Trends in biotechnology 20(12), 522–531.

Rauha, J.P., Remes, S., Heinonen, M., Hopia, A., Ka¨hko¨nen, M., Kujala, T. (2000). Antimicrobial effects of Finnish plant extracts containing flavonoids and other phenolic compounds. International Journal of Food Microbiology 56, 3–12.

Razafimandimbison, S.G., Kainulainen, K., Wikström, N., Bremer, B. (2017). Historical biogeography and phylogeny of the pantropical Psychotrieae alliance (Rubiaceae), with particular emphasis on the Western Indian Ocean Region. American Journal of Botany 104 (9), 1407–1423. Doi: 10.3732/ajb.1700116.

Razafimandimbison, S.G., Rydin, C. (2024). Phylogeny and classification of the coffee family (Rubiaceae, Gentianales): Overview and outlook. Taxon 73(3), 673–717.

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.

Razafimandimbison, S.G., Taylor, C.M., Wikström, N., Pailler, T., Khodabandeh, A., Bremer, B. (2014). Phylogeny and generic limits in the sister tribes Psychotrieae and Palicoureeae (Rubiaceae): Evolution of schizocarps in *Psychotria* and origins of bacterial leaf nodules of the Malagasy species. Am J Bot 101(7), 1102–1126. Doi: 10.3732/ajb.1400076.

Reshi, Z.A., Ahmad, W., Lukatkin, A.S., Javed, S.B. (2023). From Nature to Lab: A Review of Secondary Metabolite Biosynthetic Pathways, Environmental Influences, and In Vitro Approaches. Metabolites 13(8), 895. Doi: 10.3390/metabo13080895.

Rietveld, W.J. (1983). Allelopathic effects of juglone on germination and growth of several herbaceous and woody species. Journal of Chemical Ecology 9(2).

Rippert, P., Puyaubert, J., Grisollet, D., Derrier, L., and Matringe, M. (2009). Tyrosine and phenylalanine are synthesized within the plastids in *Arabidopsis*. Plant Physiology 149, 1251–1260.

Robbrecht, E. (1988). Tropical woody Rubiaceae. Characteristic features and progressions. Contributions to a new subfamilial classification. Opera Botanica Belgica 1, 1–272.

Robbrecht, E., Manen, J.F. (2006). The major evolutionary lineages of the coffee family (Rubiaceae, Angiosperms). Combined analysis (nDNA and cpDNA) to infer the position of *Coptosapelta* and *Luculia*, and supertree construction based on rbcL, rps16, trnL-trnF and atpB-rbcL data. A new classification in two subfamilies, Cinchonoideae and Rubioideae. Systematics and Geography of Plants 76(1), 85–145.

Rodriguez, S., Marston, A., Wolfender, J.L., Hostettmann, K. (1998). Iridoids and secoiridoids in the Gentianaceae. Current Organic Chemistry 2, 627–648.

Rodriguez, R.J., White Jr., J.F., Arnold, A.E., Redman, R.S. (2009). Fungal endophytes: diversity and functional roles. New Phytologist, 182(2), 314–330. Doi: 10.1111/j.1469-8137.2009.02773.x.

Roy, A., Khan, A., Ahmad, I., Alghamdi, S., Rajab, B., Babalghith, A., Alshahrani, Y., Islam, S., Islam, M. (2022). Flavonoids a Bioactive Compound from Medicinal Plants and Its Therapeutic Applications. BioMed Research International e5445291. Doi: 10.1155/2022/5445291.

Ryan, K.G., Swinny, E.E., Markham, K.R., Winefield, C. (2002). Flavonoid gene expression and UV photoprotection in transgenic and mutant Petunia leaves. Phytochemistry 59, 23–32.

Sabnis, R.W. (2020). Novel Tricyclic Compounds for treating bacterial infection, ACS Medicinal Chemistry Letters Journal 11, 2350–2351.

Salama, H.S., Dimetry, N.Z., Salem, S.A. (1970). On the host preference and biology of the cotton leaf worm *Spodoptera littoralis*. Zeitung für Angewandte Entomologie 67, 261–266. Doi: 10.1111/j.1439-0418.1971.tb02122.x.

Šamec D., Karalija E., Šola I., Bok V.V., Salopek-Sondi B. (2021). The role of polyphenols in abiotic stress response: The influence of molecular structure. Plants 10, 118. Doi: 10.3390/plants10010118.

Santana-Gálvez, J., Csineros-Zevallos, L., Jacono-Velázquez, D.A. (2017). Chlorogenic acid: Recent advances on its dual role as a food additive and a nutraceutical against metabolic syndrome. Molecules 22, 358.

Sanz-Biset, J., Campos-de-la-Cruz, J., Epiquién-Rivera, M. A., Cañigueral, S. (2009). A first survey on the medicinal plants of the Chazuta valley (Peruvian Amazon). Journal of Ethnopharmacology 122(2), 333–362.

Schmid, J., and Amrhein, N. (1995). The molecular organisation of the shikimate pathway in plants. Phytochemistry 39, 739–747. Doi: 10.1016/0031-9422(94)00962-S.

Scott, G. (1997). Antioxidants in Science, Technology, Medicine and Nutrition. Chichester, West Sussex: Albion Publishing.

Sefton, M.A., Skouroumounis, G.K., Massy-Westropp, R.A., Williams, P.J. (1989). Norisoprenoides in V*itis vinifera* white wine grapes and the identification of a precursor of damascenone in these fruits. Australian Journal of Chemistry 42, 2071–2084.

Seigler, D.S. (1998). Plant secondary metabolism. Kluwer Aca. Boston, Dordrecht, London, 1–759.

Shan, M., Yu, S., Yan, H., Chen, P., Zhang, L., Ding, A. (2016). A review of the Botany, Phytochemistry, pharmacology and Toxicology of *Rubiae radix* et Rhizoma, Molecules 21, 1747. Doi: 10.3390/molecules21121747.

Shikov, A.N., Pozharitskaya, O.N., Krishtopina, A.S., and Makarov, V.G. (2018). Naphthoquinone pigments from sea urchins: chemistry and pharmacology. Phytochemistry Review 17, 509–534.

Shishehbor, P., Hemmati, S.A. (2022). Investigating of secondary metabolites in bean cultivars and their impact on nutritional performance of *Spodoptera littorali*s (Lep.: Noctuidae). Bulletin of Entomological Research. 112, 378–388.

Sidibe, B., Lauge, G. (1977). Effect of warm periods and of constant temperatures on some biological criteria in *Spodoptera littoralis* Boisduval (Lepidoptera Noctuidae). Annales de la Societe Entomologique de France 13(2), 369–379.

Simkin, A.J., Guirimand, G., Papon, N., Courdavault, V., Thabet, I., Ginis, O., Bouzid, S., Giglioli-Guivarc'h, N., Clastre, M. (2011). Peroxisomal localisation of the final steps of the mevalonic acid pathway in planta. Planta 234, 903–914.

Singh, A.K., Rani, V., Singh, V., Singh, A.K., Kumar, A., Singh, R.P., Meena, R.S., Behera, T.K. (2022). Plant Secondary Metabolites as Defense Tools against Herbivores for Sustainable Crop Protection. International Journal of Molecular Science 23(5), 2690. Doi: 10.3390/ijms23052690.

Smirnoff, N. (1993). The role of active oxygen in the response of plants to water deficit and desiccation. New Phytology 125, 27–58. Doi: 10.1111/j.1469-8137.1993.tb03863.x.

Sneh, B., Schuster, S., Broza, M. (1981). Insecticidal activity of Bacillus thuringiensis strains against the Egyptian cotton leafworm *Spodoptera littoralis* (Lep.: Noctuidae). Entomophaga 26, 179–190.

Snow, D.W. (1981). Tropical Frugivorous Birds and Their Food Plants: A World Survey. Biotropica 13, 1–14.

Soleimani, S., Mashjoor, S., Yousefzadi, M., and Kumar, M. (2022). Multi-target bioactivity of summer quinones production in the Persian Gulf burrowing black-type sea urchin. Heliyon, 8, e09044.

Solis, P.N., Langat, C, Gupta, M.P., Kirby, G.C., Warhusrst, D.C., Phillipson, J.D. (1995). Bio-active compounds from *Psychotria camponutans*. Planta Medica 61(1), 62–65. Doi: 10.1055/s-2006-958001.

Stahl, E. (1967). Dünnschicht-Chromatographie: Ein Laboratoriumshandbuch (2. Aufl.) – Berlin-Heidelberg-New York: Springer. ISBN: 978-3-540-03761-3.

Stahl, E. (1888). Pflanzen und Schnecken. Biologische Studien über die Schutzmittel der Pflanzen gegen Schneckenfraß. Jenaer Zeitschrift für Medizin und Naturwissenschaften 22, 557–684. Verlag von Gustav Fischer.

Stasevych, M., Zvarych, V., Lunin, V., Kopak, N., Komarovska-Porokhnyavets, O., Deniz, N.G., Sayil, C., Ozyurek, M., Guclu, K., Vovk, M., and Novikov, V. (2018). Synthesis and investigation of antimicrobial and antioxidant activity of anthraquinonylhydrazones, Monatshefte für Chemie 149, 1111–1119.

Stermitz, F. R., Abdel-Kader, M.S., Foderaro, T.A., Pomeroy, M. (1994). Iridoid glycosides from some butterflies and their larval food plants. Phytochemistry 37(4), 997–999.

Stevens, P.F. (2001 onwards). Angiosperm Phylogeny Website. Version 14, July 2017 [and more or less continuously updated since]. Accessed Dez. 2024.

Stoessl, A. (1981). Structure and biogenetic relations: Fungal nonhost-specific, in: Durbin, R.D. (ed.), Toxins in plant disease. Academic Press, New York, pp. 109–2019.

Su, M., Liu, F., Luo, Z., Wu, H., Zhang, X., Wang, D., Zhu, Y., Sun, Z., Xu, W., Miao, Y. (2019). The Antibacterial Activity and Mechanism of Chlorogenic Acid Against Foodborne Pathogen *Pseudomonas aeruginosa*. Foodborne Pathogens and Disease 16(12), 823–830. Doi: 10.1089/fpd.2019.2678.

Sung, W.S., Lee, D.G. (2010). Antifungal action of chlorogenic acid against pathogenic fungi, mediated by membrane disruption. Pure and Applied Chemistry 82, 219–226.

Swain, T. (1977.) Secondary compounds as protective agents. Annual Review of Plant Biology 28, 479–501.

Taiz, L., Zeiger, E., Møller, I. M., & Murphy, A. S. (2018). Fundamentals of plant physiology (1st ed.). Oxford University Press 507–536. ISBN 9781605357904 (paperbound).

Takeda, Y., Mima, c., Masuda, T., Hirata, E., Takushi, A., Otsuka, H. (1998). Glochidioboside, a glucoside of (7S,8R)-dihydrodehydrodiconiferyl alcohol from leaves of *Glochidion obovatum.* Phytochemistry 49(7), 2137–2139. Doi: 10.1016/s0031-9422(98)00362-8.

Tarazona Montoya, M.d.l.A.(2023). Chemical profiling of *Eumachia microdon* (D.C.) Delprete & J.H.Kirkbr: Searching for Alkaloids. Masters's thesis, University of Vienna.

Tariq, A., Zeng. F., Graciano, C., Ullah, A., Sadia, S., Ahmed, Z., Zhang, Z. (2023). Plant Ionomics: Sensing, Signaling, and Regulation. John Wiley & Sons Ltd.; Hoboken, NJ, USA: 2023. Regulation of Metabolites by Nutrients in Plants 1–18.

Tattini, M., Galardi, C., Pinelli, P., Massai, R., Remorini, D., Agati, G. (2004). Differential acumulation of flavonoids and hydroxycinnamates in leaves of *Ligustrum vulgare* under excess light and drought stress. New Phytology 163, 547–561.

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

Taylor, C.M. (1996). Overview of the Psychotrieae (Rubiaceae) in the Neotropics. Opera Botanica Belgica 7, 261–270.

Taylor, C.M. (1997). Conspectus of the genus *Palicourea* (Rubiaceae: Psychotrieae) with the description of some new species from Ecuador and Colombia. Annals of the Missouri Botanical Garden 84(2), 224–262.

Taylor, C.M. (2001). Overview of the neotropical genus *Notopleura* (Rubiaceae: Psychotrieae), with the description of some new species. Annals of the Missouri Botanical Garden 88(3), 478–515.

Taylor, C.M. (2003). Rubiacearum Americanarum Magna Hama Pars XV. New Species and Two New Combinations in *Notopleura* (Psychotrieae) from Central and South America. Novon 13(2), 228–260.

Taylor, C.M. (2005). *Margaritopsis* (Rubiaceae, Psychotrieae) in the Neotropics. Systematics and Geography of Plants 75(2), 161–177.

Taylor, C.M. (2020). Overview of *Psychotria* in Madagascar (Rubiaceae, Psychotrieae), and of Bremekamp's foundational study of this group. Candollea 75(1), 51–70. Doi: 10.15553/c2020v751a5.

Taylor, C.M., Lorence, D.H., Gereau, R.E. (2010). Rubiacearum Americanarum Magna Hama Pars XXV: The Nocturnally Flowering *Psychotria domingensis–Coussarea hondensis* Group Plus Three Other Mesoamerican *Psychotria* Species Transfer to *Palicourea*. Novon 20(4), 481–492.

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

Taylor, C.M., Zappi, D. (2006). 162. Rubiaceae (part 5), Tribe 18. Psychotrieae (2). In: G. Harling & C. Persson (eds.), Flora of Ecuador 79, 1-113. Department of Plant and Environmental Sciences, Gothenburg University, Gothenburg.

Tholl D. (2006). Terpene synthases and the regulation, diversity and biological roles of terpene metabolism. Current Opinion in Plant Biology 9, 297–304.

Tholl, D. (2015). Biosynthesis and Biological Functions of Terpenoids in Plants. Advances in Biochemical Engineering/Biotechnology 148, 63-106. Doi:10.1007/10 _2014_295.

Thoppil, R.J., Bishayee, A. (2011). Terpenoids as potential chemopreventive and therapeutic agents in liver cancer. World Journal Hepatology 3, 228–249.

Thureborn, O., Razafimandimbison, S., Wikström, N., Rydin, C. (2022). Target capture data resolve recalcitrant relationships in the coffee family (Rubioideae, Rubiaceae). Frontiers in Plant Science 13, 967456, Doi: 10.3389/flps.2022,967456.

Treutter, D. (2006). Significance of flavonoids in plant resistance: a review, Springer-Verlag 2006, Environmental Chemistry Leters 4, 147–157 Doi: 10.1007/s10311-006-0068-8.

Tundis, R., Loizzo, M.R., Menichini, F., Statti, G.A., Menichini, F. (2008). Biological and pharmacological activities of iridoids: recent develop ments. Mini-Reviews in Medicinal Chemistry 8, 399–420.

Uenoyama, R., Miyazaki, T., Hurst, J.L., Beynon, R.J., Adachi, M., Murooka, T., Onoda, I., Miyazawa, Y., Katayama, R., Yamashita, T., Kaneko, S., Nishikawa, T., Miyazaki, M. (2021). The characteristic response of domestic cats to plant iridoids allows them to gain chemical defense against mosquitoes. Science Advances eabd9135. Doi: 10.1126/sciadv.abd9135.

Van Der Meer, I.M., Stam, M.E., Van Tunen, A.J., Mol, J.N., and Stuitje, A.R. (1992). Antisense inhibition of flavonoid biosynthesis in *Petunia* anthers results in male sterility. Plant Cell 4, 253–262.

Vasileva, E.A., Mishchenko, N.P., Zadorozhny, P.A., and Fedoreyev, S.A. (2016). New Aminonaphthoquinone from the Sea Urchins *Strongylocentrotus pallidus* and *Mesocentrotus nudus,* Natural Product Communications 11, 821–824.

Velisek, J., Davidek, J., Cejpek, K. (2008). Biosynthesis of food constituents: Natural pigments. Part 1 – a Review. Czech Journal of Food Science 25, 291–315.

Vogt, T. (2010). Phenylpropanoid Biosynthesis. Molecular plant 3(1), 2–20. Leibniz, Germany.

Wang, G., Tang, W., Bidigare, R.R. (2005). Terpenoids as therapeutic drugs and pharmaceutical agents. Natural products: Drug discovery and therapeutic medicine. In: Zhang L, Demain AL (Eds.). Totowa, NJ: Humana Press 197–227.

Wang, L., Pan, X., Jiang, L., Chu, Y., Gao, S., Jiang, X., Zhang, Y., Chen, Y., Luo, S., Peng, C. (2022). The Biological Activity Mechanism of Chlorogenic Acid and Its Applications in Food Industry: A Review. Frontiers in Nutrition 9, e943911. Doi: 10.3389/fnut.2022.943911.

Wang, C., Gong, X., Bo, A., Zhang, L., Zhang, M., Zang, E., Zhang, C., & Li, M. (2020). Iridoids: Research Advances in Their Phytochemistry, Biological Activities, and Pharmacokinetics. Molecules 25(2), 287. Doi: 10.3390/molecules25020287.

War, A.R., Buhroo, A.A., Hussain, B., Ahmad, T., Nair, R.M. and Sharma, H.C. (2020) Plant Defense and Insect Adaptation with Reference to Secondary Metabolites. In: Mérillon, J.M. and Ramawat, K., Eds., Co-Evolution of Secondary Metabolites, Reference Series in Phytochemistry, Springer, Cham, 1–28. Doi: 10.1007/978-3-319-96397-6_60.

Whitehead, S.R., Tiramani, J., Bowers, M.D. (2015). Iridoid glycosides from fruits reduce the growth of fungi associated with fruit rot. Journal of Plant Ecology 9, 357–66.

Widhalm, J.R., Rhodes, D. (2016). Biosynthesis and molecular actions of specialized 1,4-naphthoquinone natural products produced by horticultural plants. Horticulture Research 3, 16046.

Wink, M., Heinen, H.J., Vogt, H. and Schiebel, H.M. (1984). Cellular localization of quinolizidine alkaloids by laser desorption mass spectrometry (LAMMA 1000). Plant Cell Reports 3, 230–33.

Willinger, B., & Dobler, S. (2001). Selective sequestration of iridoid glycosides from their host plants in *Longitarsus* flea beetles. Biochemical Systematics and Ecology 29(4), 335–346. Doi: 10.1016/s0305-1978(00)00082-x.

Wink, M. (1997). Compartmentation of secondary metabolites and xenobiotics in plant vacuoles. Advances in Botanical Research. 25, 141–69.

Wink, M. (1988). Plant breeding: importance of plant secondary metabolites for protection against pathogens and herbivores. Theoretical an Applied Genetics 75, 225–33.

Wink, M. (1992). The role of quinolizidine alkaloids in plant insect interactions, in Insect–Plant Interactions, Vol. IV (ed. E.A. Bernays), CRC Press, Boca Raton, pp. 133–69.

Wink, M. (1993a). Allelochemical properties and the raison d'être of alkaloids, in The Alkaloids, (ed. G. Cordell), Academic Press, Orlando 43, 1–118.

Wink, M. (2010). Functions of plant secondary metabolites and their exploitation in biotechnology. In Annual plant reviews, biochemistry of plant secondary metabolism. Wiley-Blackwell 1–24.

Winkel-Shirley, B. (2001). Flavonoid biosynthesis. a colorful model for genetics, biochemistry, cell biology, and biotechnology. Plant Physiol.ogy 126, 485–493.

Winkel-Shirley, B. (2006). "The biosynthesis of flavonoids," in The Science of Flavonoids, ed E. Grotewold New York, NY: Springer 71–95.

Winterhalter, P., Sefton, M.A., Williams, P.j., (1990). Volatile C13-Norisoprenoid Compounds in Riesling Wine Are Generated from multiple precursors. American Journal of Enology and Viticulture 41, 277 LP–283.

Yang, H., Zhang, H., Yang, C., Chen, Y. (2016). Chemical constituents of plants from the genus *Psychotria*. Chemistry & Biodiversity 13(7), 807–820. Doi: 10.1002/cbdv.201500259.

Yilmaz, Y., Toledo, R.T. (2004). Health aspects of functional grape seed constituents. Trends in Food Science & Technology 15422–33.

Zappi, D. (2003). Revision of *Rudgea* (Rubiaceae) in Southeastern and Southern Brazil. Kew Bulletin 58(3), 513–596.

Zerback, R., Dressler, K., and Hess, D. (1989). Flavonoid compounds from pollen and stigma of *Petunia hybrida*: inducers of the vir region of the *Agrobacterium tumefaciens* Ti plasmid. Plant Science. 62, 83–91.

Zhang, T., Li, M., Lu, J., Wang, J., Zhang, M., Panichayupakaranant, P., & Chen, H. (2025). Insights into the Sources, Structure, and Action Mechanisms of Quinones on Diabetes: A Review. Molecules, 30(3), 665. Doi: 10.3390/molecules30030665.

Zinkel, D.F., & Russel, J. (1989). Naval stores: production, chemistry, utilization. Pulp Chemicals Association, New York.

Zollinger, H. (1987). Color Chemistry: Syntheses, Properties and Applications of Organic Dyes and Pigments. VCH Publishers.

Züst, T., Rasmann, S., and Agrawal, A.A. (2015). Growth-defense trade-offs for two major anti-herbivore traits of the common milkweed Asclepias syriaca. Oikos 124, 1404–1415. Doi: 10.1111/oik.02075.

8. Transparency Note on Language Tools and Copyright Compliance

In this present study, Gemini 2.0 Flash, Google AI (2025) and QuillBot v21.3.1, a Learneo, Inc. business was used as a supplementary writing tool to assist with paraphrasing selected sentences to improve clarity and word choice. The conceptual content and intellectual contribution of all passages remain solely that of the author.

Every effort has been made to identify and contact the copyright holders of all images used in this work to secure the necessary permission. Should any oversight be identified, I kindly request notification.

9. Appendix

Table A5 Regression parameters used for the quantification.

Compound	parameters	conc range[1]	
2-Aza-anthraquinone (**2**)	y= 15.862x+44.886; R^2= 0.9992	48.5–775.2	**2** and **3**
Harounoside (**1**)	y= 13.032x+163.24; R^2= 0.993	61.8–989.0	**1**
Rutin	y= 12057x+45.876; R^2= 0.9997	12.4–990.5	flavonols
Caffeic acid	y= 7092.2x+118.62; R^2= 0.9974	7.2–1150.0	chlorogenic acids
Asperuloside	y= 19.581x+1.1507; R^2= 0.9987	8.59–68.75	iridoids

[1] given in [µg/mL]

Table A6 Quantities of 1,4-naphthquinones, chlorogenic acids, iridoids and kaempferol derivatives. Values (mean (min; max)) are given in mg g^{-1} dry leaf mass; n.d. = not detectable. Epiphytic species are listed below the dashed line.

Taxon	6-Hydroxy-2-aza-anthraquinone (3)	2-Aza-anthraquinone (2)	Psychorubrin	Harounoside (1)	Chlorogenic acids	Iridoids	Kaempferol deriv.
N. aff. *Panamensis* (9)	0.24 (0.00; 0.66)	n.d.	n.d.	n.d.	1.70 (0.00; 4.30)	n.d.	0.50 (0.00; 1.47)
N. polyphlebia (11)	n.d.	0.83 (0.00; 6.30)	0.04 (0.00; 0.51)	8.95 (2.85; 20.89)	1.00 (0.00; 10.97)	n.d.	n.d.
N. siggersiana (1)	n.d.	n.d.	n.d.	n.d.	n.d.	0.13 (0.13; 0.13)	n.d.
N. tolimensis (3)	0.24 (0.00; 0.39)	n.d.	n.d.	0.37 (0.00; 1.10)	n.d.	n.d.	9.87 (5.60; 12.58)
N. uliginosa (13)	n.d.	0.55 (0.29; 1.06)	< 0.01 (0.00; 0.03)	1.32 (0.00; 8.50)	13.08 (0.00; 47.91)	n.d.	n.d.
N. panamensis (1)	0.95 (0.95; 0.95)	n.d.	n.d.	n.d.	n.d.	n.d.	3.03 (3.03; 3.03)
N. maxonii (9)	n.d.	n.d.	n.d.	n.d.	4.33 (0.00; 14.67)	2.54 (0.00; 5.12)	0.46 (0.00; 2.25)
N. peperomiae (9)	n.d.	n.d.	n.d.	n.d.	4.47 (0.00; 13.73)	1.96 (0.72; 3.34)	0.31 (0.00; 0.84)
N. pithecobia (4)	n.d.	n.d.	n.d.	n.d.	4.71 (0.00; 11.66)	0.51 (0.00; 0.98)	0.28 (0.00; 0.86)

Table A7 Total phenolic content in gallic acid equivalents (GAE) of the investigated taxa. The values (mean (min; max)) are given in mg mL^{-1}. The number of samples per taxon are given in parentheses.

Taxon	GAE
N. aggregata (9)	0.14 (0.07; 0.35)
N. anamothyrsa (3)	0.04 (0.02; 0.06)
N. capacifolia (1)	0.54
N. costaricensis (2)	0.02 (0.00; 0.04)
N. ex aff. *Panamensis* (9)	0.58 (0.36; 0.77)
N. maxonii (9)	0.19 (0.10; 0.37)
N. nepokroeffiae (1)	0.09
N. panamensis (1)	0.85
N. peperomiae (9)	0.25 (0.03; 0.62)
N. pithecobia (4)	0.80 (0.53; 1.03)
N. polyphlebia (13)	0.74 (0.21; 1.26)
N. siggersiana (2)	0.23 (0.19; 0.27)
N. tolimensis (6)	0.27 (0.02; 0.49)
N. tonduzii (1)	0.02
N. uliginosa (14)	0.71 (0.12; 1.54)
N. viscagoga (2)	0.47 (0.19; 0.76)

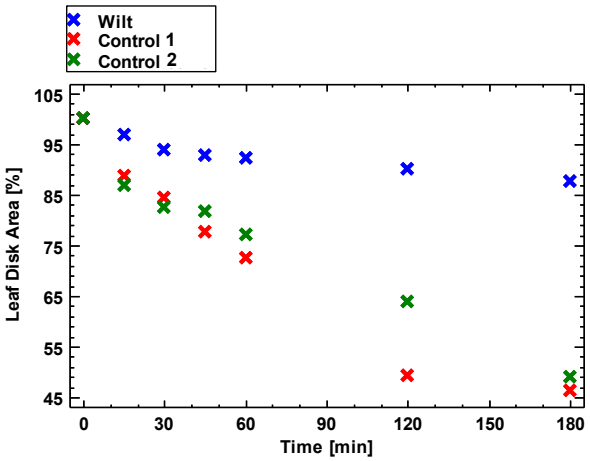


Figure A23 Biotests with *Spodoptera* caterpillars showing the change of leaf area over time. Showing the results of: Wilt (leaf disc without caterpillars and without solvent), Control 1 (leaf disc + caterpillar without solvent) and Control 2 (leaf disc + caterpillar with solvent water/acetone 80:20)

Table A8 List of all samples used in this study incl. details and dry weights, archived at University of Vienna, Department of Botany and Biodiversity Research, Divison of Systematic and Evolutionary Botany, Plant Chemodiversity.

Number	ID	Genus	Species	Plant part	Weighted sample
VF01	19021001	*Notopleura*	*polyphlebia*	leaf	39.9
VF02	1484	*Notopleura*	*siggersiana*	leaf	39.9
VF03/SCH02	1484	*Notopleura*	*siggersiana*	stem	40.5
VF04	19021001	*Notopleura*	*polyphlebia*	roots	40.0
VF05	1543	*Notopleura*	*uliginosa*	roots	39.7
VF06/Sch09	1543	*Notopleura*	*uliginosa*	stem	39.6
VF07	1507	*Notopleura*	*nepokroeffiae*	leaf	39.9
VF08	1538	*Notopleura*	*viscagoga*	leaf	39.9
VF09	7021001	*Notopleura*	*anomothyrsa*	leaf	40.2
VF10	7021001	*Notopleura*	*anomothyrsa*	stem	39.4
VF11	1538	*Notopleura*	*viscagoga*	stem	40.0
VF13	1543	*Notopleura*	*uliginosa*	leaf	39.6
VF14	No ID	*Notopleura*	*uliginosa*	leaf	40.8
VF15	file 14.3.	*Notopleura*	*uliginosa*	leaf	40.0
VF16	7021001	*Notopleura*	*anomothyrsa*	fruits	40.2
VF17/SCH05	1589	*Notopleura*	*pithecobia*	leaf	39.5
VF18	No ID	*Notopleura*	*tolimensis*	leaf	40.2
VF19/SCH06	2907081	*Notopleura*	*capacifolia*	leaf	39.5
VF20-1	1483	*Notopleura*	*polyphlebia*	stem	39.2
VF21	1491	*Notopleura*	*tonduzii*	leaf	40.0
VF22-1	1483	*Notopleura*	*polyphlebia*	leaf	40.8
VF23	1517	*Notopleura*	*tolimensis*	stem	40.1
VF24	1506	*Notopleura*	*costaricensis*	stem	39.7
VF25	1506	*Notopleura*	*costaricensis*	leaf	39.1
VF26	1463	*Notopleura*	*tolimensis*	stem	40.9
VF27-2	13031003	*Notopleura*	*tolimensis*	stem	39.9
VF28-1	2907084	*Notopleura*	*polyphlebia*	leaf	40.6
VF29-1/SCH07	5031002	*Notopleura*	*uliginosa*	leaf	40.1
VF30-1	1349	*Notopleura*	*polyphlebia*	leaf	39.6
VF31-1	1463	*Notopleura*	*tolimensis*	leaf	40.6
VF32-1	1312	*Notopleura*	*uliginosa*	leaf	40.1
VF33-1/SCH01	1539	*Notopleura*	*panamensis*	leaf	39.6
VF34-1/SCH08	1543	*Notopleura*	*uliginosa*	leaf	40.9
VF35-1	1517	*Notopleura*	*tolimensis*	leaf	39.8
VF36-1	2907086	*Notopleura*	*polyphlebia*	leaf	39.2
VF37-1/SCH04	13021007	*Notopleura*	*uliginosa*	leaf	39.7
289	289	*Notopleura*	*pithecobia*	leaf	39.7
290	290	*Notopleura*	*pithecobia*	leaf	40.1
291	291	*Notopleura*	*pithecobia*	leaf	39.7
325	325	*Notopleura*	*polyphlebia*	leaf	40.4
326	326	*Notopleura*	*polyphlebia*	leaf	40.1

327	327	*Notopleura*	*polyphlebia*	leaf	40.1
445	445	*Notopleura*	*uliginosa*	leaf	40.4
446	446	*Notopleura*	*uliginosa*	leaf	40.5
447	447	*Notopleura*	*uliginosa*	leaf	40.4
448	448	*Notopleura*	*polyphlebia*	leaf	39.9
449	449	*Notopleura*	*polyphlebia*	leaf	40.3
450	450	*Notopleura*	*polyphlebia*	leaf	40.4
493	493	*Notopleura*	*uliginosa*	leaf	39.5
494	494	*Notopleura*	*uliginosa*	leaf	39.7
495	495	*Notopleura*	*uliginosa*	leaf	39.8
514	514	*Notopleura*	*peperomiae*	leaf	39.9
515	515	*Notopleura*	*peperomiae*	leaf	40.4
516	516	*Notopleura*	*peperomiae*	leaf	39.9
556	556	*Notopleura*	*maxonii*	leaf	19.8
557	557	*Notopleura*	*maxonii*	leaf	19.8
558	558	*Notopleura*	*maxonii*	leaf	20.0
559	559	*Notopleura*	*maxonii*	leaf	19.8
560	560	*Notopleura*	*maxonii*	leaf	20.2
561	561	*Notopleura*	*maxonii*	leaf	19.8
562	562	*Notopleura*	*maxonii*	leaf	19.9
563	563	*Notopleura*	*maxonii*	leaf	20.1
564	564	*Notopleura*	*maxonii*	leaf	20.8
565	565	*Notopleura*	*peperomiae*	leaf	40.6
566	566	*Notopleura*	*peperomiae*	leaf	40.0
567	567	*Notopleura*	*peperomiae*	leaf	39.8
568	568	*Notopleura*	*peperomiae*	leaf	40.7
569	569	*Notopleura*	*peperomiae*	leaf	40.4
570	570	*Notopleura*	*peperomiae*	leaf	40.5
616	616	*Notopleura*	*aggregata*	leaf	40.1
617	617	*Notopleura*	*aggregata*	leaf	40.5
618	618	*Notopleura*	*aggregata*	leaf	39.8
619	619	*Notopleura*	*aggregata*	leaf	40.7
620	620	*Notopleura*	*aggregata*	leaf	40.3
621	621	*Notopleura*	*aggregata*	leaf	39.7
622	622	*Notopleura*	*aggregata*	leaf	39.9
623	623	*Notopleura*	*aggregata*	leaf	40.2
624	624	*Notopleura*	*aggregata*	leaf	40.4
625	625	*Notopleura*	aff. *panamensis*	leaf	40.2
626	626	*Notopleura*	aff. *panamensis*	leaf	40.4
627	627	*Notopleura*	aff. *panamensis*	leaf	40.0
628	628	*Notopleura*	aff. *panamensis*	leaf	40.7
629	629	*Notopleura*	aff. *panamensis*	leaf	40.6
630	630	*Notopleura*	aff. *panamensis*	leaf	39.8
631	631	*Notopleura*	aff. *panamensis*	leaf	39.8
632	632	*Notopleura*	aff. *panamensis*	leaf	40.5

633	633	*Notopleura*	aff. *panamensis*	leaf	39.9
643	643	*Notopleura*	*sp. (laminar stipule)*	leaf	40.0
644	644	*Notopleura*	*sp. (laminar stipule)*	leaf	40.4
645	645	*Notopleura*	*sp. (laminar stipule)*	leaf	40.3
646	646	*Notopleura*	*sp. (laminar stipule)*	leaf	39.9
647	647	*Notopleura*	*sp. (laminar stipule)*	leaf	39.9
648	648	*Notopleura*	*sp. (laminar stipule)*	leaf	39.7
649	649	*Notopleura*	*sp. (laminar stipule)*	leaf	40.0
650	650	*Notopleura*	*sp. (laminar stipule)*	leaf	40.3
651	651	*Notopleura*	*sp. (laminar stipule)*	leaf	40.2
VF12	28021003	*Geophila*	*repens*	stem	40.4
292	292	*Palicourea*	*ramonensis*	leaf	40.2
293	293	*Palicourea*	*ramonensis*	leaf	39.9
294	294	*Palicourea*	*ramonensis*	leaf	40.2
295	295	*Psychotria*	*parvifolia*	leaf	21.6
296	296	*Psychotria*	*parvifolia*	leaf	21.1
297	297	*Psychotria*	*parvifolia*	leaf	21.4
298	298	*Palicourea*	*torresiana*	leaf	40.9
299	299	*Palicourea*	*torresiana*	leaf	40.7
300	300	*Palicourea*	*torresiana*	leaf	40.0
304	304	*Psychotria*	*psychotriifolia*	leaf	39.7
305	305	*Psychotria*	*psychotriifolia*	leaf	39.8
306	306	*Psychotria*	*psychotriifolia*	leaf	40.2
307	307	*Psychotria*	*grandis*	leaf	40.0
308	308	*Psychotria*	*grandis*	leaf	39.4
309	309	*Psychotria*	*grandis*	leaf	39.5
310	310	*Psychotria*	*grandis*	leaf	39.9
311	311	*Psychotria*	*grandis*	leaf	40.2
312	312	*Psychotria*	*grandis*	leaf	39.7
313	313	*Psychotria*	*psychotriifolia*	leaf	40.0
314	314	*Psychotria*	*psychotriifolia*	leaf	39.5
315	315	*Psychotria*	*psychotriifolia*	leaf	39.9
316	316	*Palicourea*	*berteroana*	leaf	40.6
317	317	*Palicourea*	*berteroana*	leaf	39.7
318	318	*Palicourea*	*berteroana*	leaf	40.5
319	319	*Palicourea*	*berteroana*	leaf	40.3
320	320	*Palicourea*	*berteroana*	leaf	40.1
321	321	*Palicourea*	*berteroana*	leaf	39.6
322	322	*Psychotria*	*marginata*	leaf	40.4
323	323	*Psychotria*	*marginata*	leaf	40.1
324	324	*Psychotria*	*marginata*	leaf	39.6
328	328	*Palicourea*	*hondensis*	leaf	40.3
329	329	*Palicourea*	*hondensis*	leaf	39.7
330	330	*Palicourea*	*hondensis*	leaf	40.1
331	331	*Palicourea*	*hondensis*	leaf	40.2

332	332	*Palicourea*	*hondensis*	leaf	39.9
333	333	*Palicourea*	*hondensis*	leaf	39.9
334	334	*Palicourea*	*microbotrys*	leaf	39.8
335	335	*Palicourea*	*microbotrys*	leaf	39.6
336	336	*Palicourea*	*microbotrys*	leaf	39.9
337	337	*Palicourea*	*microbotrys*	leaf	40.3
338	338	*Palicourea*	*microbotrys*	leaf	40.3
339	339	*Palicourea*	*microbotrys*	leaf	40.3
343	343	*Palicourea*	*guianensis*	leaf	40.0
344	344	*Palicourea*	*guianensis*	leaf	40.3
345	345	*Palicourea*	*guianensis*	leaf	39.5
346	346	*Palicourea*	*racemosa*	leaf	39.9
347	347	*Palicourea*	*racemosa*	leaf	39.8
348	348	*Palicourea*	*racemosa*	leaf	40.0
349	349	*Palicourea*	*gracilenta*	leaf	40.3
350	350	*Palicourea*	*gracilenta*	leaf	40.5
351	351	*Palicourea*	*gracilenta*	leaf	39.9
352	352	*Palicourea*	*brachiata*	leaf	40.0
353	353	*Palicourea*	*brachiata*	leaf	40.0
354	354	*Palicourea*	*brachiata*	leaf	39.5
355	355	*Palicourea*	*tetragona*	leaf	39.6
356	356	*Palicourea*	*tetragona*	leaf	39.8
357	357	*Palicourea*	*tetragona*	leaf	40.0
358	358	*Palicourea*	*cyanococca*	leaf	40.0
359	359	*Palicourea*	*cyanococca*	leaf	40.1
360	360	*Palicourea*	*cyanococca*	leaf	40.1
361	361	*Palicourea*	*acuminata*	leaf	40.2
362	362	*Palicourea*	*acuminata*	leaf	39.9
363	363	*Palicourea*	*acuminata*	leaf	40.1
364	364	*Psychotria*	*marginata*	leaf	40.1
365	365	*Psychotria*	*marginata*	leaf	40.4
366	366	*Psychotria*	*marginata*	leaf	40.4
367	367	*Palicourea*	*longicuspis*	leaf	39.8
368	368	*Palicourea*	*longicuspis*	leaf	40.4
369	369	*Palicourea*	*longicuspis*	leaf	40.0
370	370	*Palicourea*	*longicuspis*	leaf	39.9
371	371	*Palicourea*	*longicuspis*	leaf	39.9
372	372	*Palicourea*	*longicuspis*	leaf	40.4
373	373	*Eumachia*	*haematocarpa*	leaf	39.6
374	374	*Eumachia*	*haematocarpa*	leaf	40.5
375	375	*Eumachia*	*haematocarpa*	leaf	40.2
376	376	*Palicourea*	*glomerulata*	leaf	40.3
377	377	*Palicourea*	*glomerulata*	leaf	40.0
378	378	*Palicourea*	*glomerulata*	leaf	40.1
379	379	*Palicourea*	*glomerulata*	leaf	40.3

380	380	*Palicourea*	*glomerulata*	leaf	40.3
381	381	*Palicourea*	*glomerulata*	leaf	39.5
382	382	*Psychotria*	*chagrensis*	leaf	39.6
383	383	*Psychotria*	*chagrensis*	leaf	39.8
384	384	*Psychotria*	*chagrensis*	leaf	40.4
385	385	*Psychotria*	*psychotriifolia*	leaf	39.9
386	386	*Psychotria*	*psychotriifolia*	leaf	40.2
387	387	*Psychotria*	*psychotriifolia*	leaf	39.9
388	388	*Palicourea*	*hebeclada*	leaf	40.3
389	389	*Palicourea*	*hebeclada*	leaf	39.9
390	390	*Palicourea*	*hebeclada*	leaf	40.1
391	391	*Palicourea*	*winkleri*	leaf	40.5
392	392	*Palicourea*	*winkleri*	leaf	39.9
393	393	*Palicourea*	*winkleri*	leaf	40.2
394	394	*Palicourea*	*racemosa*	leaf	40.5
395	395	*Palicourea*	*racemosa*	leaf	39.9
396	396	*Palicourea*	*racemosa*	leaf	40.1
397	397	*Palicourea*	*gracilenta*	leaf	39.9
398	398	*Palicourea*	*gracilenta*	leaf	39.9
399	399	*Palicourea*	*gracilenta*	leaf	39.4
400	400	*Psychotria*	*chagrensis*	leaf	39.9
401	401	*Psychotria*	*chagrensis*	leaf	39.8
402	402	*Psychotria*	*chagrensis*	leaf	39.7
403	403	*Palicourea*	*suerrensis*	leaf	39.9
404	404	*Palicourea*	*suerrensis*	leaf	40.0
405	405	*Palicourea*	*suerrensis*	leaf	40.0
406	406	*Palicourea*	*eurycarpa*	leaf	40.3
407	407	*Palicourea*	*eurycarpa*	leaf	40.0
408	408	*Palicourea*	*eurycarpa*	leaf	40.0
409	409	*Palicourea*	*eurycarpa*	leaf	40.3
410	410	*Palicourea*	*eurycarpa*	leaf	40.0
411	411	*Palicourea*	*eurycarpa*	leaf	40.0
412	412	*Palicourea*	*gracilenta*	leaf	39.9
413	413	*Palicourea*	*gracilenta*	leaf	39.7
414	414	*Palicourea*	*gracilenta*	leaf	39.8
415	415	*Palicourea*	*racemosa*	leaf	40.0
416	416	*Palicourea*	*racemosa*	leaf	40.4
417	417	*Palicourea*	*racemosa*	leaf	40.1
418	418	*Palicourea*	*suerrensis*	leaf	39.9
419	419	*Palicourea*	*suerrensis*	leaf	40.4
420	420	*Palicourea*	*suerrensis*	leaf	40.1
421	421	*Palicourea*	*hebeclada*	leaf	40.0
422	422	*Palicourea*	*hebeclada*	leaf	40.1
423	423	*Palicourea*	*hebeclada*	leaf	40.2
424	424	*Palicourea*	*tetragona*	leaf	40.4

425	425	*Palicourea*	*tetragona*	leaf	40.2
426	426	*Palicourea*	*tetragona*	leaf	40.3
427	427	*Palicourea*	*hebeclada*	leaf	40.1
428	428	*Palicourea*	*hebeclada*	leaf	39.8
429	429	*Palicourea*	*hebeclada*	leaf	40.4
430	430	*Palicourea*	*eurycarpa*	leaf	40.2
431	431	*Palicourea*	*eurycarpa*	leaf	40.0
432	432	*Palicourea*	*eurycarpa*	leaf	40.0
433	433	*Palicourea*	*microbotrys*	leaf	40.4
434	434	*Palicourea*	*microbotrys*	leaf	39.8
435	435	*Palicourea*	*microbotrys*	leaf	40.3
436	436	*Palicourea*	*tetragona*	leaf	39.9
437	437	*Palicourea*	*tetragona*	leaf	40.4
438	438	*Palicourea*	*tetragona*	leaf	40.3
439	439	*Eumachia*	*haematocarpa*	leaf	39.8
440	440	*Eumachia*	*haematocarpa*	leaf	40.4
441	441	*Eumachia*	*haematocarpa*	leaf	39.9
442	442	*Eumachia*	*haematocarpa*	leaf	40.2
443	443	*Eumachia*	*haematocarpa*	leaf	40.1
444	444	*Eumachia*	*haematocarpa*	leaf	39.7
451	451	*Palicourea*	aff. *hoffmannseggiana*	leaf	40.4
452	452	*Palicourea*	aff. *hoffmannseggiana*	leaf	39.8
453	453	*Palicourea*	aff. *hoffmannseggiana*	leaf	40.6
454	454	*Palicourea*	*longicuspis*	leaf	40.3
455	455	*Palicourea*	*longicuspis*	leaf	40.5
456	456	*Palicourea*	*longicuspis*	leaf	39.5
457	457	*Rudgea*	*reducticalyx*	leaf	40.2
458	458	*Rudgea*	*reducticalyx*	leaf	40.2
459	459	*Rudgea*	*reducticalyx*	leaf	39.8
460	460	*Rudgea*	*reducticalyx*	leaf	40.4
461	461	*Rudgea*	*reducticalyx*	leaf	39.4
462	462	*Rudgea*	*reducticalyx*	leaf	40.3
463	463	*Rudgea*	*reducticalyx*	leaf	39.7
464	464	*Rudgea*	*reducticalyx*	leaf	40.4
465	465	*Rudgea*	*reducticalyx*	leaf	40.5
466	466	*Palicourea*	*schunkei*	leaf	40.2
467	467	*Palicourea*	*schunkei*	leaf	39.7
468	468	*Palicourea*	*schunkei*	leaf	40.1
469	469	*Palicourea*	*schunkei*	leaf	40.5
470	470	*Palicourea*	*schunkei*	leaf	40.6
471	471	*Palicourea*	*schunkei*	leaf	39.5
472	472	*Palicourea*	*schunkei*	leaf	39.8
473	473	*Palicourea*	*schunkei*	leaf	40.1
474	474	*Palicourea*	*schunkei*	leaf	39.5
475	475	*Psychotria*	*remota*	leaf	40.5

476	476	*Psychotria*	*remota*	leaf	39.9
477	477	*Psychotria*	*remota*	leaf	39.7
478	478	*Psychotria*	*neillii*	leaf	40.7
479	479	*Psychotria*	*neillii*	leaf	40.0
480	480	*Psychotria*	*neillii*	leaf	40.2
481	481	*Psychotria*	*neillii*	leaf	40.6
482	482	*Psychotria*	*neillii*	leaf	40.4
483	483	*Psychotria*	*neillii*	leaf	39.9
484	484	*Psychotria*	*neillii*	leaf	40.2
485	485	*Psychotria*	*neillii*	leaf	39.8
486	486	*Psychotria*	*neillii*	leaf	39.9
487	487	*Palicourea*	aff. *hoffmannseggiana*	leaf	40.0
488	488	*Palicourea*	aff. *hoffmannseggiana*	leaf	39.7
489	489	*Palicourea*	aff. *hoffmannseggiana*	leaf	40.7
490	490	*Palicourea*	aff. *hoffmannseggiana*	leaf	39.8
491	491	*Palicourea*	aff. *hoffmannseggiana*	leaf	40.1
492	492	*Palicourea*	aff. *hoffmannseggiana*	leaf	40.5
496	496	*Psychotria*	*panamensis*	leaf	40.5
497	497	*Psychotria*	*panamensis*	leaf	40.6
498	498	*Psychotria*	*panamensis*	leaf	40.1
499	499	*Psychotria*	*sp.*	leaf	39.9
500	500	*Psychotria*	*sp.*	leaf	40.1
501	501	*Psychotria*	*sp.*	leaf	39.9
502	502	*Psychotria*	*sp.*	leaf	40.1
503	503	*Psychotria*	*sp.*	leaf	39.9
504	504	*Psychotria*	*sp.*	leaf	39.7
505	505	*Psychotria*	*sp.*	leaf	40.0
506	506	*Psychotria*	*sp.*	leaf	40.0
507	507	*Psychotria*	*sp.*	leaf	40.0
508	508	*Palicourea*	*valerii*	leaf	39.9
509	509	*Palicourea*	*valerii*	leaf	40.0
510	510	*Palicourea*	*valerii*	leaf	40.1
511	511	*Palicourea*	*valerii*	leaf	39.7
512	512	*Palicourea*	*valerii*	leaf	40.3
513	513	*Palicourea*	*valerii*	leaf	40.2
517	517	*Psychotria*	*remota*	leaf	40.3
518	518	*Psychotria*	*remota*	leaf	40.8
519	519	*Psychotria*	*remota*	leaf	40.0
520	520	*Psychotria*	*remota*	leaf	40.4
521	521	*Psychotria*	*remota*	leaf	40.5
522	522	*Psychotria*	*remota*	leaf	39.8
523	523	*Palicourea*	*macrocalyx*	leaf	40.3
524	524	*Palicourea*	*macrocalyx*	leaf	40.5
525	525	*Palicourea*	*macrocalyx*	leaf	39.4
526	526	*Palicourea*	*macrocalyx*	leaf	39.8

527	527	*Palicourea*	*macrocalyx*	leaf	39.6
528	528	*Palicourea*	*macrocalyx*	leaf	40.6
529	529	*Palicourea*	*macrocalyx*	leaf	40.6
530	530	*Palicourea*	*macrocalyx*	leaf	40.4
531	531	*Palicourea*	*macrocalyx*	leaf	40.2
532	532	*Palicourea*	*adusta*	leaf	40.0
533	533	*Palicourea*	*adusta*	leaf	39.9
534	534	*Palicourea*	*adusta*	leaf	40.2
535	535	*Palicourea*	*adusta*	leaf	40.3
536	536	*Palicourea*	*adusta*	leaf	40.8
537	537	*Palicourea*	*adusta*	leaf	40.3
538	538	*Palicourea*	*adusta*	leaf	40.2
539	539	*Palicourea*	*adusta*	leaf	40.7
540	540	*Palicourea*	*adusta*	leaf	39.8
541	541	*Palicourea*	*montivaga*	leaf	40.1
542	542	*Palicourea*	*montivaga*	leaf	39.7
543	543	*Palicourea*	*montivaga*	leaf	39.8
544	544	*Palicourea*	*montivaga*	leaf	40.6
545	545	*Palicourea*	*montivaga*	leaf	40.6
546	546	*Palicourea*	*montivaga*	leaf	39.4
547	547	*Palicourea*	*montivaga*	leaf	40.2
548	548	*Palicourea*	*montivaga*	leaf	40.5
549	549	*Palicourea*	*montivaga*	leaf	39.6
550	550	*Palicourea*	*elata*	leaf	39.8
551	551	*Palicourea*	*elata*	leaf	39.3
552	552	*Palicourea*	*elata*	leaf	40.3
553	553	*Palicourea*	*cf goldmanii*	leaf	40.7
554	554	*Palicourea*	*cf goldmanii*	leaf	40.1
555	555	*Palicourea*	*cf goldmanii*	leaf	40.2
571	571	*Eumachia*	*microdon*	leaf	40.1
572	572	*Eumachia*	*microdon*	leaf	40.4
573	573	*Eumachia*	*microdon*	leaf	39.8
574	574	*Eumachia*	*microdon*	leaf	40.0
575	575	*Eumachia*	*microdon*	leaf	39.9
576	576	*Eumachia*	*microdon*	leaf	39.7
577	577	*Eumachia*	*microdon*	leaf	40.0
578	578	*Eumachia*	*microdon*	leaf	39.9
579	579	*Eumachia*	*microdon*	leaf	40.0
580	580	*Psychotria*	*carthagenensis*	leaf	40.0
581	581	*Psychotria*	*carthagenensis*	leaf	39.8
582	582	*Psychotria*	*carthagenensis*	leaf	40.3
583	583	*Psychotria*	*carthagenensis*	leaf	40.3
584	584	*Psychotria*	*carthagenensis*	leaf	40.3
585	585	*Psychotria*	*carthagenensis*	leaf	40.4
586	586	*Psychotria*	*carthagenensis*	leaf	40.0

587	587	*Psychotria*	*carthagenensis*	leaf	40.1
588	588	*Psychotria*	*carthagenensis*	leaf	39.9
589	589	*Psychotria*	*monteverdensis*	leaf	40.2
590	590	*Psychotria*	*monteverdensis*	leaf	40.3
593	593	*Psychotria*	*monteverdensis*	leaf	39.7
594	594	*Psychotria*	*monteverdensis*	leaf	40.2
595	595	*Psychotria*	*monteverdensis*	leaf	40.0
596	596	*Psychotria*	*monteverdensis*	leaf	39.9
597	597	*Psychotria*	*monteverdensis*	leaf	40.0
598	598	*Psychotria*	*graciliflora*	leaf	40.2
599	599	*Psychotria*	*graciliflora*	leaf	40.6
600	600	*Psychotria*	*graciliflora*	leaf	40.3
601	601	*Psychotria*	*graciliflora*	leaf	39.9
602	602	*Psychotria*	*graciliflora*	leaf	40.3
603	603	*Psychotria*	*graciliflora*	leaf	40.0
604	604	*Palicourea*	*valerii*	leaf	40.0
605	605	*Palicourea*	*valerii*	leaf	40.6
606	606	*Palicourea*	*valerii*	leaf	40.0
607	607	*Palicourea*	*macrocalyx*	leaf	40.0
608	608	*Palicourea*	*macrocalyx*	leaf	39.8
609	609	*Palicourea*	*macrocalyx*	leaf	40.2
610	610	*Palicourea*	*macrocalyx*	leaf	39.3
611	611	*Palicourea*	*macrocalyx*	leaf	39.7
612	612	*Palicourea*	*macrocalyx*	leaf	40.5
613	613	*Palicourea*	*macrocalyx*	leaf	40.4
614	614	*Palicourea*	*macrocalyx*	leaf	40.6
615	615	*Palicourea*	*macrocalyx*	leaf	40.2
634	634	*Psychotria*	*monteverdensis*	leaf	40.2
635	635	*Psychotria*	*monteverdensis*	leaf	40.5
636	636	*Psychotria*	*monteverdensis*	leaf	39.9
637	637	*Psychotria*	*monteverdensis*	leaf	40.0
638	638	*Psychotria*	*monteverdensis*	leaf	40.1
639	639	*Psychotria*	*monteverdensis*	leaf	40.1
640	640	*Psychotria*	*monteverdensis*	leaf	40.1
641	641	*Psychotria*	*monteverdensis*	leaf	40.3
642	642	*Psychotria*	*monteverdensis*	leaf	40.6
652	652	*Psychotria*	*monteverdensis*	leaf	40.7
653	653	*Psychotria*	*monteverdensis*	leaf	40.3
654	654	*Psychotria*	*monteverdensis*	leaf	40.3
655	655	*Palicourea*	*pubescens*	leaf	39.9
656	656	*Palicourea*	*pubescens*	leaf	40.0
657	657	*Palicourea*	*pubescens*	leaf	40.5
658	658	*Psychotria*	*nervosa*	leaf	39.9
659	659	*Psychotria*	*nervosa*	leaf	39.8
660	660	*Psychotria*	*nervosa*	leaf	40.0

661	661	*Psychotria*	*nervosa*	leaf	40.5
662	662	*Psychotria*	*nervosa*	leaf	39.8
663	663	*Psychotria*	*nervosa*	leaf	39.9
664	664	*Psychotria*	*nervosa*	leaf	40.5
665	665	*Psychotria*	*nervosa*	leaf	40.6
666	666	*Psychotria*	*nervosa*	leaf	40.2
667	667	*Psychotria*	*subsessilis*	leaf	40.1
668	668	*Psychotria*	*subsessilis*	leaf	40.2
669	669	*Psychotria*	*subsessilis*	leaf	40.2
670	670	*Psychotria*	*subsessilis*	leaf	40.0
671	671	*Psychotria*	*subsessilis*	leaf	40.2
672	672	*Psychotria*	*subsessilis*	leaf	40.0
673	673	*Psychotria*	*subsessilis*	leaf	40.2
674	674	*Psychotria*	*subsessilis*	leaf	40.6
675	675	*Psychotria*	*subsessilis*	leaf	39.9
676	676	*Rudgea*	*cornifolia*	leaf	40.5
677	677	*Rudgea*	*cornifolia*	leaf	40.3
678	678	*Rudgea*	*cornifolia*	leaf	39.9
679	679	*Palicourea*	*hoffmannseggiana*	leaf	39.9
680	680	*Palicourea*	*hoffmannseggiana*	leaf	40.1
681	681	*Palicourea*	*hoffmannseggiana*	leaf	40.3
682	682	*Palicourea*	*hoffmannseggiana*	leaf	40.2
683	683	*Palicourea*	*hoffmannseggiana*	leaf	40.0
684	684	*Palicourea*	*hoffmannseggiana*	leaf	39.9
685	685	*Palicourea*	*hoffmannseggiana*	leaf	39.9
686	686	*Palicourea*	*hoffmannseggiana*	leaf	39.9
687	687	*Palicourea*	*hoffmannseggiana*	leaf	40.0
688	688	*Geophila*	*sp.*	leaf	39.6
689	689	*Geophila*	*sp.*	leaf	39.8
690	690	*Geophila*	*sp.*	leaf	39.7
691	691	*Geophila*	*sp.*	leaf	40.0
692	692	*Geophila*	*sp.*	leaf	39.6
693	693	*Geophila*	*sp.*	leaf	40.4
694	694	*Geophila*	*sp.*	leaf	39.5
695	695	*Geophila*	*sp.*	leaf	40.2
696	696	*Geophila*	*sp.*	leaf	40.3