UHPLC-Q/TOF-MS-based Ecometabolomic Study of *Nepenthaceae*

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Abstract

Nepenthes, the largest family of carnivorous plants, is found with an extensive geographical distribution throughout the Malay Archipelago, specifically in Borneo, Philippines, and Sumatra. They can be found from the lowland karangas forest up to the highland montane forest. While certain species display temperature stress tolerance in their natural habitat, our current understanding on their adaptation to the climatic conditions at the phytochemical level is limited. In the present study, we applied an ecometabolomics approach to identify temperature stressed individual metabolic fingerprints of four *Nepenthes* species: the lowlanders *N. ampullaria*, *N. rafflesiana* and *N. northiana*, and the highlander *N. minima*. We hypothesized that distinct metabolite regulation patterns exist between the *Nepenthes* species due to their adaptation towards different geographical and altitudinal distribution. Our results revealed not only distinct temperature stress induced metabolite fingerprints for each *Nepenthes* species, but also shared metabolic response and adaptation strategies. We found the interspecific responses and adaptation of certain species reflected their natural habitat niches. Moreover, our study also uncovered the potential of *N. ampullaria*, *N. rafflesiana* and *N. northiana* to inhabit highland areas, and *N. minima* to inhabit lowland areas, thus challenging our current understanding about their adaption in times of changing climate. We also use the same approach as a novel potential identification tool for *Nepenthes* species. Our study provides the first insight *Nepenthes* species classification and discrimination via intraspecies metabolic fingerprint, with 29 biomarkers identified. *Nepenthes* plant have been utilized in traditional medicine to treat several illnesses and diseases such as stomachache, wound healing and rheumatism. Our volatile phytoconstituent study via gas chromatography–mass spectrometry (GC-MS) of the methanolic leaf extracts, has discovered several phytocompounds with previously recorded bioactivities. The extracts exhibited anti-bacterial properties against *Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus epidermidis* and *Pseudomonas aeruginosa*. Our analyses also revealed that leaves of *Nepenthes* possibly assist the plant in hunting their prey by producing scents known to attract insects. Lastly, we have fortuitously discovered the mutualistic interaction between the *Nepenthes* and associated microbes and the potential of *Nepenthes* plant in bioremediation of the environmental contaminant diethylene glycol. There is much to be discovered still about this fascinating carnivorous plant which warrants further investigations.

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"Not that we are competent in ourselves to claim anything for ourselves, but our competence comes from God.- 2 Corinthians 3:5"

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May God bless you and your family, abundantly.

#no convo in the year of 2020 thanks to the COVID 19*

Declaration

I, Wong Changi, hereby declare that no portion of the work referred to in this thesis:

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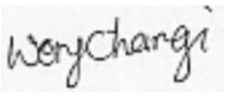

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Publication Arising from this Thesis

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Chapter 1

Introduction

Unlike mammals that can respond to threatening environmental conditions with either fight or flight, plants as sessile organisms are unable to flight. Therefore, they must fight to survive, recover, tolerate and adapt from environmental stress (Li et al. 2020; Rossoni et al. 2019; VanWallendael et al. 2019; Hasanuzzaman et al. 2019). Plant responses towards environmental stresses have been studied since decades, the acquired tolerance and adaptation are known to involve a number of physiological and biochemical changes such as alteration in membrane structure and function, global gene expression, and primary and secondary metabolite composition (Akula & Ravishankar 2011; Kaplan et al. 2004; An et al. 2018; Shi et al. 2015; Gilmour et al. 2000; Levitt 1972; Shinozaki & Dennis 2003). Some plants possess the ability to evolve unique metabolic pathways that overcome the new environmental niches- an essential aspect of plant speciation (Kliebenstein & Osbourn 2012). Carnivorous plants, especially *Nepenthes*, are one of the many examples of the adaptive radiation- a process in which an organism diversify and specify driven by the selective pressure of the environmental niches. (Darwin. 1875; Juniper et al. 1989; Chase et al. 2009; Pavlovič et al. 2011; Fukushima et al. 2017; Ellison & Adamec 2018; Thorogood et al. 2018; Murphy et al. 2020).

1.1 Nepenthes

Nepenthes, commonly known as tropical pitcher plant and locally known as periuk kera, is one of the largest families of carnivorous plants under the monotypic family of *Nepenthaceae*. The genus comprises about 151 species with the numbers still increasing, and several new species being described each year (Maarten et al. 2016; Murphy et al. 2020). Most of the species are found in the Malay Archipelago, especially Borneo, Philippines, and Sumatra (see Figure 1.1); with geographical distribution ranging from highland montane forest (warm days with cool to cold, humid nights) to steamy lowland jungles (hot and humid during both days and nights) (Cheek & Jebb 2001; McPherson 2009; McPherson 2011).

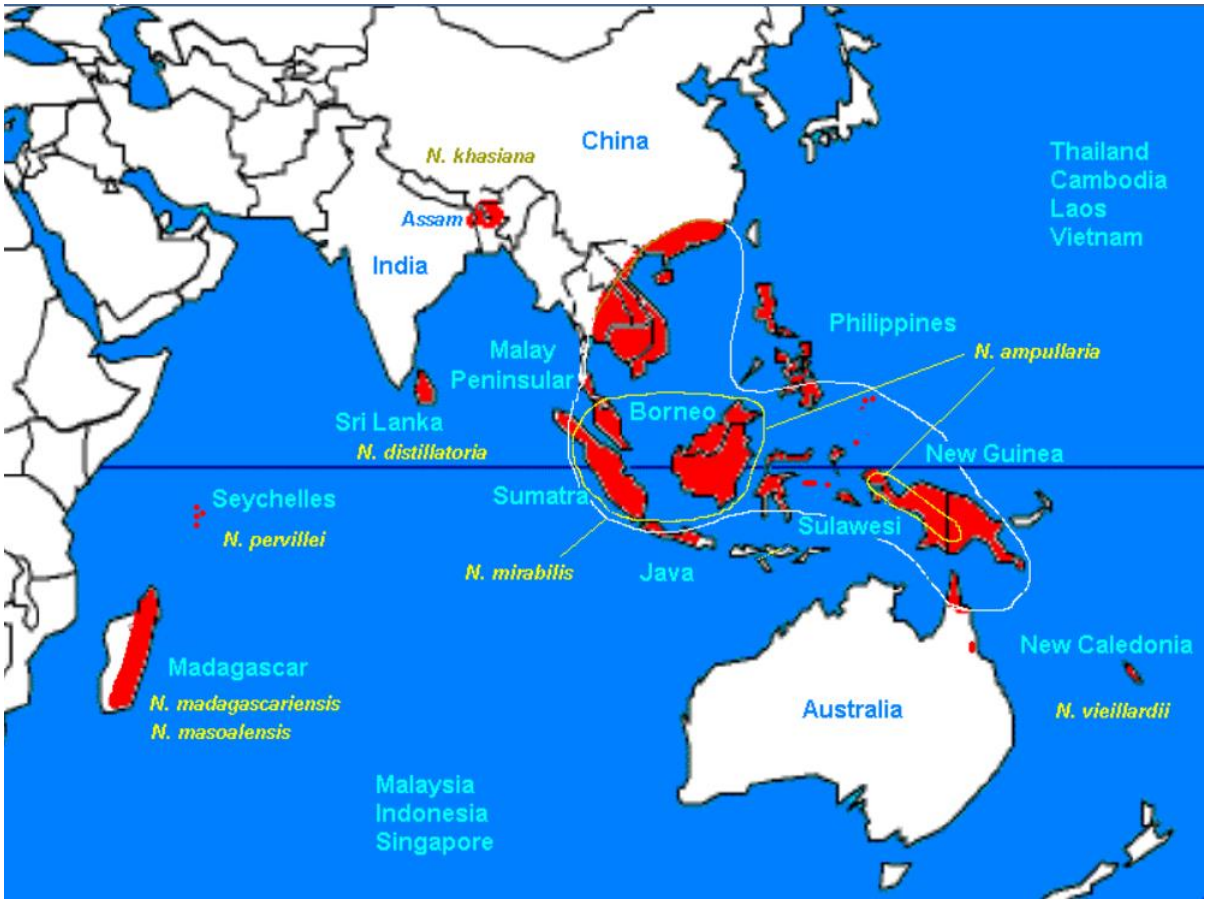


Figure 1.1 Distribution map of *Nepenthes* sp. (taken from Carnivorous Plants in the Wilderness 2012).

Unlike other carnivorous plants, *Nepenthes* possess a unique leaf (see Figure 1.2) that consists of a liquid filled tube-shaped cup (pitcher) at the end of the tendril, which functions to lure, trap, digest and obtain nutrient from their prey (Clarke & Wong 1997).

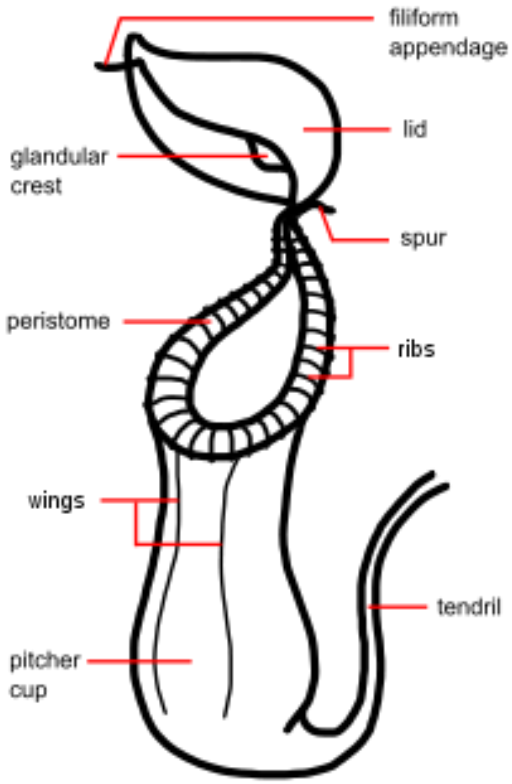


Figure 1.2 Basic structure of Nepenthes pitcher.

There is a remarkable variation in pitcher shape, color and size observed throughout the genus and even within the individual species. Each species of *Nepenthes* produces two distinct forms of pitchers: the lower pitcher and the upper pitcher. The lower pitchers arise from stems closer to the ground and have the tendency to be short and squat with darker coloration. The upper pitchers arise from the climbing stems as they vine through the canopy and has lighter coloration. Below are examples of the lower and upper pitcher of the same plant- *Nepenthes rafflesiana* (Figure 1.3; see Slack 2000 for more examples).

Figure 1.3 The two distinct forms of *Nepenthes rafflesiana* pitchers: the lower pitcher (left) and the upper pitcher (right).

1.1.1 Natural habitats

The signature carnivory characteristic of the plant grants them adaptation towards nutrient-poor substrates including peat swamp (i.e. *N. ampullaria*), sandy field (i.e. *N. rafflesiana*), limestone vegetation (i.e. *N. northiana*), and ultramafic vegetation (i.e. *N. rajah*). Besides that, there are species recorded growing as lithophytes on inselbergs (i.e. *N. pervillei*) and as epiphytes (ie. *N. veitchii*) with no soil substrates. Although not all, certain extreme habitats, i.e. limestone and ultramafic vegetation with the inhospitable soil substrate, have become an important niche habitat for certain *Nepenthes* species (Clarke & Moran 2016; Barthlott et al. 2007; Seine et al. 1996).

Nepenthes species are sub-clustered into two groups: lowlanders and highlanders. The lowlanders have altitudinal distributions below 1100 m above sea level (asl) and live within steamy lowland jungles with hot and humid conditions during both day and night. Highlanders have altitudinal distributions beyond 1100 m asl and live within highland montane forests with warm days and cool to cold, humid nights (Cheek & Jebb 2001; McPherson 2009; McPherson 2011). Apart from these two major groups, some species tend to habitat in intermediates,

between 700 -1400 m asl, but only on rare occasions do species grow beyond or below their typical altitudinal thresholds (Clarke & Wong 1997; McPherson 2009; Clarke & Moran 2016).

1.1.2 Species Identification and Infrageneric Classification of *Nepenthes*

Nepenthes has been classified by Danser (1928) into 6 infra-generic sections, which are *Insignes*, *Montanae*, *Nobiles*, *Regiae*, *Urceolate* and *Vulgatae*. Cheek and Jebb (Cheek & Jebb 2013a; Cheek & Jebb 2013b; Cheek & Jebb 2016a; Cheek & Jebb 2016b; Cheek & Jebb 2016c) have later published another five sections (*Micramphorae*, *Alatae*, *Villosae*, *Tentaculatae* and *Pyrophytae*), and an informal Danseri group (Cheek 2015), followed by a subsection Poculae-ovis of the *Montanae* (Cheek et al. 2017). The uncertainty in the evolutionary relationships has, however, caused the limited wide-scale adoption of these infra-generic classification within the plant genus. Besides that, the confliction between molecular studies and taxonomical approaches in adapting this classification have also been noticed. Moreover, Murphy et al. (2020) have suggested to modify the infra-generic classification of genus *Nepenthes*.

High levels of polymorphism of the *Nepenthes* species and population, especially in their pitcher characteristics, have led to controversy in the species delimitation (Clarke et al. 2018; Mullins 2000). New taxa descriptions have dramatically increased over the past decades, however, there are few which show stable morphological characteristics from the existing species (Clarke et al. 2018). Moreover, an increase in the numbers of segregation from the existing species has been observed in recent years, such as the separation of *N. orbiculate* from *N. mirabilis*, or *N. parvula* from *N. tenax* or *N. nebularum* from *N. robcantleyi* (Wilson & Venter 2016; Catalano 2018; Mansell & Suarez 2016).

The advent of molecular technologies has offered a sound ground on which to base species discrimination. For instance, sanger sequencing via plastid trnK intron (Meimberg et al. 2001; Schwallier et al. 2016), nuclear low copy gene PTR1 (Meimberg & Heubl 2006; Schwallier et al. 2016), plastid non-coding marker trnL–trnF (Mullins 2000), chloroplastic trnL intron (Bunawan et al. 2017), chloroplast intergenic spacer between psaA and ycf3 (PY-IGS) (Renner & Specht 2011), chloroplast matK region (Merckx et al. 2015; Biswal et al. 2018) and nuclear internal transcribed spacer (ITS) region (Mullins 2000; Renner & Specht 2011; Alamsyah & Ito 2013; Merckx et al. 2015; Schwallier et al. 2016; Bunawan et al. 2017). The sanger sequencing analyses have, however, resulted in conflicting markers, incongruence

between nuclear and plastid phylogenies, the possibility of ITS paralogues inclusion, and limited resolution, causing uncertainty in their phylogenetic (Mullins 2000; Meimberg & Heubl 2006; Meimberg et al. 2001; Nauheimer et al. 2019; Murphy et al. 2020). One of the examples is that, the phylogenetic analysis of the *Nepenthaceae* cpDNA trnK intron showed that *N. rafflesiana* is closely related to *N. ampullaria* on the Type A trnK intron, while *N. rafflesiana*, *N. ampullaria* and *N. northiana* are closely related to each other on the Type B trnK intron by (Figure 1.4a, Meimberg et al. 2006). In recent years, high throughput sequencing approaches such as DNA target capture and sequencing via 353 nuclear loci (Murphy et al. 2020) and genome skimming on 81 plastid genes with high-copy rDNA loci (Nauheimer et al. 2019) have also been applied as more robust and effective methods of resolving the taxonomy and classification. Although phylogenetic resolution and branch support have improved dramatically with superior phylogenomic data, topological incongruence was still identified and certain taxa (especially Bornean taxa) inconsistently grouped, leading to calls for further studies (Figure 1.4b and c, Nauheimer et al. 2019; Murphy et al. 2020). Please refer to the Figure 1.4 for the phylogenetic trees.

To date, there is so far no classification/ differentiation of *Nepenthes* via a metabolomic approach.

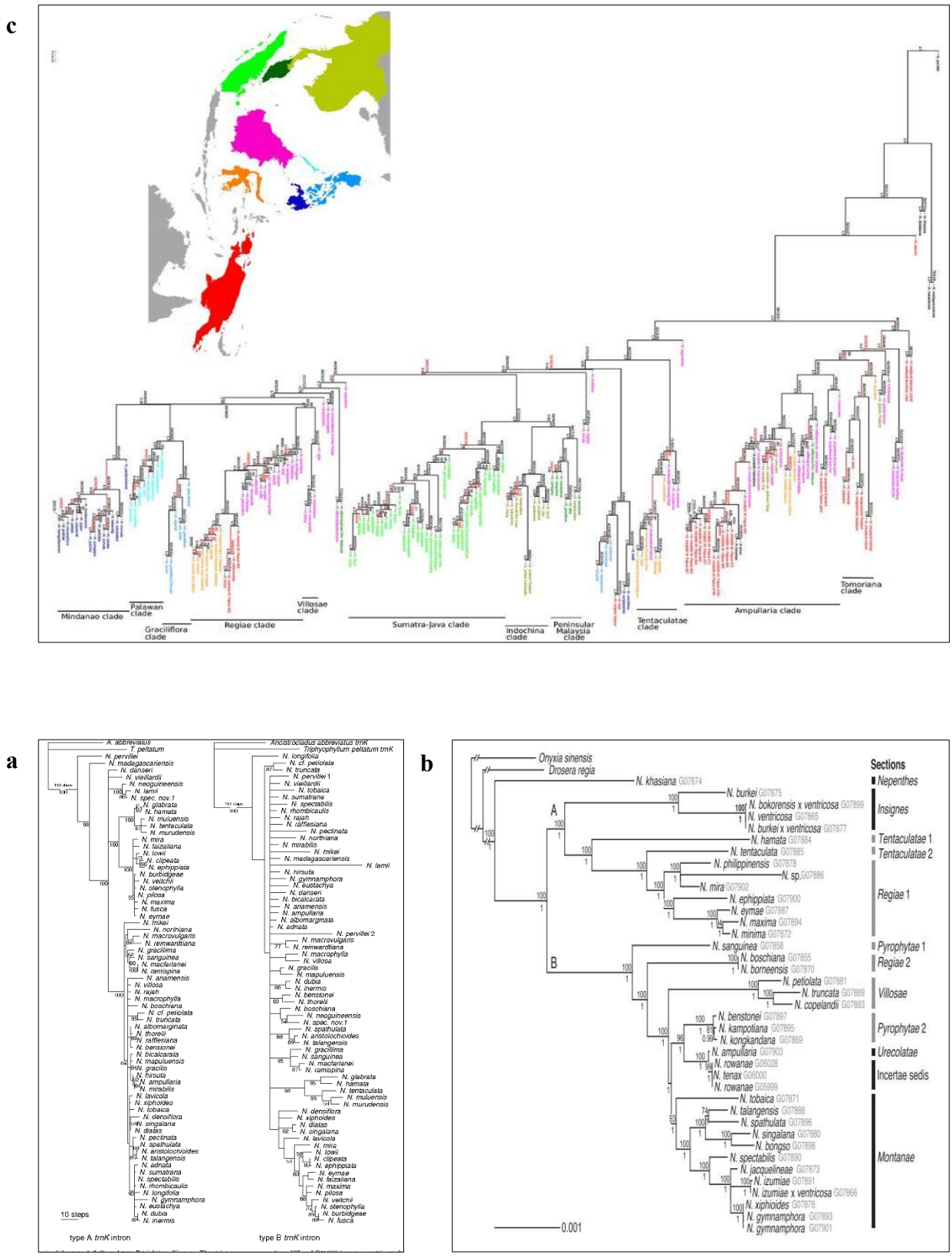


Figure 1.4 The phylogenetic trees created based on several different molecular sequencing approaches by (a) Meimberg et al. 2006 (b) Nauheimer et al. 2019, and (c) Murphy et al. 2020.

1.1.3 Ecological niches and the adaptation of *Nepenthes*

In recent years, a range of studies have demonstrated *Nepenthes* nutrient sequestration strategies and shown that the diversity of prey trapping mechanisms reflected their adaptations towards the environment and ecosystem functioning (Bonhomme et al. 2011; Bauer et al. 2012; Moran et al. 2013; Gaume et al. 2016). These are manifested in the characteristics of the pitcher morphology and the fluid inside, such as the slippery waxy layer on the inner pitcher wall, wettable peristome, viscoelastic and acidity of the pitcher fluid, narrowness and conicity of pitchers, presence of a rim of edible trichomes, emission of volatile compounds, and pitcher aperture diameter (Bohn & Federle 2004; Gaume et al. 2002; Gaume & Forterre 2007; Di Giusto et al. 2010; Moran et al. 2012; Bauer et al. 2012; Moran et al. 2013; Gaume et al. 2016). Besides that, adaption to the environment is also known to cause a variation in the prey assemblages (Adam 1997; Chin et al. 2014) and their diet: from a purely carnivorous (most of the *Nepenthes* species) to some that are partially detritivores (*N. ampullaria*; Moran et al. 2003; Pavlovič et al. 2011) and coprophagous (*N. rafflesiana, N. rajah, N. lowii* and *N. macrophylla*; Grafe et al. 2011; Greenwood et al. 2011; Chin et al. 2010).

Despite the plants' iconic ecological adaption, surprisingly no ecometabolomic study exists on the genus beyond nutrient sequestration strategies and prey trapping mechanism diversity.

1.2 Past studies

Nepenthes has been widely studied, especially on pitcher microscopy; nutrient cycling, transport, and sequestration; microbes and inquilines of the digestive fluid; proteomes of the digestive fluid; captured prey composition; the effects of anthropogenic climate change; and classification of associated endophytes. Earliest studies (Table 1.1) back to the year of 1968 (Nakayama & Amagase 1968).

Table 1.1 Overview of past studies on *Nepenthaceae*.

Study	References
Pitcher microscopy	Owen & Lennon 1999; Thornhill et al. 2008; Moran et al. 2010; Chen et al. 2016; Wang et al. 2020
Nutrient cycling and transport	Owenjr et al. 1999; Schulze et al. 1999; Lam et al. 2019; Capó-Bauçà et al. 2020
Nutrient sequestration strategy	Merbach et al. 2002; Clarke et al. 2009; Chin et al. 2010; Moran et al. 2010; Grafe et al. 2011; Wells et al. 2011; Bauer et al. 2012; Thornham et al. 2012; Pavlovič 2012; Lam et al. 2017; Lam et al. 2020
Microbe and inquiline of the digestive fluid	Shivas & Brown 1989; Adlassnig et al. 2011; Chou et al. 2014; Takeuchi et al. 2015; Chan et al. 2016; Kanokratana et al. 2016; Sickel et al. 2016; Lam et al. 2017; Bittleston 2018; Lam et al. 2019; Gilbert et al. 2020a; Gilbert et al. 2020b; Lam et al. 2020
Proteome study of the digestive fluid	An et al. 2002; Athauda et al. 2004; Takahashi et al. 2005; Stephenson & Hogan 2006; Hatano & Hamada 2008; Thornhill et al. 2008; Hatano & Hamada 2012; Buch et al. 2014; Lee et al. 2016; Rottloff et al. 2016; Yilamujiang et al. 2017; Saganová et al. 2018; Senevirathna et al. 2019; Zakaria et al. 2019
Captured prey composition and the mechanism	Moran 1996; Moran et al. 1999; Moran et al. 2001; Merbach et al. 2001; Merbach et al. 2002; Bohn & Federle 2004; Gaume & Forterre 2007; Di Giusto et al. 2008; Adlassnig et al. 2009; Bauer et al. 2009; Gaume & Di Giusto 2009; Raj et al. 2011; Moran et al. 2013; Bazile et al. 2015
Anthropogenic climate change effect on the plant genus	Gray et al. 2017
Plant's endophytes	Li et al. 2012; Bhore et al. 2013; Lee et al. 2014; Lii et al. 2017; Wong et al. 2018; Naseem & Kayang 2018; Chu et al. 2019

Mutualism was found between the plant genus and other organisms such as insects and small mammals. For instance, a Bornean native ant (*Camponotus schmitzi*) was found to inhabit the swollen tendrils (domatia) of *N. bicalcarata*, and in return, the ant protects the host plant from weevils (*Alcidodes* sp.) damage (Merbach et al. 2007). Hardwicke's woolly bats (*Kerivoula hardwickii*) are known to take roost in the aerial pitcher of *N. rafflesiana*, and in return, the bat provides foliar nitrogen via their faeces to the host plant (Grafe et al. 2011). Two little mammals, which are summit rat (*Rattus baluensis*) and mountain treeshrew (*Tupaia montana*), were found to feed on sugary exudates produced by the glands on the lid of *N. rajah* and in return they provide nutritious faeces (Greenwood et al. 2011). Similar mutualism with mountain treeshrew (*Tupaia montana*) were also observed in *N. lowii* and *N. macrophylla* (Chin et al. 2010).

1.2.1 *Nepenthes* pitcher as treats

Nepenthes plants are served as delicious traditional glutinous rice snacks throughout Malaysian Borneo, especially among indigenous Bidayuh or Kadazandusun tribes. The pitcher is cleaned, filled with glutinous rice and coconut milk, and then steamed to produce the sticky rice snack (Figure 1.5) (Schwallier et al. 2015).

Figure 1.5 The delicious traditional glutinous rice snacks cooked using the pitcher of *N. ampullaria*.

1.2.2 Role of *Nepenthes* in (Ancient) Medicine

Ever since the Paleolithic age, mankind has used plants as medicine with the earliest known medical document dated 4,000 years ago. The practice has continued as the most affordable and effortlessly accessible source, especially for communities with poor access to primary health care systems (Hosseinzadeh et al. 2015). *Nepenthes* has been used by several communities to treat diseases and illnesses. Traditionally the fluid from the unopened pitcher of *Nepenthes khasiana* is used for diabetes and night blindness treatment by the indigenous peoples of the North-Eastern region of India (Rao 1981; Sudhir 2002). The same species is used to treat cholera (stem extracts) and cataract by indigenous people of the Nokrek Biosphere Reserve,

Meghalaya, India (Singh et al. 2014). Moreover, it is used in Nagaland to treat cuts and injuries (Ramashankar & Sharma 2015). In the Dayak Seberuang community, the people use the fluid from the unopened pitcher to treat cough and stomachache (Setiawan et al. 2015). The plant is also used by the Kalanguya tribe of Tinoc, Ifugao, Luzon, Philippines, to treat nocturnal polyuria (excessive urination at night) (Balangcod & Balangcod 2011). The Dayak Iban community from Dusun Meliau Desa Melemba Kalimantan Barat, Indonesia, uses the fluid from unopened pitchers to treat goitre, and the community from other region of the same country also use the unopened pitcher fluid as eye drop to treat eye diseases, and as medicine to cure cough and itchy throat (Sinaga et al. 2016). The decoction of the root of *N. ampullaria* is used by the Jakun community in Kampung Peta, Johor, Malaysia, for asthma and rheumatism treatment (Sabran et al. 2016). The same decoction is also used to treat malaria, as well as the crushed leaf is used as astringent in the country (Burkill 1966; Perry & Metzger 1980).

1.2.3 Phytochemical studies of *Nepenthes*

Phytochemicals are plant-derived constituents that control the biological functions of plant cells, and protect plants from biotic and abiotic stresses (Velu et al. 2018). They are known to exhibit pharmacological activities with therapeutic potential such as anti-leukemic, antioxidant, anti-tumor, anti-viral and antimicrobial (Molyneux et al. 2007; Mendoza and Silva 2018; Kuspradini et al. 2018). For the genus of *Nepenthes*, one of the earliest phytochemical studies can be traced back to the year of 1980, during which 4 quinones - together with plumbagin, droserone, and hydroxydroserone- were isolated from the roots part of the plant (Cannon et al. 1980). Tables 1.2 and 1.3 show an overview of metabolites found in *Nepenthes* plants, as well as the biological activities of the extracts.

Table 1.2 Overview of metabolites found in *Nepenthes* plants.

Nepenthes species	Metabolites	References
Nepenthes albomarginata	the free form of β-amyrin and α-amyrin	Wan et al. 1972
	the esterified form of obtusifoliol, cycloeucalenol, citrostadienol, cycloartenol and 24-methylenecycloartanol	
	both free and esterified forms of cholesterol, campesterol, stigmasterol, sitosterol, isofuctosterol	
Nepenthes sanguinea	sitosterol	Wan et al. 1972
Nepenthes rafflesiana	Droserone, plumbagin, Nepenthone (A, B, C, D and E) hydroxydroserone,	Cannon et al. 1980
Nepenthes thorelii	isoshinanolone, octadecyl caffeate, 2-methylnaphthazarin, plumbagin, droserone	Likhitwitayawuid et al. 1998
Nepenthes rajah	quercetin, Kaempferol, luteolin, cyanidin	Adam et al. 2002
Nepenthes burbidgeae	quercetin, Kaempferol, ellagic acid, phenolic acid	
Nepenthes ×Alisaputrana	quercetin, Kaempferol, ellagic acid, luteolin, cyanidin, phenolic acid	
Nepenthes muluensis	quercetin, Kaempferol, ellagic acid, luteolin,	
Nepenthes tentaculata	Kaempferol, ellagic acid, phenolic acid	
Nepenthes ×Sarawakiensis	quercetin, Kaempferol, ellagic acid, luteolin, phenolic acid	
Nepenthes mirabilis	quercetin, Kaempferol, ellagic acid, cyanidin, phenolic acid	Adam et al. 2002
	leucocyanidin	Adam & Wilcock 1995
	nepenthosides (A and B), koaburaside, leonuriside A, (-)-heimiol A, phenylethyl-β-D-glucopyranoside, icariside D1 , phenethyl rutinoside, 4-hydroxy-2,6-dimethoxyphenyl 6'-O-vanilloyl-β-D-glucopyranoside, syringaresinol, pinoresinol-4-O-β-D-glucopyranoside, lupeone and syringaresinol-4'-O-β-D-glucopyranoside	Van Thanh et al. 2015a
	nepenthones (F and G), 2-methoxy-7-methyljuglone, droserone, 3-methoxy-7-methyljuglone, nepenthone C, cis-isoshinanolone, quercetin, (-)-epicatechin, quercetin 3-O-β-D-glucuronide, plumbagin, quercitrin and kaempferol-3-O-α-L-rhamnoside	Van Thanh et al. 2015b
Nepenthes gracilis	quercetin, cyanidin, phenolic acid	Adam et al. 2002
	isoshinanolone, Plumbagin, shinanolone, epishinanolone, kaempferol, quercetin	Aung et al. 2002
	leucocyanidin	Adam & Wilcock 1995
Nepenthes x Ghazallyiana	quercetin, Kaempferol, ellagic acid, cyanidin, phenolic acid	Adam et al. 2002

	leucocyanidin	Adam & Wilcock 1995
Nepenthes ventricosa x maxima	plumbagin	Shin et al. 2007b
Nepenthes khasiana	droserone, 5-O-methyldroserone	Eilenberg et al. 2010
Nepenthes alata	Plumbagin	De et al. 2019
Nepenthes miranda	N-benzylnicotinamide, benzo[c][2,7]naphthyridinone, plumbagin, 11-Decyldocosane, n-hexadecanoic acid, Erucamide, sitosterol, 2-Ethyl-3-methylnaphtho[2,3-b]thiophene-4,9-dione, Stearic acid, 1,2-ethanediol dimethacrylate, phenylethyl alcohol and cyclohexane-1,3,5-triyltribenzene	Huang et al. 2020

Table 1.3 Overview of the biological activities of the extracts of *Nepenthes* plant.

Nepenthes species	**Solvent**	**Plant Parts**	**Bioactivities**	**References**
Nepenthes mirabilis	Methanol	Leaf	anti-bacterial against *Staphylococcus aureus*	Wiart et al. 2004
	Methanol	Branch, Leaf	anti-osteoporotic and antioxidant	Van Thanh et al. 2015b
	Methanol	Branch, Leaf	anti-inflammatory	Thao et al. 2016
Nepenthes ventricosa x maxima	Hexane	Leaf	anti-fungal against *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, *Rhizopus stolonifer* var. *stolonifer*, *Alternaria alternata*, *Bipolaris oryzae*, *Fusarium oxysporum*, *Phytophthora capsici*, *Aspergillus niger*	Shin et al. 2007a; Shin et al. 2007b
	Ethyl Acetate	Aerial part	antiproliferative	Tang et al. 2019a
Nepenthes khasiana	N/A	Chitin-Induced Pitcher Liquid	Anti-fungal against *Candida glabrata*, *Candida albicans* CBS 562, *Candida krusei*, *Candida albicans* mas, *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus*	Eilenberg et al. 2010
	Methanol	Leaf	antioxidant, hepatoprotective	Uriah et al. 2015
	Methanol	Pitcher	anti-diabetic effect in rats	Shil et al. 2010, Shil et al. 2014

Nepenthes bicalcarata		Leaf	anti-bacterial against *Bacillus subtilis* (ATCC6633), *Staphylococcus aureus* (ATCC25923) and *Bacillus spizizenii* (ATCC6633), anti-frungal against *Candida albicans* (ATCC10231) and *Saccharomyces cerevisiae* (ATCC2601), anti-diabetic effect in rats, antioxidant, cytotoxic against brine shrimp (*Artermia salina*)	Ismail et al. 2015
Nepenthes alata	Dichloromethane	Root	anticancer activity against MCF- cells	De et al. 2019
Nepenthes thorellii x (*ventricosa x maxima*)	Ethyl Acetate	Aerial Part	antitumor activity against breast cancer cells	Ou-Yang et al. 2019
Nepenthes miranda	Methanol, Ethanol, Acetone	Leaf, Stem, Pitcher	antioxidant, anti-bacterial against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, and *cytotoxicity against* melanoma cell	Huang et al. 2020
Nepenthes ventricosa x sibuyanensis	Ethyl Acetate	Twig, Leaf	anti-proliferation against oral cancer cells	Tang et al. 2019a
Nepenthes adrianii x clipeata	Ethyl Acetate	Twig, Leaf	anti-proliferation against oral cancer cells	Tang et al. 2019b
Nepenthes thorelii	N/A	N/A	potential anti-plasmodial against *Plasmodium falciparum*	Likhitwitayawuid et al. 1998

1.3 Abiotic stress

Abiotic stresses result from non-living organisms and /or unfavorable stressful environmental conditions such as nutrient limitation, drought, flood, salinity, pesticide contamination, heavy metals exposure, and extreme temperatures including high and low temperature exposure (Negi et al. 2015; Hong et al. 2016; Liu et al. 2019; Sharma et al. 2019). Biotic stresses are result of damage by other living organisms such as herbivory, parasites and pathogens (Fujita et al. 2006; Atkinson and Urwin 2012; Hong et al. 2016). The abiotic stress is usually interrelated, and multiple stresses can co-occur simultaneously or individually (Kráľová et al. 2012; Bowne et al. 2018; Lamaoui et al. 2018). Abiotic stress is known to affect the productivity in plants or reduce the biomass formation below optimal levels (Farooq et al. 2011; Daryanto et al. 2016; Farooq et al. 2017). Besides that, it is also known to affect plant species distribution (Normand et al. 2009).

The genus *Nepenthaceae* is known to be exposed abiotic stress such as nutrient limitation, toxic substrates, low temperature (as low as 10°C) exposure when the night temperature drops, high temperature (up to 38°C) exposure during dry season. Besides that, habitats such as grasslands are prone to seasonal burning. However, the abiotic stresses do not affect them as much. The highlanders can tolerate cold stress, and the lowlanders are able to tolerate heat stress. Besides that, re-growing from the plant rootstock after the wildfires was observed (Cheek & Jebb 2016; D'amato 2013; Jebb & Cheek 1997; Kaul 1982). Apart from the ecological niches and the adaptation of *Nepenthes* nutrient sequestration strategies and the diversity of prey trapping mechanism mentioned above (1.1.2), our current understanding on the adaptation or survival mechanisms acquired by the different *Nepenthes* species to their climatic conditions at the phytochemical level is, however, limited.

1.3.1 Heat and cold Stresses

Among the abiotic stress, heat and cold stress are the main issues around the world, especially during plant development and crop productivity (Thakur et al. 2010; Bita & Gerats 2013; Rahman 2013; Lamaoui et al. 2018; Yadav et al. 2020). Heat and cold stress are defined as the increase or decrease, respectively, of the temperature beyond a threshold level for a minimal period that leads to permanent harm to the growth and development of the plant (Balestrasse et al. 2010; Lamaoui et al. 2018). Heat stress has been shown to increase respiration, reduce photosynthesis, disrupt plant cellular structures and defensive mechanisms, and elevate stress

metabolite production in plants (Ding et al. 2016; Djanaguiraman et al. 2010; Wahid et al. 2007). Low temperature stress, on the other hand, can aggravate the balance between the energy source and the metabolic sink, induce floral abortion, reduce enzymatic activities, and affect the photosynthesis rate of the plant (Pareek et al. 2017; Allen & Ort 2001; Paul & Foyer 2001). Both stresses will cause overproduction of reactive nitrogen species (RNS) and reactive oxygen species (ROS), thus causing oxidative stress in plant (Samanta et al. 2011; Sharma et al. 2012; Airaki et al. 2012; Kuk et al. 2003).

1.3.2 Plant defense mechanisms against heat and cold stresses

Plants have evolved through various mechanisms to survive through the temperature stresses. When plant receptors are stimulated by the by stress signals, stress-responsive genes are activated and specialized metabolites are subsequently biosynthesized to adapt to the environmental stresses (Jian et al. 2020; Abdelrahman et al. 2020). The strategies are classified into two different adaptations, which are long-term phenological and morphological evolutionary adaptation, and short-term stress acclimation and avoidance mechanism (Choudhary et al. 2015). Both strategies involve morphological, physiological, and biochemical mechanisms (Xalxo et al. 2020; Alhaithloul et al. 2020; Wani & Kumar 2020; Wani & Herath 2018; Chen et al. 2016; Hu et al. 2016). For example, the increased accumulation of plant hormone jasmonic acid in Arabidopsis plants (*Arabidopsis thaliana*) have been observed to tolerate the heat stress (Balfagón et al. 2019). Besides that, the alteration of lipid composition and saturation level has also been observed in heat stressed and acclimated creeping bentgrass (*Agrostis stolonifera*) (Higashi & Saito 2019). Under cold stress exposure, the increased membrane rigidification and production of reactive oxygen species scavengers, configurational alteration in proteins, activation/deactivation of kinases and phosphatases, and transduction of signal into biochemical processes via the Ca^{2+} secondary messengers are often observed in plants (Monroy et al. 1998; Alcázar et al. 2010; Thakur & Nayyar 2013). Table 1.4 shows examples of the plant adaptation strategies.

Table 1.4 Overview of plant adaptation strategies under temperature stresses exposure.

Abiotic stress	Plant adaptation strategy	References
Heat stress	· Osmotic activity regulation and cellular structures protection (by buffering the cellular redox potential, and by maintaining cell membrane integrity and cell water balance) via soluble sugars, proline, and glycine betaine accumulation. · High carbohydrate availability (such as glucose and sucrose)	Souri et al. 2020 Sadiq et al. 2020 Abdelrahman et al. 2020 Khan et al. 2020 Mishra et al. 2020

	· Reactive oxygen species (ROS) detoxification	Yadav et al. 2020
	· ROS scavenger production (such as ascorbate peroxidase)	Chandran et al. 2020
	· Phytohormones regulation and signaling (such as abscisic acid, auxin, cytokinin, ethylene, jasmonic acid, nitric oxide, and salicylic acid)	Liu et al. 2019 Yu et al. 2019 He et al. 2018
	· Secondary metabolites synthesis protects the plant against oxidative damage	Hamayun et al. 2018 Abd El-Daim et al. 2014
	· Phenolic compounds accumulation	Bita & Gerats 2013
	· Anthocyanin, flavonoids, and carotenoids accumulation	Hubbard et al. 2012
	· Plant-growth-promoting rhizobacteria association	Dobra et al. 2010
	· Endophytes association	Frank et al. 2009
	· Heat-shock proteins induction	Rampino et al. 2009
	· Hydrogen sulphide regulation	Allakhverdiev et al. 2008
	· Changing leaf orientation	Wahid 2007
	· Antioxidant enzymes synthesis (such as glutathione peroxidase, superoxide dismutase, catalase, guaiacol peroxidase, glutathione reductase, peroxiredoxins, ascorbate peroxidase, monodehydroascorbate reductase, and dehydroascorbate reductase)	Kotak et al. 2007 Musatenko et al. 2003 Rivero et al. 2001 Liu & Huang 2000
	· Alterations in the membrane lipid composition	Blum & Ebercon 1981
	· Cooling via transpiration	
Cold stress	· Plant-growth-promoting rhizobacteria association	Chandran et al. 2020
	· Alterations in the membrane lipid composition	Chen & Raji 2020
	· Glycine betaine accumulation	Liu et al. 2019
	· Total soluble sugars accumulation	Saini et al. 2019
	· Proline and glutamic acid accumulation	He et al. 2019
	· Phenolic compounds accumulation	Zhang et al. 2019
	· Flavonols and flavonoids accumulation	Valitova et al. 2019
	· Maintaining cell membrane fluidity	Sharma et al. 2019
	· Secondary metabolites synthesis protects the plant against oxidative damage	Zhao et al. 2019 Hu et al. 2017
	· Endophytes association	Pareek et al. 2017
	· Cold responsive proteins production via CBF／DREB responsive pathway	Miura & Tada 2014 Zhu et al. 2013
	· Dehydrins accumulation	Kosová et al. 2012
	· Cell membrane stabilization	Turan et al. 2012
	· Protection of proteins from denaturation	Barka et al. 2006
	· Heat shock protein accumulation (such as HSP90, HSP70, small HSPs and chaperonins 60 and 20) which serves as strong cryoprotective effect, contributing towards the membrane protection, the refolding of denatured proteins and the aggregation prevention	Nayyar et al. 2005 Renaut et al. 2004 Renaut et al. 2006 Timperio et al. 2008 Kim et al. 2002 Sakamoto & Murata 2002
	· Monosaccharides and disaccharides accumulation	Rivero et al. 2001

· Energy · Increased activities of catalase, ascorbate peroxidase and glutathione reductase · Fatty acid accumulation · total waxes and cutin monomer accumulation · Alteration of stability and fluidity of membrane by accumulating unsaturation of fatty acids in membrane lipids · Phytohormones regulation and signaling (such as cytokinin, auxin, gibberellins, jasmonate, nitric oxide, salicylic acid and jasmonic acid) · Ca^{2+} signaling · Putrescine synthesis

1.4 Metabolomics

Metabolites are small molecules found within a biological system which are required for growth, maintenance and regular cellular functions (Gargallo-Garriga et al. 2014; Sarabia et al. 2018; van Dam & van der Meijden 2018). Such molecules include the products and substrates of cellular metabolism such as sugars, lipids, peptides, organic acids, carbohydrates, amino acids, thiols, vitamins, and nucleic acids (Jones et al. 2013; Zhang et al. 2012). The metabolites are known to be highly responsive to external and internal stimuli and are key components in both biochemical and ecological processes. For organisms to adapt and survive in their natural habitats, they need to be able to alter their physiological characteristics at molecular level in response to the ecological stresses, including biotic and abiotic stresses. These physiological alterations can be studied using metabolomic techniques (Gargallo-Garriga et al. 2014; Sarabia et al. 2018; Castro-Moretti et al. 2020; Melandri et al. 2020; Feng et al. 2020; Gargallo-Garriga et al. 2020; Liu et al. 2020).

Metabolomics allow the simultaneous detection, identification, and quantitation of a wide range of biochemical compounds. That includes the assessment of an organism's reproductive, energetic, defensive and oxidative, as well as their health statuses and dynamics, thus providing an instantaneous image of the metabolome (the complete set of metabolites present within a cell or tissue or even one organism) of a biological system (Penuelas and Sardans 2009; Sarabia et al. 2018; Lu et al. 2020). The first paper published on metabolomics was in the year of 1971 (Pauling et al. 1971). Since then, the platform has been successfully applied to different applications including, but not confined to, studies of heart diseases drug toxicity

and gene function, veterinary medicine, cancer, microbiology, ecotoxicology, nutrition, and plant sciences (Barderas et al. 2011; Gauthier et al. 2020; Carlos et al. 2020; Jones et al. 2013).

Metabolomics can be classified into several specific areas, including general metabolomics, targeted analyses, untargeted analyses, metabolite profiling, metabonomics and metabolite fingerprinting. The terms are often used interchangeably, the modelling and analytical procedures are, however, the same (Goodacre et al. 2004; Nicholson & Lindon 2008). Below are the classifications of metabolomic techniques.

- General metabolomics: Comprehensive analysis of the entire metabolome under a provided set of conditions (Goodacre 2007)
- Targeted metabolomics: Works on monitoring the specific metabolome of interest (Goodacre et al. 2004).
- Untargeted metabolomics: Focuses on a global analysis of the detected metabolome interest (Alonso et al. 2015).
- Metabonomics: Focuses on the fingerprint of biochemical perturbations measurement caused by toxin, drugs, and diseases (Nicholson & Lindon 2008).
- Metabolic profiling: Quantitative analysis focuses on a specific group of metabolome (Dettmer et al. 2007).
- Metabolite fingerprinting: Unbiased, analyzes and identifies global patterns of metabolome in response to a given condition (Dettmer et al. 2007).

Apart from the classification mentioned above, there is a new term gaining interest in the last decade. It is called “eco-metabolomics” or “ecometabolomics” (Kuzina et al. 2009; Sardans et al. 2011; Rivas-Ubach et al. 2012; Rivas-Ubach et al. 2013; Leiss et al. 2016; Peters et al. 2018; Rivas-Ubach et al. 2019; Pabbathi et al. 2020).

1.5 Ecometabolomics

Ecometabolomic is defined as the study of metabolic fluxes against environmental stoichiometry that focuses on the interactions between plants and the environment, or other organisms across different temporal and spatial scales (Sardans et al. 2011; Peters et al. 2018; Jan & Ahmad 2019). It is an emerging application with promising potential in the field of ecology and has been used to understand the growth and phenotypic responses of the plants, and their morphological adaptations towards environmental changes (Kaplan et al. 2004; Gray & Heath 2005; Turner et al. 2016; Paupière et al. 2017; Sarabia et al. 2018; Wu et al. 2018; Antunes et al. 2019; Ma et al. 2019; Coleine et al. 2020; Feng et al. 2020; Griffiths et al. 2020; Lu et al. 2020; Melandri et al. 2020). It has also been used to evaluate the responses of the plants towards other organisms such as herbivores, pathogens, competitors, symbiotic organisms and parasite at coarser scales of spatiotemporal complexity (Allwood et al. 2010; López-Gresa et al. 2010; Balmer et al. 2013; Song et al. 2017; Ichihashi et al. 2018; Peters et al. 2018; van Dam et al. 2018; Galati et al. 2019; Castro-Moretti et al. 2020; Willett et al. 2020). The techniques employed are either targeted or untargeted analysis, with latter method being more suitable to identify the variation in the unknown terrain of a metabolome, often leading to novel scientific discoveries (Peters et al. 2018; Jan & Ahmad 2019).

1.6 Identification of cryptic species and strains via metabolomics

Taxonomists often classify organisms based on similar biological traits and/or taxonomic criteria. However, there are several draw-backs to these identification tools. For instance, such identification methods require highly experienced researchers and misidentification is common (Sweeney et al. 2011). Besides that, species with extremely different ecological properties are found in a broad taxonomic group, and similar ecological attributes can be found in two distantly related species (Sevenster 1996; Wilson 1999; Mallet et al. 2007; Bickford et al. 2007; Bai 2014). In the modern era, new tools such as molecular techniques (via DNA sequencing of gene regions or full genome sequencing), have emerged to detect and differentiate morphologically similar species (Adams et al. 2005; Rajaniemi et al. 2005; Stuart et al. 2006; Bickford et al. 2007; Geml et al. 2008; Caminer & Ron 2014; Liede-Schumann et al. 2020). However, there are disadvantages using molecular techniques such as high costs, laborious, and huge amount of data required to be analyzed for full genome sequencing. Besides that, several gene sequences are often required to increase the accuracy of species

discrimination, especially in the kingdom of *Plantae*, which is known to be problematic to discriminate between species (Nolan & Cribb 2005; Cameron et al. 2006; Kress & Erickson 2007; Martinez-Murcia et al. 2011; Hollingsworth et al. 2011; Roger et al. 2012; Ruhsam et al. 2015; Bronski et al. 2020; Rezasoltani et al. 2020).

Neither taxonomic nor molecular techniques have the capability to identify differences at intraspecific level. For example, both approaches cannot identify between resistant and non-resistant strains within a species, or detect ecological niche adaptation of the same species (Cameron et al. 2006). A metabolomic approach could be used as an identification tool and also provide insights into the environmental conditions of the plants. This is supported by Wink (2003) and the successful intra- and interspecies differentiation in plants and other organisms, as well as understanding their adaptive characteristics, via their metabolic fingerprinting (Davey et al. 2008; Huseby et al. 2012; Nagler et al. 2018; Yang et al. 2018; Harrison et al. 2020; Shi et al. 2020).

1.7 Aims of the Present Study and Dissertation Outline

The first part of the present study investigates the impact of heat and cold stress on four *Nepenthes* species representing different ecological systems and altitudinal distributions. The species studied represent lowlanders (*N. ampullaria*, *N. rafflesiana* and *N. northiana*) and highlanders (*N. minima*). Using a Mass Spectro-based non-targeted approach, the aim is to understand whether and how the underlying ecological adaptation of the plant species influences their metabolite regulation upon heat and cold exposure.

The second aim is to evaluate the usability of metabolomics for the discrimination and infrageneric classification between different *Nepenthes* species (*N. ampullaria*, *N. rafflesiana*, *N. northiana* and *N. minima*).

The third aim is to confirm the traditional uses of the *Nepenthes* as a medicinal plant via identification of metabolites with recorded medicinal properties, followed by the examination of their anti-bacterial activity against Gram-positive and Gram-negative bacteria.

The objectives of the different chapters are:

The 1st chapter provides the literature background and introduction of the topics of the present study.

The 2nd chapter summarizes the methods utilized in the present study.

The 3rd chapter identifies stress adaption strategies applied by the 4 *Nepenthes* species (*Nepenthes ampullaria*, *northiana*, *rafflesiana* and *minima*) towards heat and cold stress exposure and the impact assessment of ecological niches towards the metabolic response (niche-specific adaptation and response), as well as the determination of universal adaptation and responses among the 4 *Nepenthes* species.

The 4th Chapter focus on the species discrimination and infrageneric classification among the selected *Nepenthes* species via their metabolite fingerprints and identification of potential biomarkers for species discrimination.

The 5th Chapter focuses on the identification of volatile phytoconstituents of *N. rafflesiana* and *N. ampullaria* from the methanolic leaf extracts and other of volatile metabolites with previously recorded medicinal properties, as well as the investigation of anti-bacterial activity against Gram-positive and Gram-negative bacteria.

The last chapter summarizes the key findings and outlook for future studies.

Chapter 2

Methodology

The current chapter covers the general information of the four *Nepenthes* species, materials, and instruments, as well as the general methodology of the present study. Please refers to the Chapter 3, 4 and 5 for the detail information.

2.1 The four *Nepenthes* species used in the study.

Four *Nepenthes* species namely *N. minima*, *N. ampullaria*, *N. northiana* and *N. rafflesiana* were used in the present study. The *Nepenthes* species were chosen based on their different ecology and altitudinal distribution (please refer to section **2.1.1**; **2.1.2**; **2.1.3** and **2.1.4**). It is hypothesized that distinct metabolite regulation pattern can be distinguished between the *Nepenthes* species based on their adaptation towards different geographical and altitudinal distribution.

2.1.1 *Nepenthes ampullaria*

Nepenthes ampullaria (Figure 2.1), under the section of *Urceolatae* (Danser 1928), is one of the unique lowland species within the plant genus, with very wide distribution that includes Borneo, Peninsular Malaysia, New Guinea, Singapore, Thailand and many more. Besides that, this species also inhabits a wide range of ecology including damp, shady forest, open microphyllous vegetation, secondary forests, swamp grassland and swamp forests with the elevations from 0 to 2100 m above sea level (asl) (Jebb & Cheek 1997; Clarke 2001; McPherson 2009). Moreover, it is one of the rare species that evolved as partial detritivores (Moran et al. 2003; Pavlovič et al. 2011), which collects and digests the fallen leaf litter in the pitcher.

Figure 2.1 The pitcher of *Nepenthes ampullaria* plant.

2.1.2 *Nepenthes rafflesiana*

Nepenthes rafflesiana (Figure 2.2), under the section of *Insignes* (Danser 1928), is a widespread lowland species that can be found growing in Borneo, Peninsular Malaysia (particularly in the state of Johor), Singapore and Sumatra (between Indrapura and Barus). This species can be found growing at the open area, shady forest, sandy area and offshore, with the elevations from 0 to 1000 m asl (Jebb & Cheek 1997; Clarke 2001; McPherson 2009). *N. rafflesiana* have been observed growing in open areas with direct exposure to sunlight and heat (anecdotal observations in Matang area of Kuching, Sarawak, Malaysia). It is known to be a coprophagous species which has been recorded to mutualize with Hardwicke's woolly bats (*Kerivoula hardwickii hardwickii*). The bat roosts in its aerial pitchers and provides foliar nitrogen via the faeces, in return (Grafe et al. 2011).

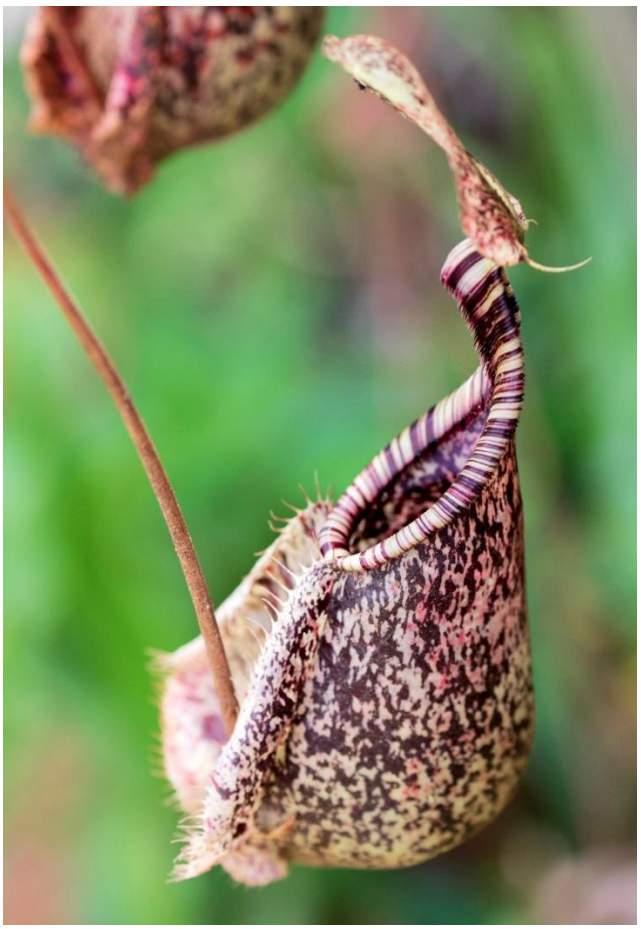


Figure 2.2 The pitcher of *Nepenthes rafflesiana* plant.

2.1.3 *Nepenthes northiana*

Nepenthes northiana (Figure 2.3), under the section of *Insignes* (Danser 1928), is endemic to Borneo (only at Bau district, Sarawak), and restricted to limestone vegetation - a harsh environment composed of calcium carbonate, alkaline pH and highly susceptible to drought (Cheek & Golos 2019). This lowland species can be found growing at the elevation from 0 – 500 m asl (Jebb & Cheek 1997; Clarke 2001; McPherson 2009).

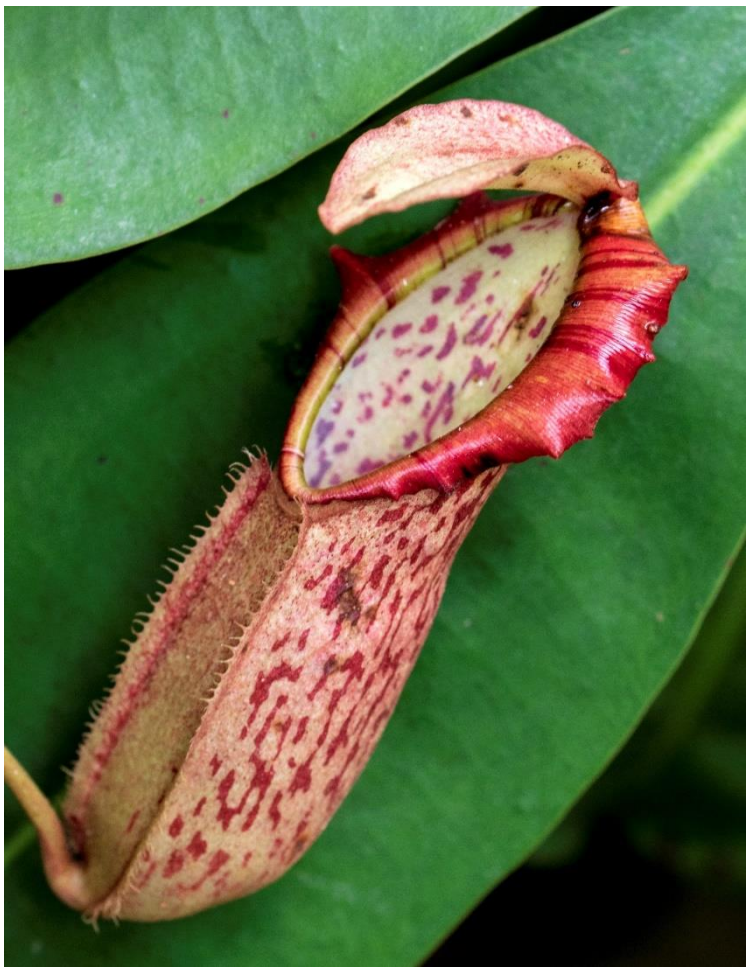


Figure 2.3 The pitcher of *Nepenthes northiana* plant.

2.1.4 *Nepenthes minima*

Nepenthes minima (Figure 2.4), a newly named species, was previously known as the miniature form of *N. maxima* (Evans 2009). The species is under the section of *Regiae*, and is restricted to the highland (sub-montane) grasslands of Central Sulawesi, with grey-yellow clay as substrate. The habitat is known to experience temperatures up to 38 °C, dry season with little or no rain for several weeks. This highland species is found growing at elevations from 1000 – 1700m asl. It is known to be a pyrophytic species that experiences seasonal burning, with the re-growing from the plant rootstock observed after the wildfires (Cheek & Jebb 2016). While most of the highland species fail to survive under similar condition, this is one of the rare species that adapts well in the greenhouse under lowland condition (anecdotal observations). *N. maxima*, have been brought into cultivation during the late 1970s until early '80s (Evans 2009). The plant samples used in the present study were individual seed grown plants, via the horticulture produced seeds obtained as the miniature form of *N. maxima*.

Figure 2.4 Miniature form of *Nepenthes maxima* (*Nepenthes minima*).

2.2 Sampling Sites

Nepenthes ampullaria, *N. minima*, *N. rafflesiana* and *N. northiana* plant samples were collected at different sites across Sarawak. Table 2.1 shows Global Positioning System (GPS) coordinates, and Figure 2.5 shows GPS location via Google Map:

Table 2.1 GPS coordination for the *Nepenthes* plant samples collected.

Nepenthes species	**Global Positioning System (GPS)**
N. ampullaria	1°36'01.4"N 110°14'09.0"E
N. rafflesiana	1°36'01.4"N 110°14'09.0"E
N. northiana	1°23'48.8"N 110°07'51.9"E
N. minima	1°31'56.8"N 110°21'28.2"E

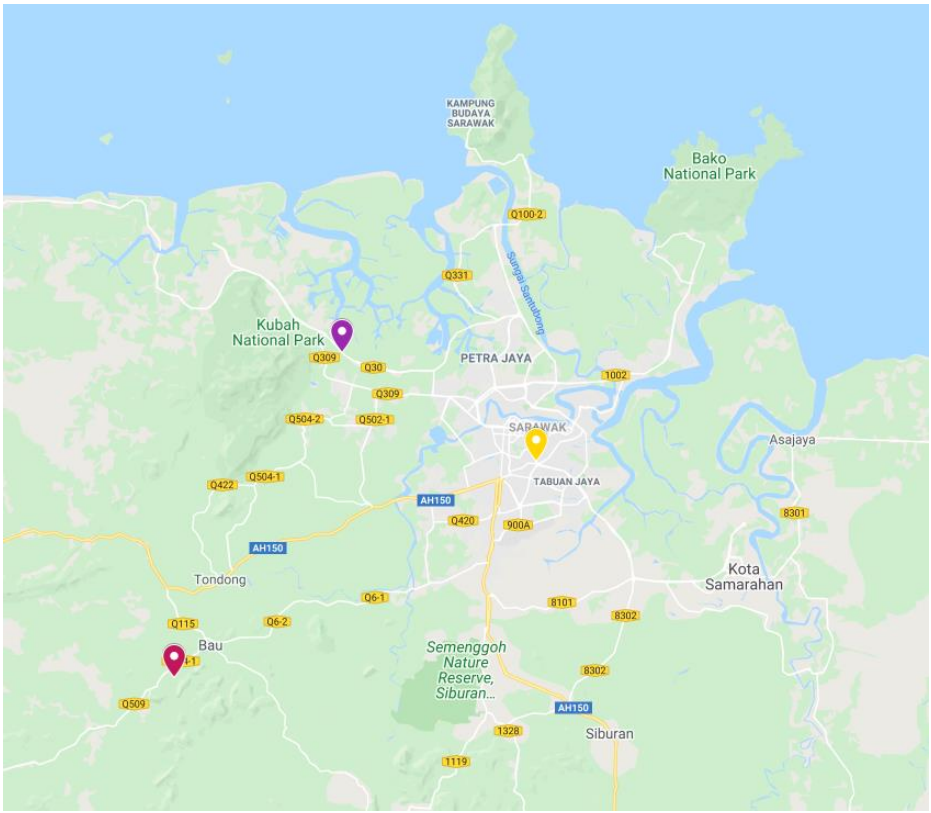


Figure 2.5 Plant collection sites indicated by red point for *N. northiana*, yellow point for *N. minima* and purple point for *N. ampullaria* and *N. rafflesiana* (Source: Google Map).

2.3 Plant Specimen and Preparation of the Extracts

2.3.1 Preparation of Plant Extracts for Ultra-High Performance Liquid Chromatography-Quadrupole Time-of-Flight Mass Spectrometry (Chapter 3 and 4)

All plants were grown in a Pol-Eko-Aparatura climatic chamber with phytotron system (Model: KK 750 FIT P) in a mixture of cocopeat and perlite (at a ratio of 10 : 0.5; g/g). All the plants were grown under a 12 hours light and 12 hours dark photoperiod.

The plants were exposed to:

(a) lowland conditions (33 °C day / 28 °C night)

(b) intermediate conditions (25 °C day / 18 °C night)

(c) highland conditions (23 °C day / 8 °C night)

for 7 days before being harvested.

The targeted temperature ranges were adopted based on their natural environment temperature exposure (Cheek & Jebb 2016; D'amato 2013; Jebb & Cheek 1997; Kaul 1982).

It is important to immediately freeze the plant samples to prevent post-sampling hydrolysis of the compounds (Sardans et al. 2011). The growing tips (Figure 2.6), the active growing part of the plant, were harvested and immediately frozen using liquid nitrogen, and thereafter freeze-dried (Labconco Freezone 6 Freeze Dryer System). The fully dried plant samples were then stored at -80ºC for further use.

Figure 2.6 The red arrow indicates the growing tips of the plant harvested.

One (±0.1) mg freeze-dried plant samples was weighed, ground, and exhaustedly extracted with 600 µL of solvent mixture of methanol : chloroform : ultrapure with 1% sodium chloride added (1:1:1 v/v/v). The mixture was vortexed for 30 minutes at room temperature, followed by 30 minutes centrifugation at 3000× *g* maintained at 4 °C. The layers were then transferred into a new borosilicate tube and vacuum dried using a speed concentrator. Dried extracts were then reconstituted using 400 µL of methanol and filtered using 0.2 µm PTFE membrane filter before subjected to Liquid Chromatography and Mass Spectrometry analysis. Figure 2.7 shows the schematic flowchart of the plant extracts preparation for ultra-high performance liquid chromatography-quadrupole time-of-flight mass spectrometry.

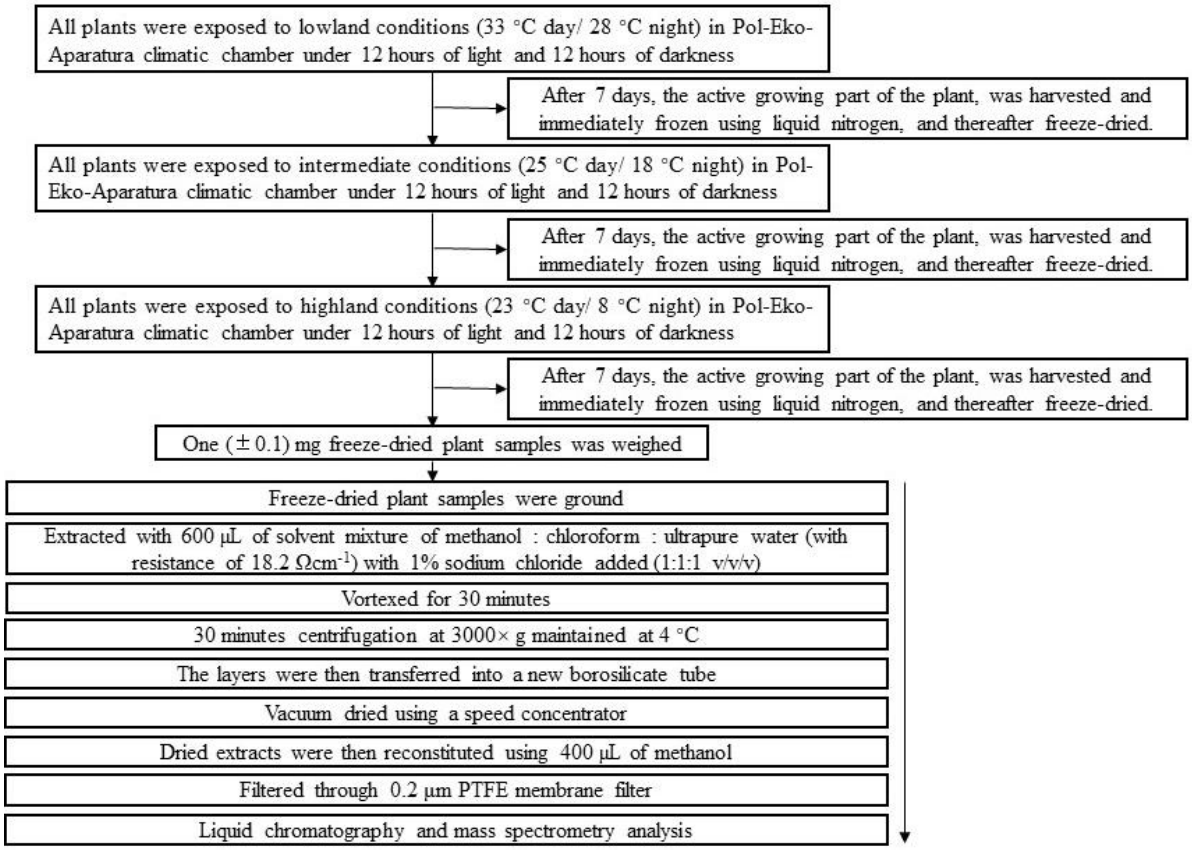


Figure 2.7 A schematic flowchart of the plant extracts preparation for ultra-high performance liquid chromatography-quadrupole time-of-flight mass spectrometry.

2.3.2 Plant Extracts Preparation for Gas Chromatography-Mass Spectrometry (Chapter 5)

Nepenthes ampullaria and *N. rafflesiana* were freshly harvested and kept cold on ice in a polystyrene box during transport to Swinburne Sarawak laboratories. The plants were washed under running tap water and leaf samples were harvested and freeze dried. The freeze-dried leaf samples were extracted with methanol. The extraction was performed by preparing 500 g of crushed freeze-dried leaf samples in a Schott bottle with enough methanol to completely cover and left standing for 24 hours. The extracts were filtered through Whatmann No. 1 filter and concentrated using a rotary vacuum evaporator (Figures 2.8 and 2.9), then freeze dried into solid form. One mg of dried extracts were weighted and reconstituted using 1 mL of methanol, then filtered using 0.2 µm PTFE membrane filter before being subjected to Gas chromatography mass spectrometry analysis. Figure 2.10 shows the schematic flowchart of the plant extracts preparation for Gas Chromatography-Mass Spectrometry.

Figure 2.8 The methanolic leaf extracts of *N. ampullaria* in a rotary evaporator. Note the green color of the extracts.

Figure 2.9 The methanolic leaf extracts of *N. rafflesiana* in a rotary evaporator. Note the red color of the extracts.

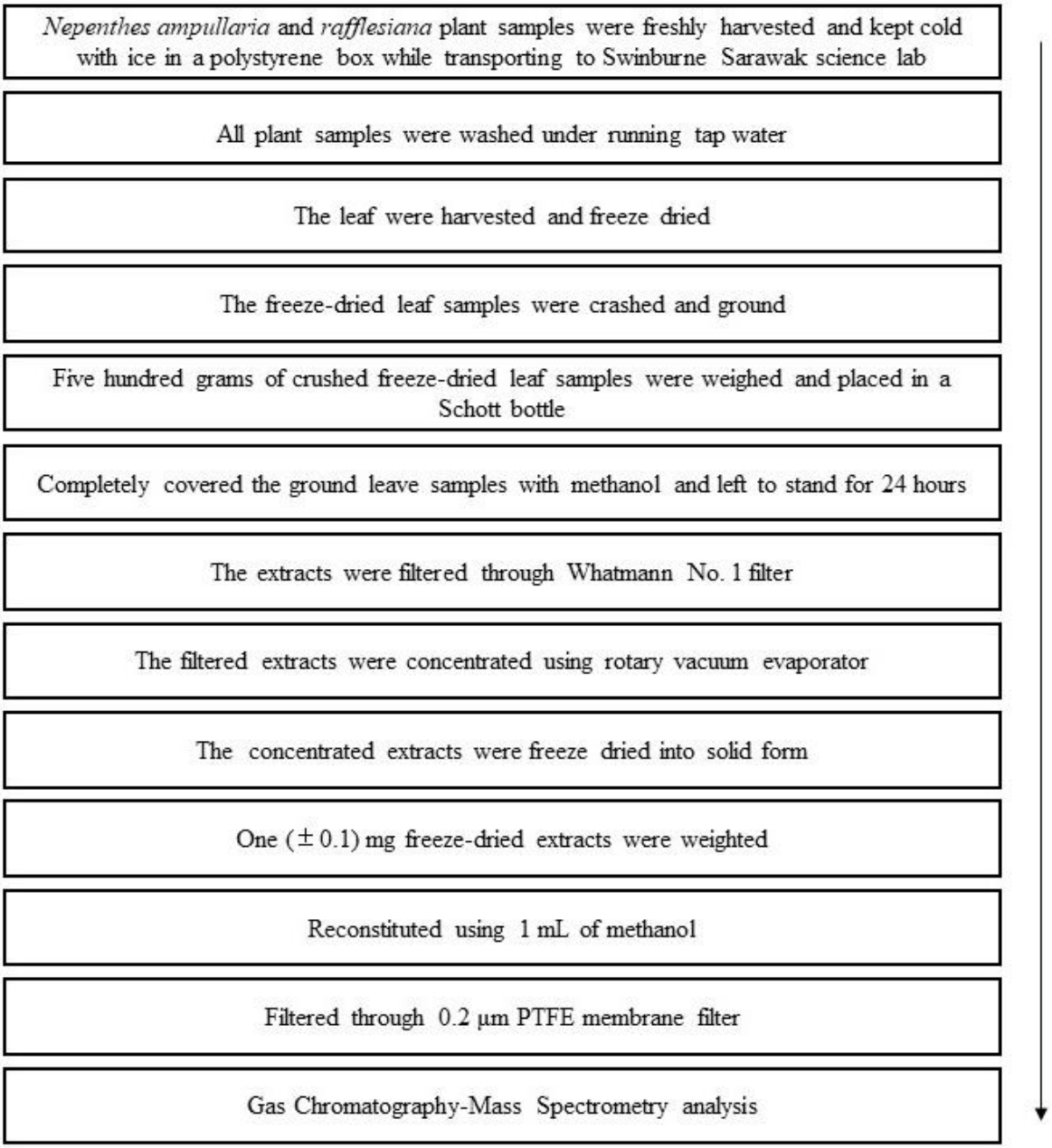


Figure 2.10 A schematic flowchart of the plant extracts preparation for Gas Chromatography-Mass Spectrometry.

2.4 Metabolite Fingerprinting

Development in analytical instrumentation have improved the metabolite coverage for metabolomics. Nuclear Magnetic Resonance (NMR) spectroscopy, Fourier Transform-Infrared spectroscopy (FT-IR), and Mass Spectrometry (MS) coupled with chromatographic separation such as such as high/ultra-high performance liquid chromatography (HPLC/UHPLC), Gas Chromatography (GC), or Capillary Electrophoresis (CE), are some of the common analytical techniques that are useful for metabolomic studies (Sardans et al. 2011). Among those, Liquid Chromatography-Mass Spectrometry (LC-MS) and Gas Chromatography-Mass Spectrometry (GC-MS) are the most powerful techniques for metabolite separation and analyses covering a wide range of metabolomes (Sardans et al. 2011; Matich et al. 2019).

2.4.1 Liquid Chromatography-Mass Spectrometry (LC-MS)

LC-MS is particularly important in studying a great number of metabolic pathways because it analyses a very wide range of chemical species, including the thermolabile, non-volatile, polar, and nonpolar metabolites, with small sample size and minimal sample preparation is required. The technique uses the soft ionization sources- electrospray ionization (ESI) and is usually acquired in both positive and negative modes. Ion trap (IT), triple quadrupole (QQQ), time of flight (TOF), and quadrupole time-of-flight (QTOF) are some of the mass analyzer instruments used in the combination with several chromatographic separation techniques such as HPLC, UHPLC and CE. UHPLC coupled with QTOF is best known to be able to produce extremely high chromatographic resolution with excellent sensitivity, high mass accuracy (<5 ppm) and fast data acquisition. Such combination has been used extensively in metabolomics research, especially in metabolite profiling/fingerprinting (Gika et al. 2014; Vorkaset et al. 2015; Makita et al. 2016; Núñez et al. 2020; Shi et al. 2020), as well as ecometabolomics (Sardans et al. 2011; Martinez et al. 2016; Zhang et al. 2017; Tortosa et al. 2018; Oliveira et al. 2020; Li et al. 2020).

2.5 Ultra-High Performance Liquid Chromatography-Quadrupole Time-of-Flight Mass Spectrometry (Chapter 3 and 4)

The extracted samples were profiled based on a previously published method (Ling et al. 2018). Briefly, 10 µL of the sample was injected into Kinetex F5 (2.1 × 100 mm, 2.6 µm; Phenomenex, Torrance, CA, USA) for chromatographic separation via Vanquish^{TM} Horizon UHPLC system (Thermo Fisher Scientific, USA). During analysis, the column was maintained at 40 °C with the flow rate of 600 µL/min. The mobile phase was composed of 2 solvents; solvent A (H_2O - 0.1 % HCOOH – 1 % 10 mM NH_4OAc) and solvent B (acetonitrile/methanol [6:4 v/v] – 1 % of 0.1 % HCOOH – 1 % 10 mM NH_4OAc). The gradient elution program was initiated from 1 % to 40 % solvent B in 5 min, followed by 100 % solvent B from 5.1 to 8 min and maintained for 2 min. Before injecting the next sample, the initial gradient was employed to condition the column for 3 minutes. UHPLC system was coupled with electrospray ionization Impact II QToF-mass spectrometry system (Bruker Daltonic, Germany). Mass-to-charge ratio (*m/z*) was set between 50 and 1500 for data acquisition. The heated electrospray ionization (ESI) was deployed at 4,200 V for positive. Ion source gas temperature and flow rate was set at 300°C and 12 L /min respectively.

Mass calibration solution, 10 mM sodium formate was introduced to the post-column through a 6-port valve diverted between 0.1 - 0.3 minutes. The acquired *m/z* was calibrated against the introduced sodium formate, and then subsequently converted into a mzXML file format.

2.5.1 Metabolomics Data Processing via MZmine 2

During data processing, raw data was exported as .mzXML format prior to MZmine 2 analysis (Pluskal et al. 2010). The software provides noise filtering, peak detection, normalization, alignment, and gap-filling and exported as .csv format (Table 2.2 shows the Processing steps and parameters of Mzmine, and Figure 2.11 shows the example of aligned and gap-filled peak list). Exported .csv files were introduced to multivariate analysis via MetaboAnalyst 4.0 (Chong et al. 2019). By using the open source metabolomic analytical tool, metabolite features with the missing value > 45% were removed, and the missing values were imputed using K-nearest neighbors (Do et al. 2018). The data was then log transformed and pareto scaled. Metabolite features (ANOVA $P < 0.01$) between the *Nepenthes* species under 3 environmental conditions were further subjected to compound matching and analysis.

The analysis conducted in 5 biological replicates and focuses only on the polar layer as the non-polar layer demonstrated no significant difference. All statistical analyses were performed on the positive ion data sets.

Table 2.2 Processing steps and parameters of Mzmine.

Processing steps		**Criteria**	**Parameter setting**
Raw data methods			
Filtering	Baseline correction	MS level	1
		Use m/z bins	✓
		m/z bin width	1×10^{-1}
		Correction method	Asymmetric baseline corrector
		Smoothing	1×10^{5}
		Asymmetry	1×10^{-3}
Peak detection	Mass detection	Mass detector	Centroid
		Noise level	1×10^{3}
		MS level	1
	Chromatogram builder	Minimum time span (min)	1×10^{-1}
		Minimum height	3×10^{3}
		m/z tolerance	0.005 m/z or 1.0 ppm
		MS level	1
Peak list methods			
Peak detection	Chromatogram deconvolution	Algorithm	Baseline cut-off
		Minimum peak height	1×10^{3}
		Peak duration range (min)	0.10 - 0.30
		Baseline level	1×10^{3}
Isotopes	Isotopic peaks grouper	m/z tolerance	0.001 m/z or 5.0 ppm
		Retention time tolerance	10.0 relative %
		Maximum charge	2
		Representative isotope	Lowest m/z
Filtering	Duplicate peak filter	m/z tolerance	0.001 m/z or 1.0 ppm
		RT tolerance	0.2 absolute (min)

		Require same identification	✓
Normalization	Linear normalizer	Normalization type	Total raw signal
		Peak measurement type	Peak area
Alignment	RANSAC aligner	m/z tolerance	0.001 m/z or 5.0 ppm
		RT tolerance	50.0 relative %
		RT tolerance after correction	50.0 relative %
		RANSAC iterations	0
		Minimum number of points	60%
		Threshold value	1 $x10^{-1}$
		Linear model	✓
		Require same charge state	✓
Filtering	Peak list rows filter	Minimum peaks in a row	5
Gap filling	Same RT and m/z range gap filler	m/z tolerance	0.001 m/z or 1.0 ppm

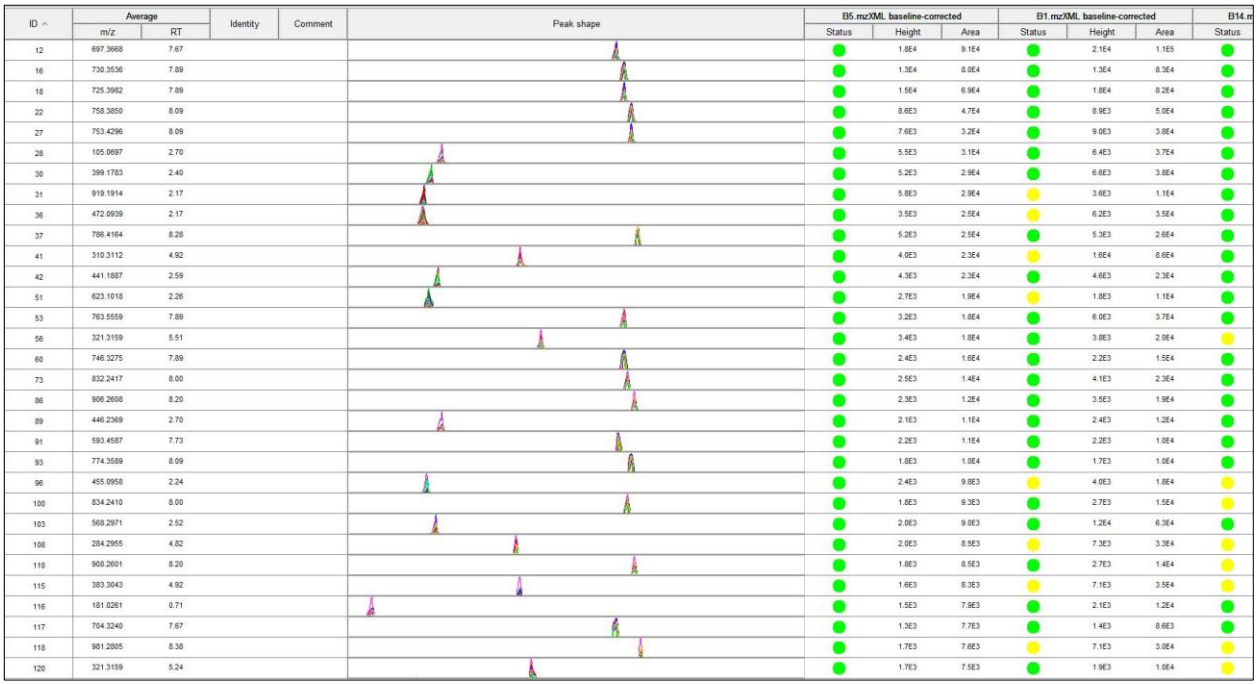


Figure 2.11 Screenshot of Mzmine 2.29 showing the aligned and gap-filled peak list. Each row represents the metabolic feature and the corresponding m/z with the extracted ion chromatogram. The columns with colored circles represent individual samples: green color- detected features and yellow color- gap-filled.

2.5.2 Metabolite Annotation and Identification

Metabolite features, including accurate m/z, possible chemical formula, and the fragmentation pattern, were queried against biological databases (highest priority is given to the database KEGG, followed by PubChem, and other databases, such as ChEBI and ChemSpider) by the application of in silico fragmenter MetFrag (Ruttkies et al. 2016). The precursor mass search was set at 5 ppm. Higher value of precursor mass search (up to 25 ppm) was used if there was no candidate retrieved using 5 ppm. The fragmentation settings were Mzppm = 5, Mzabs = 0.01, Mode = [M+H]+; [M+NA]+; [M+NH4]+; [M+K]+, and Tree depth = 2. In addition to the dashboard scoring, the MetFrag scoring terms such as Spectral Similarity (MoNA), Exact Spectral Similarity (MoNA), Statistical Scoring was activated.

The candidate was chosen based on the following criteria:

(a) highest score with at least 80 % matched of the major fragment ions towards the databases.

(b) lowest relative mass deviation error when compared to the theoretical value.

(c) lowest relative mass deviation error from the fragment ions matched.

To increase the accuracy of the identified metabolites, the matched compounds were cross checked with earlier literature on similar compound especially found in *Nepenthes* or in other plants. Pathway Tools Omics Viewer, developed by the Plant Metabolic Network (PMN) were used to identify the highly correlated metabolites and visualize the biosynthetic pathways (Caspi et al. 2011).

2.5.3 Multivariate Statistical Analysis

Multiple comparison of means tests and pie chart were performed using Microsoft Excel. The data were pre-transformed using generalized logarithm transformation method via MetaboAnalyst 4.0 and two-way ANOVA were performed using PAST software. Multivariate analyses including analysis of variance (ANOVA), principal component analysis (PCA), partial least squares–discriminant analysis (PLS-DA), hierarchical cluster analysis and heat map were performed using MetaboAnalyst 4.0 (Chong et al. 2019). Venn diagram is created using Venny 2.1- developed at Bioinformatics for Genomics and Proteomics (Oliveros 2007). Highly correlated metabolites in different biosynthetic pathways was obtained through Omics Viewer (Caspi et al. 2011). Figure 2.12 shows the schematic diagram summarizing the UHPLC-Q/TOF-MS analysis workflow.

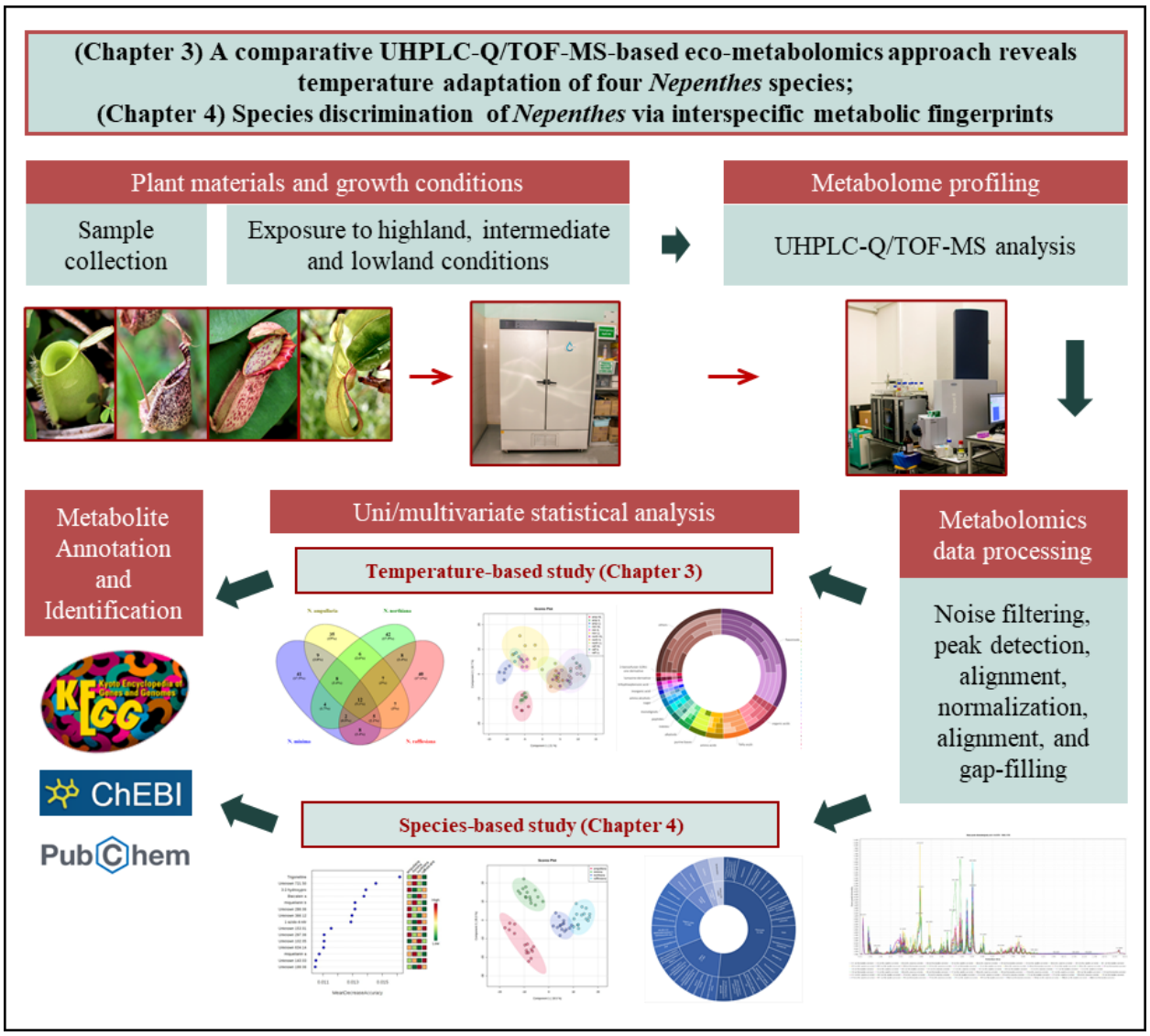


Figure 2.12 Schematic diagram summarizing the UHPLC-Q/TOF-MS analysis workflow.

2.6 Gas Chromatography-Mass Spectrometry (Chapter 5)

GC-MS is a robust tool for the study of volatile compounds (Sardans et al. 2011). It provides high chromatographic resolution, permitting the separation of structurally similar compounds, reproducible retention times, and is easy to operate. The technique requires the analyte to be volatile and thermally stable, thus on some occasions, a chemical derivatization step will be used prior to the chromatographic separation to yield volatile and thermostable compounds. Nearly all GC-based metabolomic applications combine GC with MS detection using hard ionization sources - electron ionization (EI), which allows for in-source fragmentation and identification of molecular ion through extensive databases such as Wiley/NBS Mass Spectral Database and the NIST/EPA/NIH Mass Spectral Database (Vinaixa et al. 2016; Gross 2017).

GC-MS has been widely applied and presented in different fields of metabolomics research such as microbiology, food-, cardiovascular-, medical-, and plant science (Barderas et al. 2011; Koek et al. 2011; Hussain and Maqbool 2014).

Analysis was performed using Shimadzu GC-MS (Model QP-2010, Shimadzu Co., Kyoto, Japan), equipped with Rtx-5MS capillary column (inner diameter of 0.25 mm, length of 30 m, and film thickness of 0.25 μm). The GC-MS condition was modified from the method by Song et al. (2015). Both injector and ion source temperature were set at 200 ºC. The oven temperature was initially programmed at 50 °C (isothermal for 1 min), then increased to 280 °C at 10 °C/min (isothermal for 5 min). The carrier gas helium was used at the flow rate of 1 mL/min and an injector volume of 1 μL, using a 10:1 split ratio. Electron impact at 70 eV was used as the ionization mode. Data was collected in the full scan mode (40-400 m/z). The methanolic extracts (1 mg/mL) were prepared, filtered (0.20 μm syringe filter), and injected onto the GC-MS. The mass spectra of compounds obtained from GC-MS were tentatively identified based on mass spectral data from the Wiley library (7^{th} edition). Only the candidates with the spectral data matched of 80% and above were selected, and the selected candidates were further verified via earlier literature on similar compound found in *Nepenthes* or in other plants.

2.6.1 Anti-bacterial activity via disk diffusion assay

Anti-bacterial activities of the samples were evaluated against eight bacteria species, including Gram-positive (*Bacillus subtilis*, *Bacillus cereus*, *Streptococcus salivarius* and *Staphylococcus epidermidis*) and Gram-negative bacteria (*Salmonella typhimurium*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*). The antimicrobial activity was studied using the disk diffusion assay by a protocol modified by Murray et al. (1995). The freeze-dried plant extract was re-dissolved in methanol to yield solution containing 50 mg of extract per mL. The sterilized discs (Whatman grade A filter paper discs of 6 mm diameter) were loaded with 10 μL of the extracts, with methanol as negative control. The loaded discs, including the negative control, were air dried and placed on the testing bacteria inoculated agar plate- prepared by inoculating the overnight broth bacteria cultures onto nutrient agar plate (20 mL of nutrient agar per petri dish) using sterile cotton swabs. The agar plates were incubated at 37ºC for 24 hours. The inhibition zones were measured and recorded in millimeter. All assays were performed in 5 replicates. Figure 2.13 shows the schematic diagram of GCMS analysis workflow.

2.6.2 Statistical Analyses

Statistical analyses including two-way analysis of variance (ANOVA) and tukey's post-hoc test was performed using PAST software. The multivariate analyses including hierarchical cluster analysis, principal component analysis (PCA) and heat map were performed using MetaboAnalyst 4.0 (Chong et al. 2019), with the missing values imputed using K-nearest neighbors (Do et al. 2018). The data was log transformed and pareto scaled. The .csv file with the identified metabolite features is provided in the supplementary file. Venn diagram was created using Venny 2.1- developed at Bioinformatics for Genomics and Proteomics (Oliveros 2007).

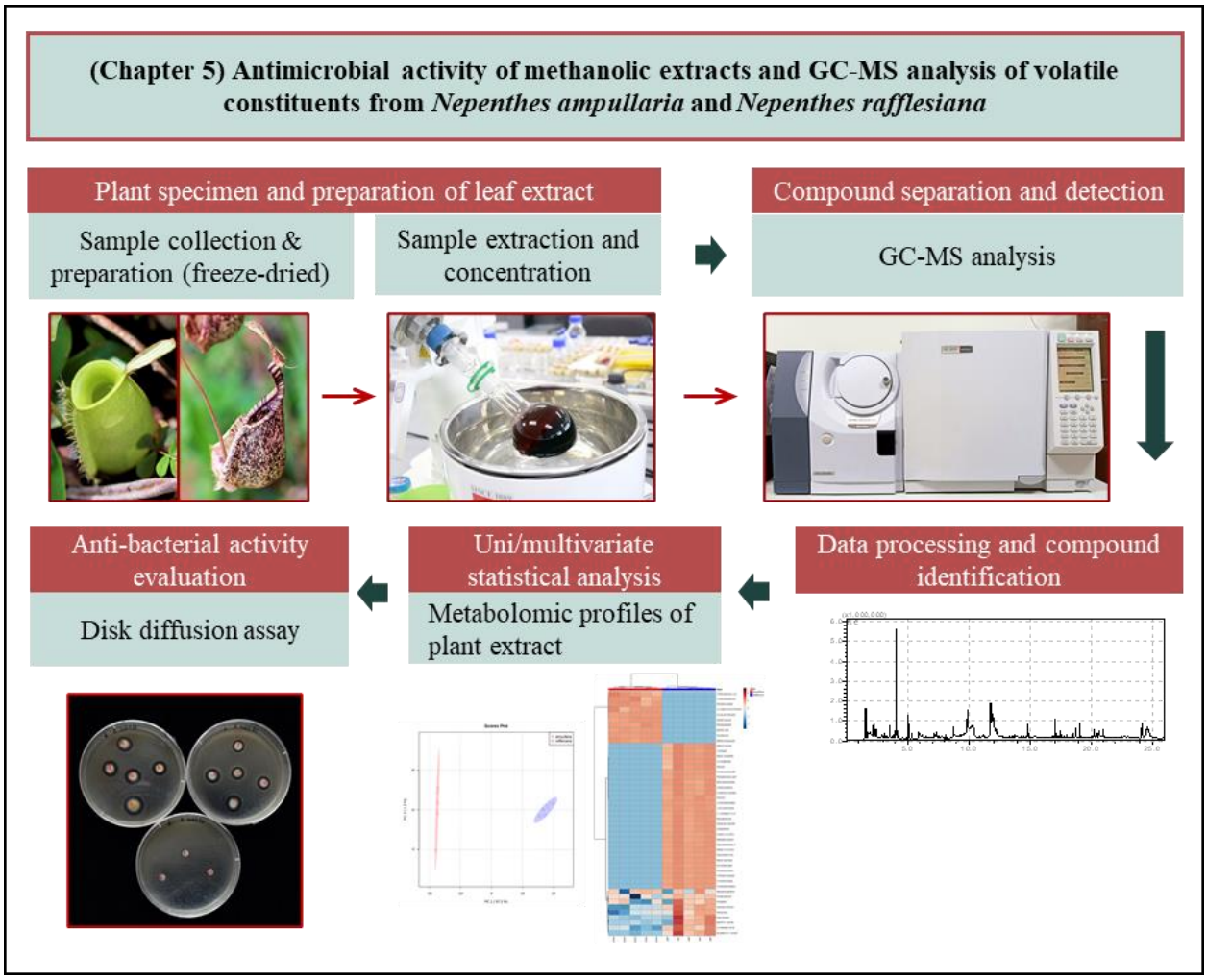


Figure 2.13 Schematic diagram of GCMS analysis workflow.

Chapter 3

A comparative UHPLC-Q/TOF-MS-based ecometabolomics approach reveals temperature adaptation of four *Nepenthes* species

Abstract

Nepenthes, as the largest families of carnivorous plants, is found with an extensive geographical distribution throughout the Malay Archipelago, specifically in Borneo, Philippines, and Sumatra. Highland species are able to tolerate cold stress and lowland species heat stress. Our current understanding on the adaptation or survival mechanisms acquired by the different *Nepenthes* species to their climatic conditions at the phytochemical level is, however, limited. In this study, we applied an ecometabolomics approach to identify temperature stressed individual metabolic fingerprints of four *Nepenthes* species: the lowlanders *N. ampullaria*, *N. rafflesiana* and *N. northiana*, and the highlander *N. minima*. We hypothesized that distinct metabolite regulation patterns exist between the *Nepenthes* species due to their adaptation towards different geographical and altitudinal distribution. Our results revealed not only distinct temperature stress induced metabolite fingerprints for each *Nepenthes* species, but also shared metabolic response and adaptation strategies. The interspecific responses and adaptation of *N. rafflesiana* and *N. northiana* reflected their natural habitat niches. Moreover, our study also uncovered the potential of lowlanders, especially *N. ampullaria* and *N. rafflesiana*, to inhabit highland areas, and the highlander *N. minima* to inhabit lowland areas, thus providing a new way of thinking about the plant genus and future adaption in times of changing climate.

3.1 Introduction

Nepenthes (*N.*), the sole genus under the family *Nepenthaceae*, is one of the largest families of carnivorous plants, with an extensive geographical distribution across the Malay Archipelago, specifically in Borneo, Philippines, and Sumatra. To date, 151 species have been documented, with most species displaying high degrees of endemism and often restricted to single areas, i.e. *N. villosa*, *rajah* and *burbidgeae* which can only be found in Mount Kinabalu

and the neighboring Mount Tambuyukon in Borneo (Jebb & Cheek 1997; McPherson 2009; Murphy et al. 2020). The characteristic pitcher and their adaptation to nutrient poor soils has been well documented (Clarke 1997; Clarke & Moran 2016; Barthlott et al. 2007; Seine et al. 1996).

Nepenthes can be clustered into two groups: lowlanders (with altitudinal distributions below 1100 m above sea level (asl) – hot and humid jungles) which can tolerate heat stress and highlanders (with altitudinal distributions beyond 1100 m asl such as highland montane forests with warm days and cool to cold, humid nights) which can tolerate cold stress (Cheek & Jebb 2001; McPherson 2009; McPherson et al 2011). There are some exemptions such as *Nepenthes ampullaria* and *N. rafflesiana*, even though categorized as lowland species, both were recorded in highland environments but only very rarely (Jebb & Cheek 1997; Clarke & Wong 1997; McPherson 2009; Clarke & Moran 2016). Besides that, *N. minima* was the only highlander species able to grow well at our greenhouse under lowland conditions. Our current understanding on the adaptation or survival mechanisms acquired by the different *Nepenthes* species to their climatic conditions at the phytochemical level is, however, limited.

Heat stress has been shown to increase respiration, reduce photosynthesis, disrupt plant cellular structures and defensive mechanisms, and elevate stress metabolites production in plants (Ding et al. 2016; Djanaguiraman et al. 2010; Wahid et al. 2007). Low temperature stress, on the other hand, can aggravate the balance between the energy source, cause cellular DNA damage, disrupt physiological functions of plant cell and the metabolic sink, and affect the photosynthesis rate of the plant (Wang et al. 2020; Liu et al. 2018; Allen & Ort 2001; Paul & Foyer 2001). Both stresses will cause overproduction of reactive nitrogen species (RNS) and reactive oxygen species (ROS), thus causing oxidative stress in plants (Samanta et al. 2011; Sharma et al. 2012; Airaki et al. 2012; Kuk et al. 2003).

Metabolomics can contribute significantly to our understanding of stress responses in plants by identifying the involved metabolites in response to endogenous and exogenous stressor (see for example Wei et al. 2020; Jian et al. 2020; Guy et al. 2008; Shulaev et al. 2008). Applying a non-targeted metabolome approach, combined with high-resolution Mass Spectrometry (MS) and high-resolution chromatography, we can discover true dynamics of biological systems in response to specific perturbations (Weckwerth 2003).

In the current study, we investigated the impact of heat and cold stress on four *Nepenthes* species, representing lowland (*N. ampullaria*, *N. rafflesiana* and *N. northiana*) and highland species (*N. minima*). Using a MS-based non-targeted approach, we aim to understand how the underlying ecological adaptation of the plant species influences their metabolite regulation upon heat and cold exposure. Do they share (a) similar response toward the provided environmental conditions; or (b) are different strategies been applied by each species?

3.2 Methods

3.2.1 *Nepenthes*

Four *Nepenthes* species namely *Nepenthes minima*, *Nepenthes ampullaria*, *Nepenthes northiana* and *Nepenthes rafflesiana* were pre-adapted at lowland greenhouse for a period of 6 months, at least, before being subjected into climatic chamber with control environment conditions. The *Nepenthes* in this study represented highland and lowland climatic conditions (Table 3.1; for morphological details of the plant, please refer to Jebb and Cheek (1997) and Adam et al. (1992)). We hypothesized that distinct metabolite regulation patterns can be distinguished between the *Nepenthes* species due to their adaptation towards different geographical and altitudinal distribution.

Table 3.1 Four *Nepenthes* species employed in this study.

***Nepenthes* (*N.*) species**	**Habitat**	**Altitudinal distribution**	**Environmental niche assigned**
N. northiana	Limestone hills	0 - 500	Lowlander
N. rafflesiana	Open area, shady forest, offshore	0 - 1500	Lowlander
N. ampullaria	Damp, shady forest, swamp forest	0 - 2000	Lowlander
N. minima	Open grassland, with grey-yellow clay as substrate (highland grasslands of Central Sulawesi (Celebes)	1000 - 1700 with most localities lying above 1400m asl.	Highlander

3.2.2 Plant materials and growth conditions

All plants were grown in a Pol-Eko-Aparatura climatic chamber with phytotron system (Model: KK 750 FIT P) in a mixture of cocopeat and perlite (at a ratio of 10 : 0.5; g/g). All plants were exposed to 12 hours of light and 12 hours of darkness.

The plants were exposed to (a) lowland (33 °C day / 28 °C night), (b) intermediate (25 °C day / 18 °C night), and (c) highland conditions (23 °C day / 8 °C night) for 7 days before being harvested. The growing tips, the active growing part of the plants, were harvested and freeze-dried (Labconco Freezone 6 Freeze Dryer System), and metabolites extracted.

3.2.3 Sample preparation

One (±0.1) mg freeze-dried plant samples was weighed, ground, and exhaustedly extracted with 600 µL of solvent mixture of methanol:chloroform:ultrapure water (with resistance of 18.2 Ωcm^{-1}) with 1% sodium chloride added (1:1:1 v/v/v). Mixtures were vortexed for 30 minutes at room temperature, followed by 30 minutes centrifugation at 3000 × *g* maintained at 4°C. The lower layer was then transferred to a new borosilicate tube and vacuum dried using a speed concentrator. Dried extracts were then reconstituted using 400 µL of methanol and filtered using 0.2 µm PTFE membrane filter before being subjected to Liquid Chromatography and Mass Spectrometry analysis.

3.2.4 Metabolome profiling

The extracted samples were profiled based on previously published method (Ling et al. 2018). Briefly, 10 µL of the sample was injected into Kinetex F5 (2.1 × 100 mm, 2.6 µm; Phenomenex, Torrance, CA, USA) for chromatographic separation via Vanquish^{TM} Horizon UHPLC system (Thermo Fisher Scientific, USA). During analysis, the column was maintained at 40°C with the flow rate of 600 µL/min. The mobile phase was composed of 2 solvents; solvent A (H_2O - 0.1 % HCOOH – 1 % 10 mM NH_4OAc) and solvent B (acetonitrile/methanol [6:4 v/v] – 1 % of 0.1 % HCOOH – 1 % 10 mM NH_4OAc). The gradient elution program was initiated from 1% to 40% solvent B in 5 min, followed by 100% solvent B from 5.1 to 8 min and maintained for 2 min. Before injecting the next sample, the initial gradient was employed to condition the column for 3 minutes. UHPLC system was coupled with electrospray ionization Impact II QToF-mass spectrometry system (Bruker Daltonic, Germany). Mass-to-charge ratio (*m/z*) was set between 50 and 1500 for data acquisition. The heated electrospray ionization (ESI) was

deployed at 4,200 V for positive. Ion source gas temperature and flow rate was set at 300°C and 12 L /min respectively.

Mass calibration solution, 10 mM sodium formate was introduced to the post-column through a 6-port valve diverted between 0.1 - 0.3 minutes. The acquired *m/z* was calibrated against the introduced sodium formate, and then subsequently converted into a mzXML file format.

3.2.5 Metabolomics data processing

Raw data was exported in .mzXML format prior to MZmine 2 analysis (Pluskal et al. 2010). The software provides noise filtering, peak detection, alignment, normalization, alignment, and gap-filling and exported data in .csv format. Exported .csv files were used for multivariate analyses with MetaboAnalyst 4.0 (Chong et al. 2019). Metabolite features with missing values > 45% were removed, and missing values imputed using K-nearest neighbors (Do et al. 2018). The data was log transformed and pareto scaled. Metabolite features (ANOVA $P < 0.01$) between the *Nepenthes* species under 3 environmental conditions further subjected to compound matching and analysis. The .csv file with significantly changed metabolite features is provided in the supplementary file. The current analysis focused on the polar layer only as the non-polar layer demonstrated no significant difference (data excluded). All statistical analyses were performed on the positive ion data sets.

3.2.6 Metabolite Annotation and Identification

Metabolite features, including accurate m/z, possible chemical structures, and the fragmentation pattern, were queried against biological databases (highest priority was given to the database KEGG, followed by PubChem, and other databases, such as ChEBI and ChemSpider) using in silico fragmenter MetFrag (Ruttkies et al. 2016). The candidate was chosen based on the following criteria: (a) highest score with at least 80% match of the major fragment ions towards the databases (b) lowest relative mass deviation error when compared to the theoretical value (c) lowest relative mass deviation error from the fragment ions matched. To increase the accuracy of the identified metabolites, we cross checked the matched compound with earlier literature on similar compounds especially in *Nepenthes* or in other plants. Pathway Tools Omics Viewer, developed by the Plant Metabolic Network (PMN), was used to identify highly correlated metabolites and to visualize the biosynthetic pathways (Caspi et al. 2011).

3.2.7 Statistical Analyses

Multiple comparison of mean tests, two-way ANOVA and pie chart were performed using Microsoft Excel. The data were pre-transformed using generalized logarithm transformation method via MetaboAnalyst 4.0 and Post hoc analyses were performed using PAST software. Multivariate analyses including analysis of variance (ANOVA), principal component analysis (PCA), partial least squares–discriminant analysis (PLS-DA), hierarchical cluster analysis and heat map were performed using MetaboAnalyst 4.0 (Chong et al. 2019). Venn diagram was created using Venny 2.1- developed at Bioinformatics for Genomics and Proteomics (BioinfoGP) (Oliveros 2007). Highly correlated metabolites in different biosynthetic pathways was obtained through Omics Viewer (Caspi et al. 2011).

3.3 Results

We determined the individual metabolite fingerprints of four (4) *Nepenthes* species in response to highland, intermediate and lowland growing conditions. The applied workflow allowed the determination of 125 significantly altered metabolites under the experimental conditions from which 89 could be identified (Supplementary Table 1). The identified metabolites were grouped under 16 categories, with the majority of them being flavonoids, followed by organic acids, fatty acyls, amino acids, purine base, alkaloids, etc. (Figure 3.1). Fourteen of the identified metabolites were found to be involved in 32 metabolic pathways (Table 3.2).

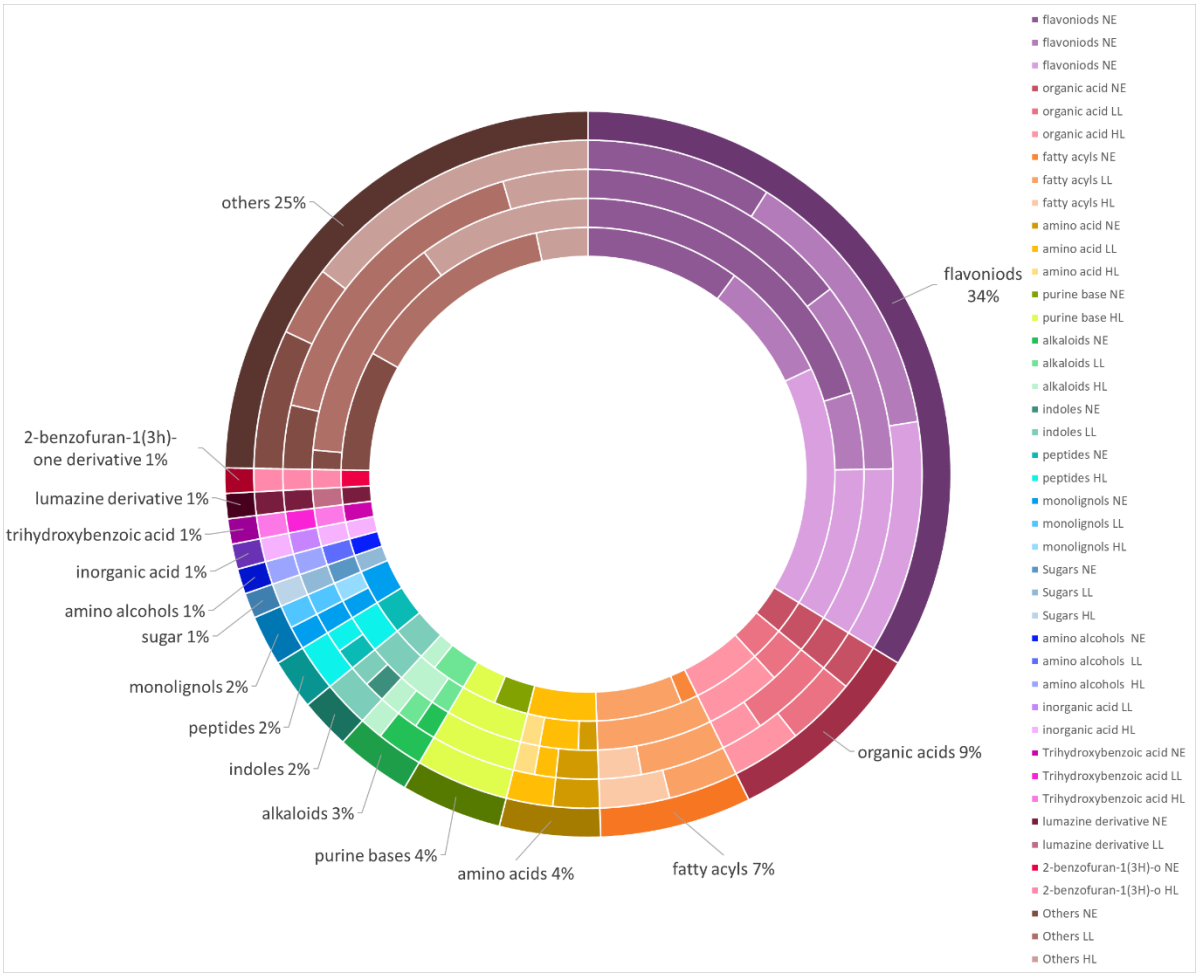


Figure 3.1 Pie chart depicting percentages of the metabolite group of the 89 putatively identified metabolites, as well as the regulation of the metabolites of each group in response to the different temperature conditions. Each layer indicates a *Nepenthes* species, from inner layer to outer layer: *N. minima*, *ampullaria*, *northiana*, *rafflesiana*. NE: no effect/ particular pattern, LL: metabolites expressed highest at lowland condition, HL: metabolites expressed highest at highland condition.

Table 3.2 Summary of highly correlated metabolites in different biosynthetic pathways. Letter(s) after the pathways and metabolites indicate the involved metabolites in the same pathway. Enrichment analysis was carried out using the Fisher Exact statistical test.

Biosynthetic pathways	P-value	Metabolites
Phenylpropanoid derivatives biosynthesis[a]	2.56e-05	Quercetin[a,b,c,d,e,f,g,j,q]
Superpathway of flavones and derivatives biosynthesis[b]	4.65e-04	Luteolin[a,d,g,j,n]
Flavonols biosynthesis[c]	7.48e-04	Syringin[a,j,k]
Flavonoids biosynthesis[d]	1.18e-03	Kaempferol-3-glucoside[a,b,c,d,j]
Rutin biosynthesis[e]	2.95e-03	Rutin[a,b,c,d,e,j]
Quercetin glycoside biosynthesis (Arabidopsis)[f]	5.07e-03	Benzoate[a,j,m,p]
Flavonoid Biosynthesis (In Equisetum)[g]	7.72e-03	Coniferin[a,j,k,p,r]
Proteinogenic amino acids biosynthesis[h]	2.26e-03	Quercetin 3-O-
Amino acids biosynthesis[h]	6.54e-03	rhamnoside[a,b,c,d,f,j]
Proteinogenic amino acids degradation[i]	8.72e-03	L-Arginine[h,i,p,s]
Amino acids degradation[i]	1.07e-02	L-Tryptophan[h,i,j,p]
Secondary metabolites biosynthesis[j]	1.29e-02	Adenine[h,l,p,t]
Lignins biosynthesis[k]	2.31e-02	L-Isoleucine[h,i]
S-methyl-5'-thioadenosine degradation ii[l]	2.59e-02	Adenosine[h,i,o,p,t]
Benzoyl-β-d-glucopyranose biosynthesis[m]	2.59e-02	L-Isoleucine[p]
Benzoate degradation[m]	3.86e-02	
Benzoate degradation II (aerobic and anaerobic)[m]	3.86e-02	
Chrysoeriol biosynthesis[n]	3.23e-02	
Adenine and adenosine salvage VI[o]	3.23e-02	
Degradation/utilization/assimilation[p]	3.35e-02	
Methylquercetin biosynthesis[q]	3.86e-02	
Phenylpropanoid derivatives degradation[r]	4.49e-02	
Coniferin metabolism[r]	4.49e-02	
Putrescine biosynthesis I[s]	4.49e-02	
Adenine and adenosine salvage II [t]	8.13e-04	
Adenine and adenosine salvage[t]	1.72e-03	
Purine Nucleosides Salvage II (Plant)[t]	2.95e-03	
Cytokinins degradation[t]	3.43e-03	
Purine nucleotide salvage[t]	7.72e-03	
L-methionine salvage[t]	2.19e-02	
L-methionine biosynthesis[t]	3.06e-02	
Purine nucleotide biosynthesis[t]	4.04e-02	

3.3.1 Individual metabolomic fingerprints of the four *Nepenthes* species

The metabolomes of the four *Nepenthes* species, subjected to varying temperature regimes, displayed significant differences ($P < 0.01$; Supplementary Table 2). A dendrogram based on Pearson distances and average clustering showed very distinct grouping of *N. ampullaria* and *N. minima* under all three temperature conditions compared to *N. northiana* and *N. rafflesiana* (Figure 3.2a). Principle components 1 and 2 derived from the PLS-DA, showed the total variance among the species at 39.7% (Figure 3.2b). Despite grouping distinctly on its own, PLS-DA indicated greater temperature-related variability of *N. ampullaria* compared to the other species (Supplementary Table 3). The PLS-DA model was well-validated using a permutation test with $p < 0.001$ after 1000 permutations (Supplementary Figure 1).

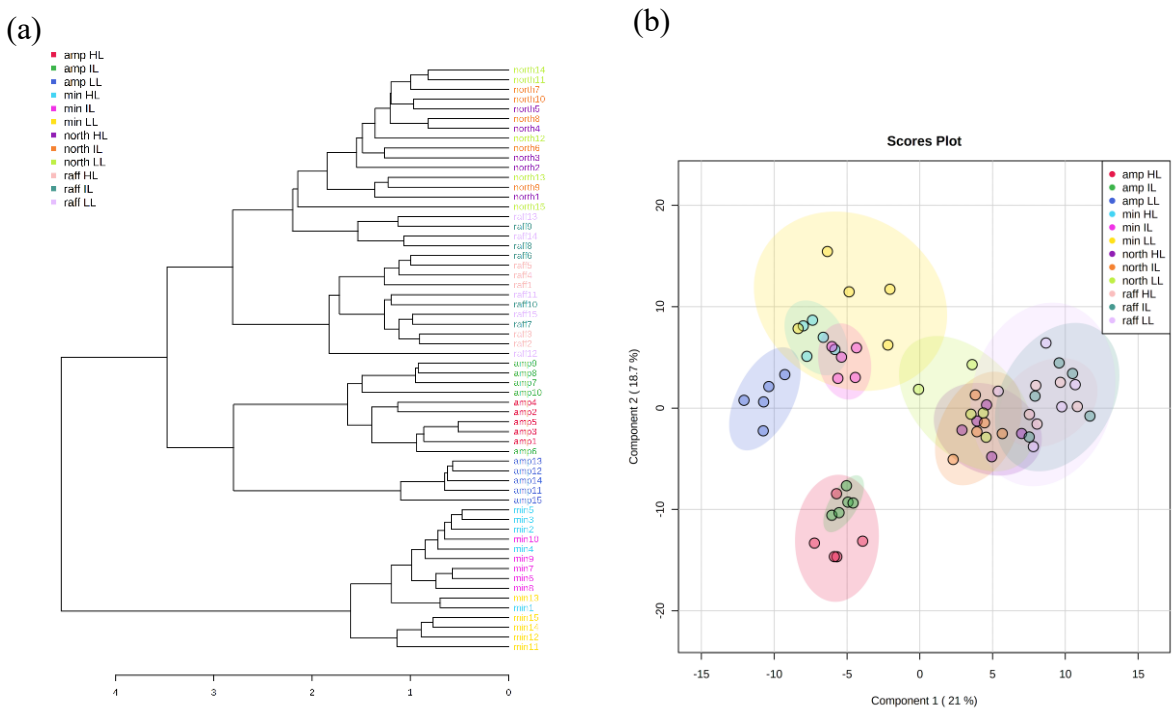


Figure 3.2 Metabolite profiles of the four *Nepenthes* species as influenced by the three different growing conditions. (a) Dendrogram shows the relationship among the samples using Pearson distances and average clustering. (b) Principal Component Analysis (PCA) score plot showing the spatial separation between the *Nepenthes* species and the provided growing conditions. Abbreviations: amp represents *N. ampullaria*, min represents *N. minima*, north represent *N. northiana*, and raff represents *N. rafflesiana*. The numbering after the species name represents the biological replicates. HL represent highland condition, IL represent intermediate condition, and LL represent lowland condition.

3.3.2 Universal Metabolite Response to temperature stress

Our result showed that the metabolites were greatly affected by temperature stress under lowland and highland conditions. While the metabolites expressed differed significantly among the four species (Figure 3.3), high or lowland stress also led to a similar response in metabolite regulation across all our species. Adenine, berberastine baicalein (isomers included) and 1-naphthoic acid were, for example, were the most expressed compounds under highland cold stress, whereas L-tryptophan, 18-oxononadecanoic acid, olealdehyde and indole-3-acrylic acid were the most expressed compounds under lowland heat stress (Figure 3.3; Supplementary Table 4). Interestingly, a flavone baicalein together with its isomers showed the highest accumulation at both highland and lowland conditions (Supplementary Table 4). Among the identified compounds, certain groups showed consistent expression among the *Nepenthes* sp. such as purine bases (highest expression at highland condition), fatty acyls, amino acids, and indoles (highest expression level at lowland conditions).

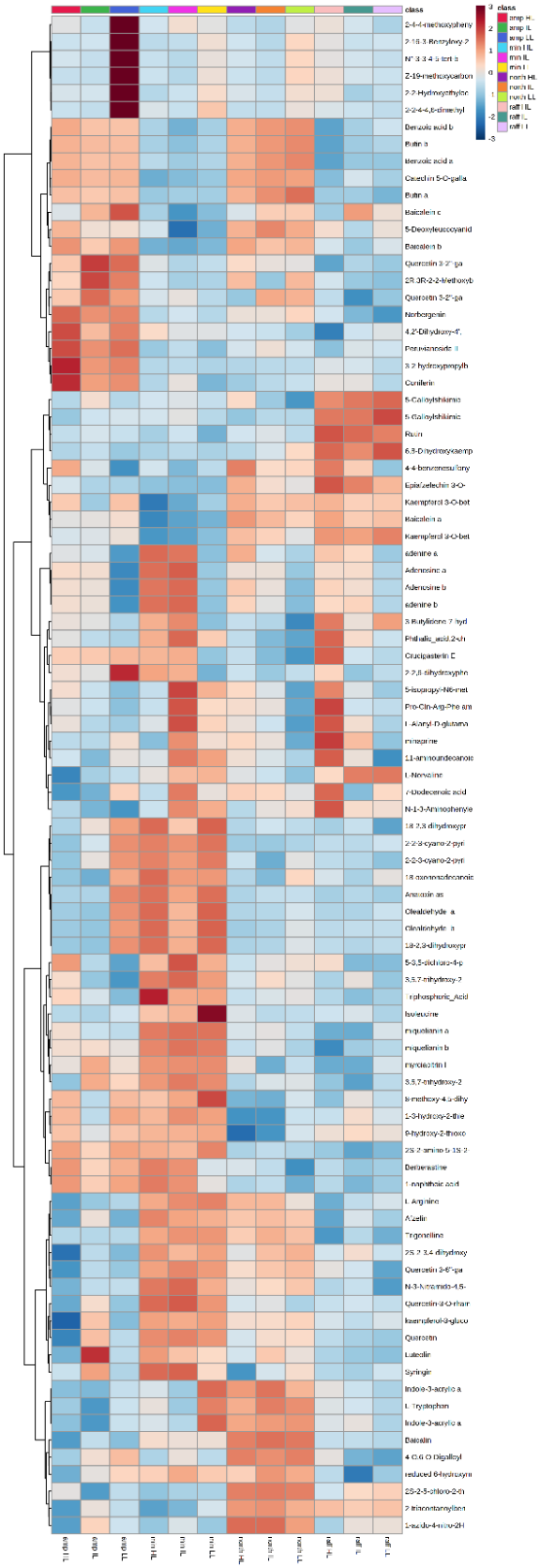


Figure 3.3 Heatmap of 89 identified metabolites from the 4 *Nepenthes* species based on Euclidean distances and Ward clustering. The concentration of metabolites is presented on a log scale.

3.3.3 Species-specific Metabolite Response to temperature stress

Our results (Figure 3.4) also revealed unique combinations of metabolite responses towards heat and cold stress at interspecific level. For instance, *N. ampullaria* had the highest accumulation of alkaloid trigonelline and amino acid isoleucine under highland cold stress exposure while *N. minima* had these compounds at highest concentrations at lowland heat stress. *N. northiana* displayed high accumulation of norvaline under cold stress. Besides that, within the identified groups, most of the detected flavonoids had the highest expression under lowland conditions for *N. northiana* and *rafflesiana*, and highland condition for *N. minima*, while most of the flavonoids showed no change in response in *N. ampullaria*. Organic acids were recorded with the highest expression under highland conditions for *N. minima* and *ampullaria*, and lowland condition for *N. northiana*.

Apart from the universal and species-specific metabolite response mentioned above, we were able to observe the pattern of metabolites accumulation that were consistent for a subset of the species. For examples, under lowland heat stress, syringin exhibited the highest accumulation for *N. northiana* and *N. rafflesiana*, while the highest accumulation of adenosine were shown in *N. ampullaria*, *N. northiana* and *N. rafflesiana* under highland cold stress. A complete list of the metabolites expressed for each species under differing temperature conditions can be found in the Supplementary Table 4.

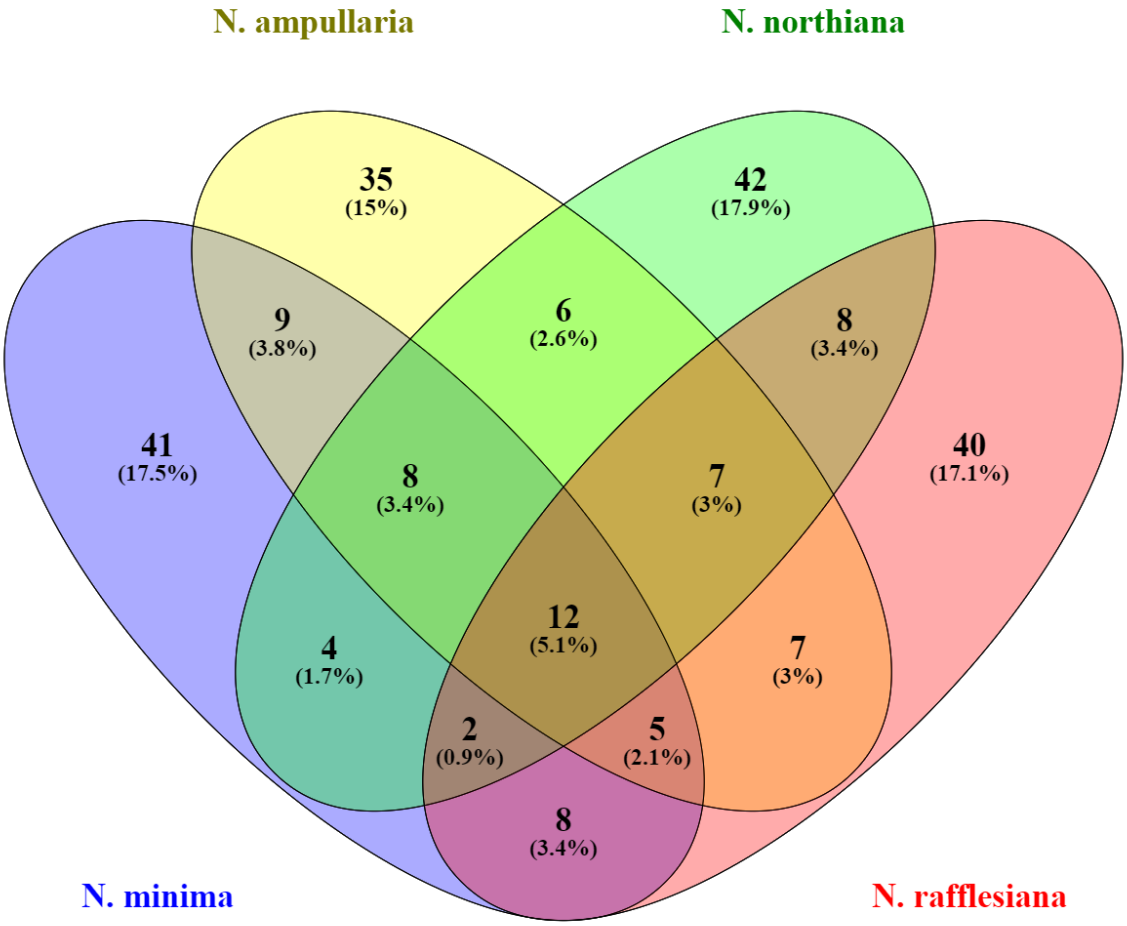


Figure 3.4 Venn diagram based on similarly expressed metabolites (highest expression recorded on either highland or lowland condition of the identified metabolites) among the 4 *Nepenthes* species in response to the three temperature conditions. Please refer to the supplementary file (Supplementary Table 4) for the metabolites detail.

3.3.4 Biosynthetic pathways and metabolic networks

We identified 14 metabolites that are involved in 32 metabolic pathways, including biosynthesis of phenylpropanoid and flavones derivatives, flavonols, flavonoids, amino acids, secondary metabolites and lignins, as well as coniferin metabolism (Table 3.2). A metabolic network was created to summarize the major heat and cold stresses adaptation strategies applied by the 4 *Nepenthes* species (Figure 3.5).

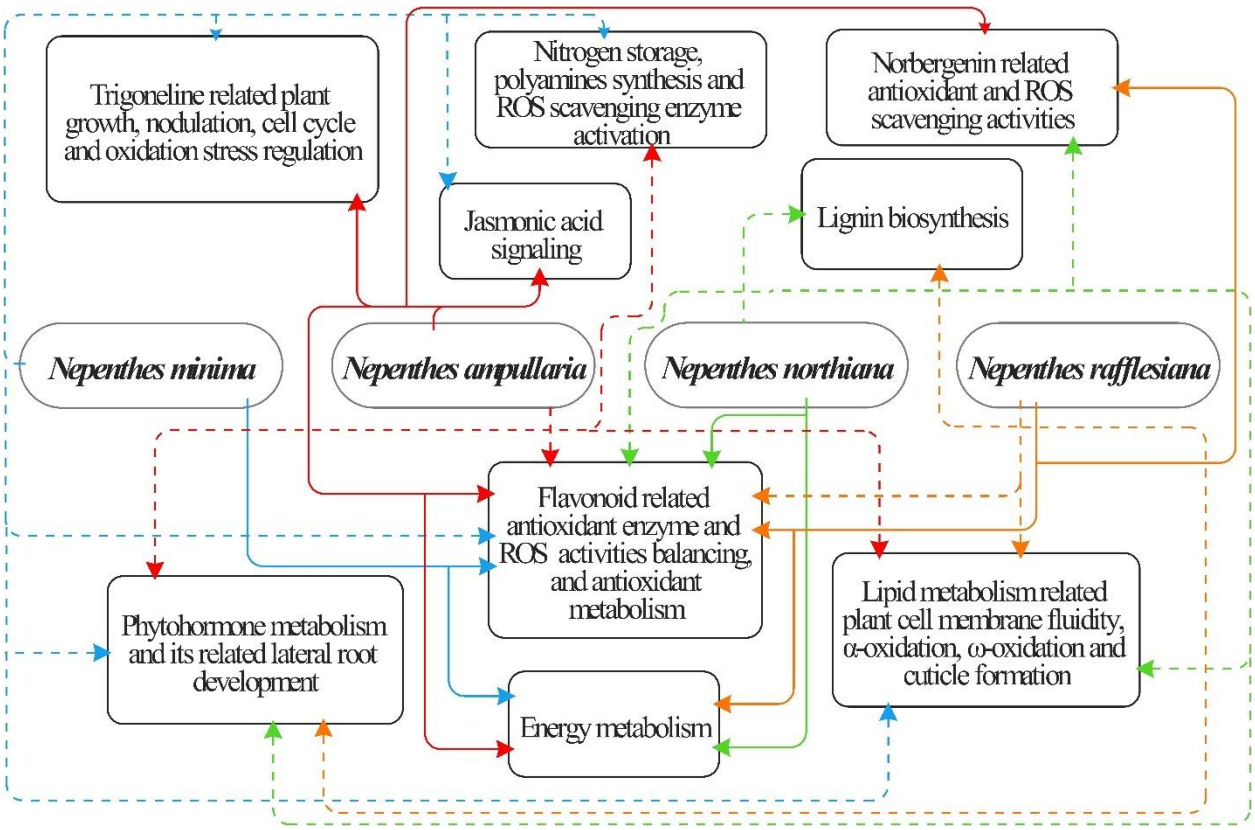


Figure 3.5 Adaptation strategies applied by different *Nepenthes* species in response to different environmental conditions. dot lines: strategies used under highland cold stress exposure; solid lines: strategies used under lowland heat stress exposure.

3.4 Discussion

Till today, we have no clear understanding on the adaptation or survival mechanisms acquired by different *Nepenthes* species to their climatic conditions. The distinctiveness of *N. ampullaria* and *N. rafflesiana* (capable of inhabiting lowland and highland altitudes), and *N. minima* (unique highlander that is able to grow at lowland conditions), as well as *N. northiana* from an extreme habitat (limestone vegetation), made them the target species for the present study. We aimed to shed light on their unique adaptation mechanisms by studying their metabolomes in response to different environmental conditions. For the purpose of this study, we considered temperature to be the main environmental variable between the highland (with temperatures down to 8 °C during the night) and lowland (up to 33 °C during the day) and exposed all four *Nepenthes* to temperatures representative of lowland, intermediate and highland conditions. *Nepenthes* are known to produce a wide range of metabolites but their metabolites have so far only been studied for their enzymatic properties and pharmacological potential (Van Thanh et al. 2015b; Saganová et al. 2018;

Sanusi et al. 2017). The role of metabolites as regulatory/signaling agents, or in defense against biotic and abiotic stresses (such as temperature changes), has been described in other plant species such as *Arabidopsis*, *Triticum*, and *Vitis* (Kaplan et al. 2004; Hassanein et al. 2013; Cohen et al. 2008). Our study provides the first insight into the response (at metabolome level) of *Nepenthes* plants originating from different climatic niches to temperature stress.

3.4.1 Universal adaptation strategy

Plants possess various acclimatization strategies to survive through the temperature stresses, which includes the accumulation of flavonoids, alterations in the membrane lipid composition and signaling, phytohormones regulation and signaling, increased in transpiration, ROS scavenger accumulation and many more (Das & Roychoudhury 2014; Takahashi et al. 2003; Tangmouo et al. 2009; Qi et al. 2010; Higashi & Saito 2019; Balfagón et al. 2019; Souri et al. 2020; Abdelrahman et al. 2020; Chen & Raji 2020). Similar strategies were also observed in *Nepenthaceae* (Figures 3.1, 3.3, 3.4 and 3.5).

Under lowland high temperature condition, *Nepenthes* apply several strategies to overcome the heat stress such as increased phytohormone metabolism and related lateral root development. L-tryptophan is known to be involved in auxin indole-3-acetate synthesis (Mano & Nemoto 2012; Zhao 2012), while isoleucine is known to be the key amino acid that activates endogenous phytohormone jasmonic acid (Staswick & Tiryaki 2004). Our results demonstrated the importance of auxin in *Nepenthes* plants to tolerate heat through enhanced lateral root system development to reduce the water loss via transpiration, thus providing better cooling effect for the plant. Similar strategies were found in *Arabidopsis thaliana*, where high temperature increased water loss via transpiration and enhanced leaf cooling capacity (Crawford et al. 2012). Besides that, the importance of α-oxidation (olealdehyde) and ω-oxidation (18-oxononadecanoic acid) in response to heat stress were also observed in *Nepenthes* plants (Buchhaupt et al. 2014; Rizzo 2014). Both oxidation processes, which involve aldehyde dehydrogenase as the key enzyme, are known to yield an unsaturated fatty acid (α-oxidation) and a dicarboxylic fatty acid (ω-oxidation) in which the unsaturated fatty acid is responsible in maintaining the fluidity of plant cell membrane lipids and dicarboxylic acid is essential for the cuticle formation in the plants (Hu et al. 2018; Kurdyukov et al. 2006). This can be further supported by the increased expression of aldehyde

dehydrogenase genes in *Arabidopsis* plant under heat stress exposure (Zhao et al. 2017). The amino acid norvaline was previously recorded in cold and drought stress responses (Kaplan et al. 2004; Shi et al. 2015), and our results showed that it was involved in both cold and heat stress responses (Figure 3.3; Supplementary Table 4).

Our results highlighted the utilization of energy metabolism by *Nepenthes* plants to overcome the stress caused by exposure to low temperature. Both adenine and adenosine, which were expressed at the highest level under cold conditions, are known for their importance for proper functioning of plant cell, nucleic acid synthesis and energy metabolism (Haferkamp et al. 2011; Ashihara et al. 2018). Similar effects were also observed in other plants. For example, increase of energy in the form of ATP from the cold acclimation of winter rape plants when temperatures dropped down to 5^{o}C (Sobczyk & Kacperska-Palacz 1978) and winter weeds (*Triticum aestivum* L. cv. Frederick) when temperature went down to 2^{o}C (Perras & Sarhan 1984). This phenomenon is, however, still poorly understood. The authors suggested possibility of the energy utilization through the accumulation of ATP during the initial plant acclimation, to synthesize glycoprotein or other protective substances of protein that could increase the cold hardiness of the plant membrane (Perras & Sarhan 1984). Besides plants, similar ATP increase under cold condition has been reported in ice worms (*Mesenchytraeus solifugus*) (Napolitano et al. 2004), psychrophilic microorganisms (Napolitano & Shain 2005) and bacteria (Amato & Christner 2009) with strong evidence of linear relationship between intracellular ATP concentration and cold tolerance (Morrison & Shain 2008).

Reactive oxygen species scavenging activity via the secondary ROS scavenging system flavonoids was also observed in *Nepenthes* plants, in defense against oxidative damage induced by heat and cold stresses. Flavonoids have been recorded to play different roles in different plants under one genus (Gharibi et al. 2016), and sometimes one flavonoid may have different responses in a single plant species with different origins (Goh et al. 2016). Our study showed distinctive flavonoid modification of the 4 *Nepenthes* species in response to the different environmental conditions provided. Among the identified flavonoids, our results have shown the interesting distinct biological activity of baicalein isomers in assisting the plant to overcome the environmental stress. This can be further supported by several studies (Mueller & Boehm 2011; Böhm et al. 2002; Muller et al. 2011; Xu et al. 2012), in which different flavonoid isomers resulted in various antioxidant activities.

Together with its glucuronide form baicalin, both flavonoids play important roles in the balance between antioxidant enzyme and ROS activities in adaptive responses to temperature stress (Yuan et al. 2011). Limited studies are available on the role of proanthocyanidin in response to cold stress, however, An et al. (2018) have shown the importance of the R2R3-MYB transcription factor MdMYB23 in accumulation of proanthocyanidin accumulation under cold temperature (4°C) exposure in apple (*Malus* x domestic). Our study lends further support to the involvement of proanthocyanidin (Epiafzelechin 3-O-gallate-(4beta->6)-epigallocatechin 3-O-gallate) in response to cold stress (Supplementary Table 4).

3.4.2 Species-specific adaptation strategies

The metabolite regulation patterns clearly showed that responses to temperature changes are linked to the plant habitats. Thermal specialization in tropical plant species indicate further inabilities for highland *Nepenthes* plants to adapt to changing environments (Addo-Bediako et al. 2000; Ghalambor et al. 2006; Araújo et al. 2013). The pyrophytic species *Nepenthes minima* is, however, unique as it adapted well in the greenhouse under lowland conditions. Its habitat is known to experience high temperatures up to 38 °C and prone to seasonal burning, with re-growing observed from the plant rootstock after the wildfires (Cheek & Jebb 2016). This could be the reason for the heat tolerance (up to 33 °C) in that species. Our temperature metabolome study revealed the ability of this species to manipulate production of amino acids and phytohormones in their heat stress adaptation. We discovered that this highland species possesses the heat adaptation strategy similar to that of the lowlander *N. rafflesiana*, such as increased nitrogen storage, and polyamides synthesis via L-arginine accumulation (Okumoto et al. 2016). Nitrogen storage is known to be crucial in heat shock protein production which plays a vital role in surviving heat stress (Heckathorn et al. 1996), while polyamines and L-arginine play a major role in activating ROS-scavenging enzymes under abiotic stress (Okumoto et al. 2016; Yu et al. 2014).

Apart from the increased production of the phytohormone auxin, a strategy employed by all 4 *Nepenthes* species studied, *N. minima* upregulated the production of two other phytohormones: jasmonic acid and trigonelline. The importance of the endogenous phytohormone jasmonic acid for thermal toleration in plants have been previously recorded (Ruan et al. 2019; Sharma & Laxmi 2016; Du et al. 2013). The trigonelline was previously

recorded with various regulatory roles in relation to plant cell cycle regulation, nodulation, oxidative stress, as well as the growth of the plant (Ghosh et al. 2020; Garg 2016; Boivin et al. 1990). Interestingly, similar manipulation of the two hormones can also been observed in the lowlander *N. ampullaria*. However, instead of the lowland condition, *N. ampullaria* utilized them to adapt to highland cold stress.

As a lowlander, *N. ampullaria* was found to be capable of inhabiting a wide altitude range-including highland environments (up to 2100m asl; Jebb & Martin 1997). That is to say, the species even as a lowlander, is capable of tolerating low temperatures. Besides the two phytohormones mentioned above, the species also utilizes norbergenin, which possesses both antioxidant and ROS scavenging activities, to protect themselves from the cold stress induced oxidative damage (Takahashi et al. 2003; Tangmouo et al. 2009). Similar protection strategy was also observed in *N. rafflesiana*, which has been recorded growing at 1500m asl according to Adam et al. (1992).

While two metabolites involved in lignin biosynthesis pathways (coniferin and syringin) were detected in all four species, two of the lowland species (*N. northiana* and *rafflesiana*) displayed significantly higher accumulation of syringin under lowland conditions. *N. northiana* is commonly found on limestone hills, a harsh environment composed of calcium carbonate, alkaline pH and highly susceptible to drought (Cheek & Golos 2019), while *N. rafflesiana* can be found in open habitats such as degraded, dry laterite and podsols (Clarke & Wong 1997; Phillipps & Lamb 1988). In Matang, Kuching, Sarawak, Malaysia, *N. rafflesiana* has also been observed in open areas with direct exposure to sunlight and heat (anecdotal observations). Based on our data, it seems that in response/adaptation to the sun, heat, and drought, both *N. northiana* and *rafflesiana* developed a self-protection strategy by increasing lignification to inhibit water loss from plant tissues (Yoon et al. 2020). Similar adaptation mechanisms have been shown for Norway spruce, *Ctenanthe setosa*, and wheat (Mandre 2002; Terzi et al. 2013; Bala et al. 2016).

3.4.3 Survival in a changing climate

Past studies revealed the importance of ecological adaptation of *Nepenthes* as the key determining factor driving, not only the diversification of pitcher morphology and their prey trapping mechanisms, but also the evolution of nutrient sequestration strategies (Bauer et al. 2012; Moran et al. 2013). In this study, we demonstrated the individual adaptation strategies

applied by four *Nepenthes* species towards high and low temperature heat stress. Some responses, such as the lignification, are clearly linked to the habitat niches (Figure 3.5).

Nepenthes is known to be susceptible towards climate change. Due to the narrow endemism geographical distribution of certain species, especially the highlanders confined to single mountain summits, they are at particularly high risk of species extinction (Moran et al. 2013; Gray et al. 2017). Previously ecological niche modeling and maxent modeling have determined the climatically suitable area (habitat) for some species such as *N. rafflesiana*, *N. tentaculate*, *N. macrophylla* and *N. lowii*, via application of the climatic (such as rainfall and temperature) and edaphic (such as landform, soil association, soil parent material and soil suitability) variables (Gray et al. 2017; Maycock et al. 2011). The present ecometabolomic study has demonstrated the capability of the plant genus to adapt to environmental thermal stress. Thus, the future for selected species might not be so bleak and we suggest that the variability of their metabolomes should be put into consideration, as well.

3.4.4 Discovery of fungal and bacterial xenobiotic metabolites, natural occurring cyanobacteria toxin within the plant extracts

Apart from the metabolites described above, a fungal and bacterial xenobiotic metabolite, 1-naphthoic acid (Supplementary Table 1), was also detected in the plant extracts. Previous studies have shown the *Nepenthes* plant are consisted of endophytes (Wong et al. 2018; Bhore et al. 2013; Lee et al. 2014). We also found anatoxin which is produced by cyanobacteria (Supplementary Table 1; Metcalf & Bruno 2016). This finding was surprising but other studies have indeed shown that cyanobacteria are found in the fluid of *Nepenthes* pitcher (Takeuchi et al. 2015). Our detection of the toxin in the plant tissue, shows that the toxin can be transferred to the plant through its pitcher. Toxins can act similar to pesticides and insecticides (Metcalf & Bruno 2016), hence we hypothesize that this could be part of the mutualism between the cyanobacteria and their host plant, by supplying natural toxin to protect their host plant from biotic stress.

3.5 Conclusion and Final Remark

Our ecometabolomic study on the impact of lowland heat stress and highland cold stress revealed different metabolic fingerprints and species adaptation strategies based on their different ecological niches. *Nepenthes* responses to thermal stress clearly depend on genotypic

parameters, as certain genotypes show unique ways of tolerating the abiotic stress. Moreover, our study also demonstrated universal (shared across all four species studied) responses and adaptation strategies towards heat and cold stress. Importantly, our study revealed the potential of selected lowlanders, *N. ampullaria* and *N. rafflesiana*, to inhabit highland areas, as well as the highlander *N. minima* to inhabit lowland areas. This offers hope for future adaption towards changing climatic conditions. Lastly, we suggest more studies on plant metabolomes to achieve a better understanding of the adaption of *Nepenthes* (and other plant) species to their habitats.

Chapter 4

Species discrimination of *Nepenthes* via interspecific metabolic fingerprints

Abstract

Nepenthes is one of the largest carnivorous plant families that possess unique liquid filled tube-shaped cups with the main function to attract, trap, kill, and digest the prey for their nutrition. The high levels of polymorphism of *Nepenthes* species and populations, especially in their pitcher characteristics, have led to difficulties in the species delimitation. Advanced molecular technologies, such as sanger sequencing method and high throughput sequencing, have also been used in the species discrimination. Topological incongruence was, however, still identified, and especially Bornean taxa showed inconsistent grouping. In the present study, we applied a non-targeted metabolomics approach (using UHPLC-QTOF-MS) to evaluate intraspecific metabolite fingerprints of four (4) *Nepenthes* species: *Nepenthes ampullaria*, *Nepenthes rafflesiana*, *Nepenthes northiana* and *Nepenthes minima*. The aim was to use this approach as a novel potential identification tool for *Nepenthes* species. Our study provides the first insight classification and discrimination of *Nepenthes* species via intraspecies metabolic fingerprint.

4.1 Introduction

Nepenthes pitcher plant is one of the largest carnivorous plant families that possess a unique liquid filled tube-shaped cup (pitcher) at the end of the leaf. The pitcher has the main function to produce nectar for attracting their prey and to trap, kill, and digest them for their nutrition (Clarke 1997). The plant consists of 151 species described, and can be found across the Malay Archipelago, particularly in Sumatra, Philippines, Indochina, Palawan, Mindanao, Peninsular Malaysia and Borneo (Murphy et al. 2020).

High levels of polymorphism of the *Nepenthes* species and population, especially in their pitcher characteristics, have led to difficulties in the species delimitation (Clarke et al. 2018; Mullins 2000). New taxa descriptions have greatly increased over the past decades with few

describing stable morphological characteristics (Clarke et al. 2018). An increase in new segregations from existing species has also been observed (Wilson & Venter 2016; Catalano 2018; Mansell & Suarez 2016). The use of advanced molecular technologies, such as the sanger sequencing method via plastid trnK intron, nuclear low copy gene PTR1, plastid non-coding marker trnL–trnF and others (Meimberg et al. 2001; Schwallier et al. 2016; Meimberg & Heubl 2006; Mullins 2000; Merckx et al. 2015; Biswal et al. 2018; Renner & Specht 2011; Alamsyah & Ito 2013; Merckx et al. 2015; Schwallier et al. 2016; Bunawan et al. 2017), has aided in *Nepenthes* species discrimination over the past decades, but the phylogeny still remains largely uncertain. This is mainly due to the incongruence between nuclear and plastid phylogenies, conflicting markers, the possibility of Internal transcribed spacer paralogues inclusion, and limited phylogenetic resolution (Mullins 2000; Meimberg & Heubl 2006; Meimberg et al. 2001). In the last two years, Nauheimer et al. (2019) and Murphy et al. (2020) have used a high throughput sequencing approach, such as DNA target capture and sequencing via 353 nuclear loci and genome skimming on 81 plastid genes with high-copy rDNA loci, as a more robust and effective method to resolve the taxonomy and classification. The new superior phylogenomic data have resulted in greatly improved phylogenetic resolution and branch support, however, topological incongruence was still observed and certain taxa (especially Bornean taxa) showed inconsistent grouping, highlighting the need for further studies (Nauheimer et al. 2019; Murphy et al. 2020).

Metabolomics is an advanced 'omics' platform that contributes significantly to the field of plant science, especially in ecometabolomics which focuses on the interactions between plants and the environment, or other organisms across different temporal and spatial scales (Sardans et al. 2011; Peters et al. 2018; Jan & Ahmad 2019). This advanced platform allows the simultaneous detection, identification, and quantitation of a wide range of biochemical compounds. Metabolomics approach has been successfully used in the intra- and interspecies differentiation of plants and other organisms (Davey et al. 2008; Hoffmann et al. 2017; Huseby et al. 2012; Nagler et al. 2018; Yang et al. 2018; Li et al. 2020; Shi et al. 2020). Therefore, we hypothesize that this approach could be used as an identification tool for *Nepenthes* species.

In Chapter 3, we applied a non-targeted metabolomics approach combined with Ultra High-Resolution Liquid Chromatography and high-resolution Mass Spectrometry to study metabolite fingerprints of four species of *Nepenthes*: *Nepenthes ampullaria*, *Nepenthes rafflesiana*, *Nepenthes northiana* and *Nepenthes minima*. In the present study, we re-analyze

the raw data and aim to discriminate and classify the *Nepenthes* species based on their individual metabolic fingerprints and identify their potential interspecific biomarker.

4.2 Methods

4.2.1 *Nepenthes*

Four *Nepenthes* species, namely *Nepenthes minima*, *Nepenthes ampullaria*, *Nepenthes northiana* and *Nepenthes rafflesiana*, from different habitat, altitudinal distribution and infrageneric classification were targeted in the present study. (Table 4.1; Jebb and Cheek (1997) and Adam et al. (1992)). Plants are known to possess different metabolic profiles, even at the intraspecies level, therefore we hypothesized that distinct metabolite fingerprints can be distinguished between the *Nepenthes* species. Similar plant materials, growth conditions, sample preparation and metabolome profiling methods were archived as in chapter 3, and the raw data was differently analyzed (Tables 4.2.1 – 4.2.5).

Table 4.1 Four *Nepenthes* species employed in this study.

***Nepenthes* (*N.*) species**	**Habitat**	**Altitudinal distribution (m)**	**Environmental niche assigned**	**Infrageneric classification**	**Country distribution**
N. northiana	Limestone hills	0 – 500	Lowlander	*Insignes*; Danser (1928)	widespread, can be found growing in Borneo, Peninsular Malaysia, New Guinea, Singapore, Thailand and many more (Jebb & Cheek 1997)
N. rafflesiana	Open area, shady forest, offshore	0 - 1500	Lowlander	*Insignes*; Danser (1928) *Pyrophytae*; Clarke et al. (2018)	widespread, can be found growing in Borneo, Peninsular Malaysia Singapore and Sumatra (Jebb & Cheek 1997)
N. ampullaria	Damp, shady forest, swamp forest	0 - 2000	Lowlander	*Urceolate*; Danser (1928) and Clarke et al. (2018)	Endemic to Borneo (Jebb & Cheek 1997)
N. minima	Open grassland, with grey-yellow clay as substrate (highland grasslands of Central Sulawesi (Celebes)	1000 – 1700 with most localities lying above 1400m asl.	Highlander	*Regiae*; Cheek and Jebb (2016) and Murphy et al. (2020)	Endermic to Central Sulawesi (Cheek & Jebb 2016d)

4.2.2 Metabolomics data processing

Raw data was exported in .mzXML format prior to MZmine 2 analysis (Pluskal et al. 2010). The software provides noise filtering, peak detection, alignment, normalization, alignment, and gap-filling and exported data in .csv format. Exported .csv files were used for multivariate analyses with MetaboAnalyst 4.0 (Chong et al. 2019). Metabolite features with missing values > 80% were removed, and missing values imputed using K-nearest neighbors (Do et al. 2018). The data was log transformed and pareto scaled. Metabolite features (ANOVA $P < 0.01$) between the 4 *Nepenthes* species further underwent compound matching and analysis. The current analysis focused on the polar layer only as the non-polar layer demonstrated no significant difference (data excluded). All statistical analyses were performed on the positive ion data sets.

4.2.3 Metabolite Annotation and Identification

Metabolite features, including accurate m/z, possible chemical formula, and the fragmentation pattern, were queried against biological databases (highest priority was given to the database KEGG, followed by PubChem, and the others such as ChEBI and ChemSpider) using in silico fragmenter MetFrag (Ruttkies et al. 2016). The candidate was chosen based on the following criteria: (a) highest score with at least 80% match of the major fragment ions towards the databases (b) lowest relative mass deviation error when compared to the theoretical value (c) lowest relative mass deviation error from the fragment ions matched. To increase the accuracy of the identified metabolites, we cross checked the matched compound with earlier literature on similar compounds, especially in *Nepenthes* or in other plants.

4.2.4 Screening of important metabolic biomarkers

A biomarker, or marker compound, in contrast, is generally thought of as a constituent characteristic for, or specific to, a distinct species (Crockett and Khan 2003). In the present study, any metabolite with a threshold value of over 1 in VIP score and the top 15 metabolites of the mean decrease accuracy (MDA) values from Random forest (RF) analysis were targeted as potential biomarkers (Liu et al. 2017; Park et al. 2019).

4.2.5 Statistical Analysis

Multiple comparison of mean tests and pie chart were performed using Microsoft Excel. The data were pre-transformed using generalized logarithm transformation method via MetaboAnalyst 4.0 and two-way ANOVA performed using PAST software. Multivariate analyses including analysis of variance (ANOVA), Principal Component Analysis (PCA), Partial Least Squares–Discriminant Analysis (PLS-DA), hierarchical cluster analysis, heat map and Random Forest analysis were performed using MetaboAnalyst 4.0 (Chong et al. 2019). Venn diagram was created using Venny 2.1- developed at Bioinformatics for Genomics and Proteomics (BioinfoGP) (Oliveros 2007).

4.3 Results

In the present study, we determined the individual metabolite fingerprints of *N. ampullaria*, *N. rafflesiana*, *N. northiana* and *N. minima* and discovered 249 metabolites that differed significantly among 4 species ($p < 0.01$; Supplementary Figure 2) from which 86 could be identified (see Supplementary Table 5). The identified metabolites were grouped under 16 categories, with most of them being flavonoids, followed by organic acids, alkaloids, amino acids, fatty acyls, indoles, etc. (Supplementary Table 5). Twenty-nine potential identified biomarkers under several groups, including flavonoids, alkaloids, amino acids, organic acids, monolignols and others, were discovered based on variable importance in projection (VIP) in PLS-DA analysis and mean decrease accuracy (MDA) values from Random Forest (RF) analysis.

4.3.1 Individual metabolomic fingerprints of the four *Nepenthes* species

The metabolomes of the four *Nepenthes* species displayed significant differences ($P < 0.01$; Supplementary Table 6). A dendrogram based on Pearson distances and average clustering showed distinct grouping of the 4 *Nepenthes* species, with the *N. rafflesiana* and *N. northiana* are more closely related than the rest of the species (Figure 4.1a). *Nepenthes minima*, as a highlander, was observed to be vastly different and more isolated in another group compared to the 3 lowlanders.

Correlation matrix of the samples by deriving a Spearman's correlation coefficient between each replicate sample of *Nepenthes* species showed the excellent grouping among the different *Nepenthes* species (Figure 4.1b). Principle components 1 and 2 derived from the PCA and

PLS-DA, showed the total variance among the species at 40.3% and 39.8%, respectively (Figure 4.1c and 4.1d). The PLS-DA has the score plot with R2Y = 0.95 and Q2= 0.93, and the permutation was significant (Supplementary Figures 3 and 4). The key compounds separating the *Nepenthes* species based on variable importance in projection (VIP) in PLS-DA analysis have been provided in Supplementary Table 6.

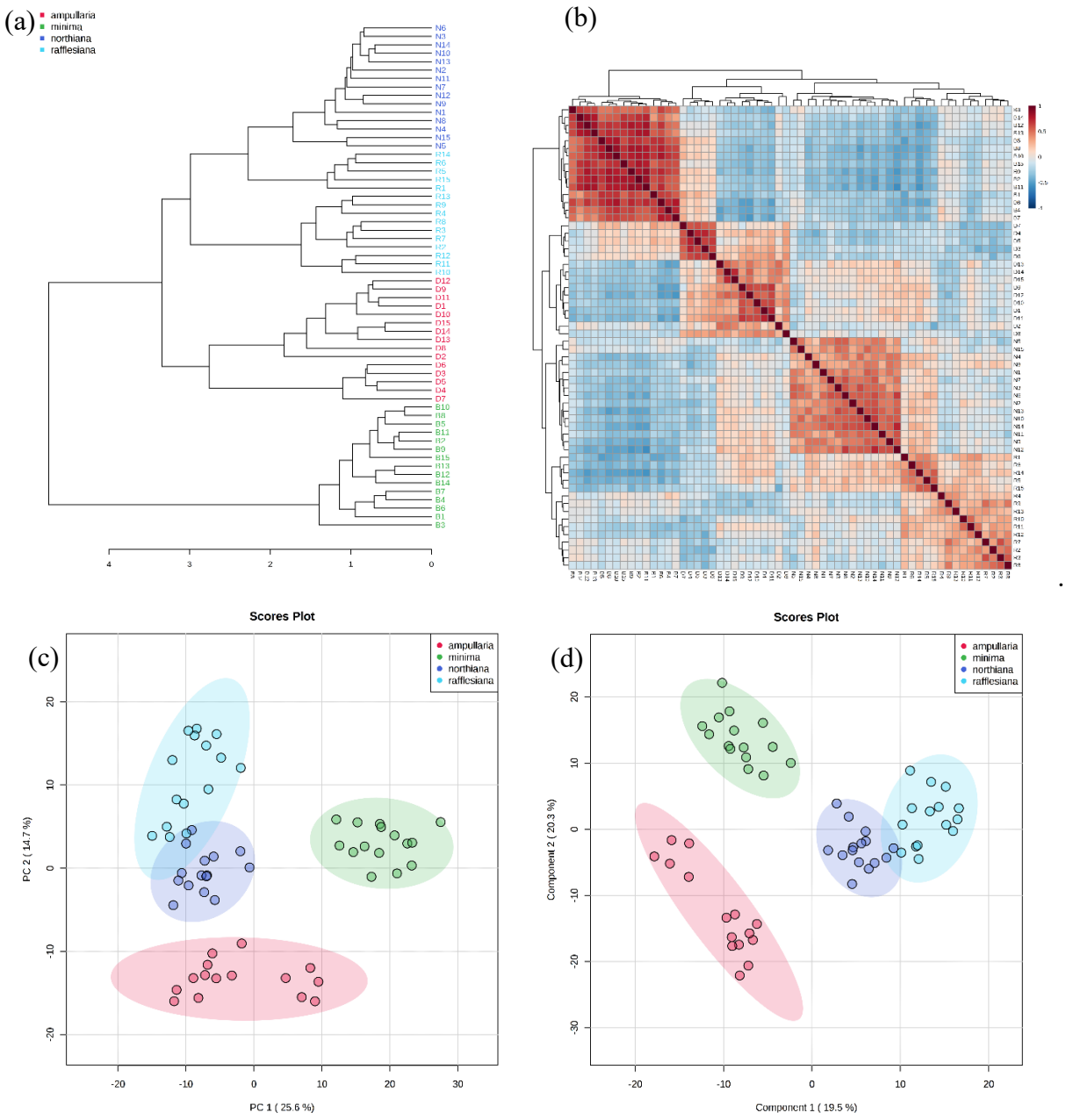


Figure 4.1 The metabolite profiles influenced by different *Nepenthes* species. (a) Dendrogram shows the relationship among the samples using Pearson distances and Average clustering. (b) Correlation matrix of the samples by deriving a Spearman's correlation coefficient between each replicate sample of *Nepenthes* species. (c) Principal Component Analysis (PCA) and (d) Partial Least Squares–Discriminant Analysis (PLS-DA) score plots show the spatial separation among the ***Nepenthes*** species.

4.3.2 Potential species biomarkers

Although all 4 plant species are grouped under the same genus of *Nepenthes*, differences in the abundance of each metabolite were observed (Supplementary Figure 2). For instance,

indole-3-acrylic acid and L-tryptophan were found more abundant in *N. northiana* than in the others, while the monolignols coniferin and alkaloids trigonelline were found more abundant in *N. ampullaria* and *N. minima,* respectively, than in the others. Besides that, this study also showed that all 4 amino acids (L-arginine, L-tryptophan, isoleucine and norvaline) were found highly abundant in *N. minima*.

The top 15 key metabolites based on the PLS-DA separation were flavonoids, amino acids and organic acids (Figure 4.2a; Figure 4.3), while the top 15 key metabolites involved in the RF analysis were flavonoids, alkaloids, and organic acids (Figure 4.2b; Figure 4.3). Among the 77 key metabolites (Supplementary Table 7) with VIP scores over 1 and 15 key metabolites from RF analysis, we were able to identify a total of 29 metabolites with mostly grouped under flavonoids (51.72%), followed by organic acids (17.24 %), amino acids (3.45 %), monolignols (3.45 %), alkaloids (6.90 %), and others (12.74 %) (Figure 4.3).

Metabolite driving separation in the PLS-DA model with the 77 key metabolites with VIP scores over 1 (Supplementary Table 7) was used to cross reference with the top 15 metabolites involved in the classification as mean decrease accuracy (MDA) values from Random forest (RF) analysis (Figure 4.2b). An organic acid (3,2-hydroxypropylbenzoic acid) and a flavonoid (miquelianin) were highlighted metabolites in both VIP and RF analysis (Supplementary Table 7 and Figure 4.2b). The important metabolites, including both VIP and MDA values, were summarized and presented in a sunburst chart and heat map (Figures 4.3 and 4.4).

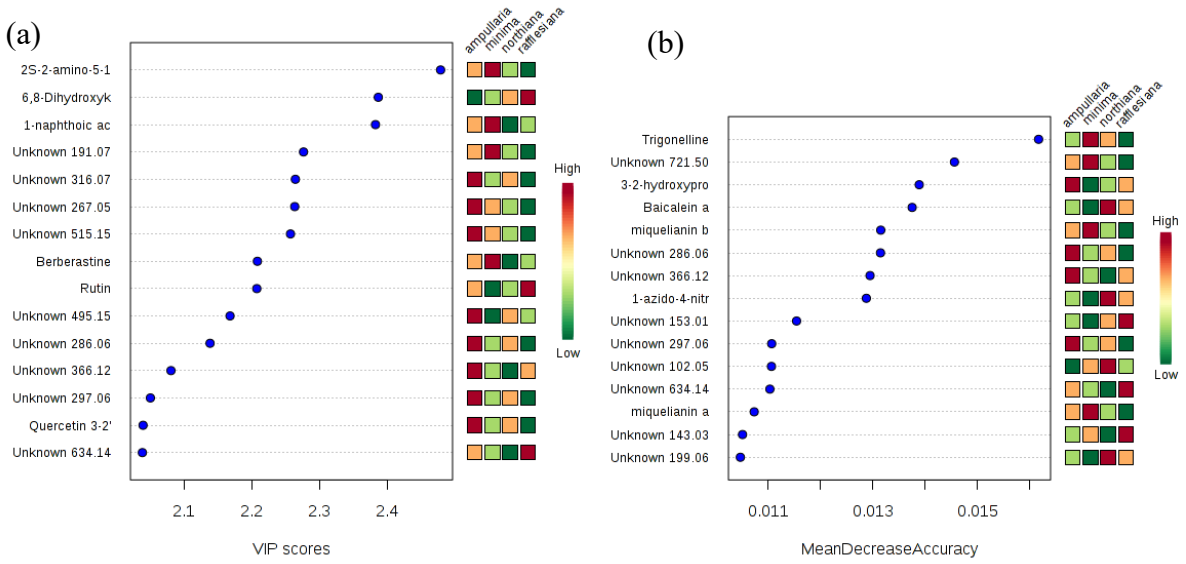


Figure 4.2 (a) Top 15 key metabolites involved in the separation of PLS-DA model. (b) Top 15 key metabolites involved in the classification as MDA values based on Random Forest (RF) analysis.

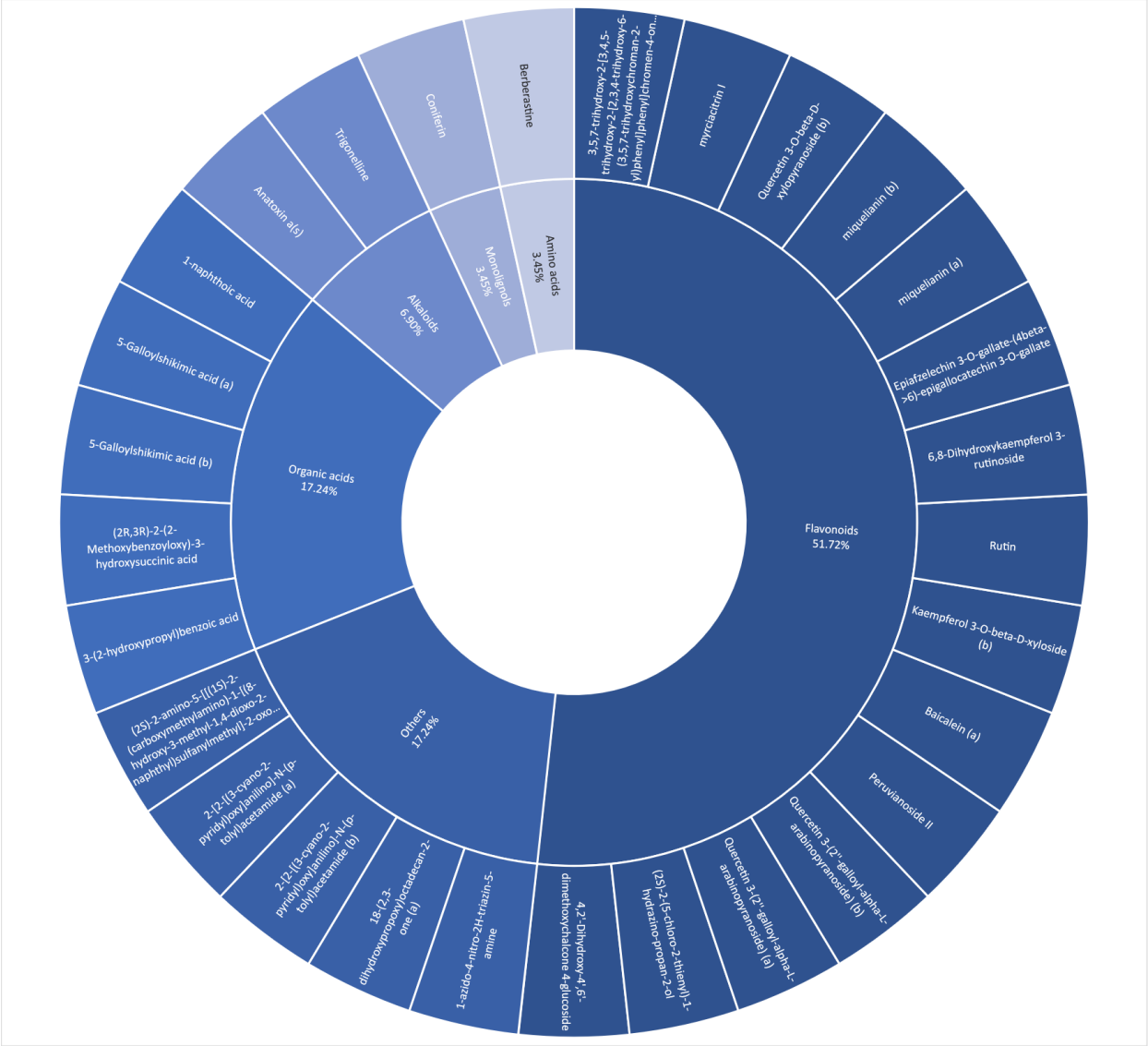


Figure 4.3 Sunburst chart depicting percentages of 8 important metabolite groups of the 29 identified biomarkers. Inner layer: Metabolite groups; Outer layer: Identified biomarkers. Note: unknown metabolites were excluded.

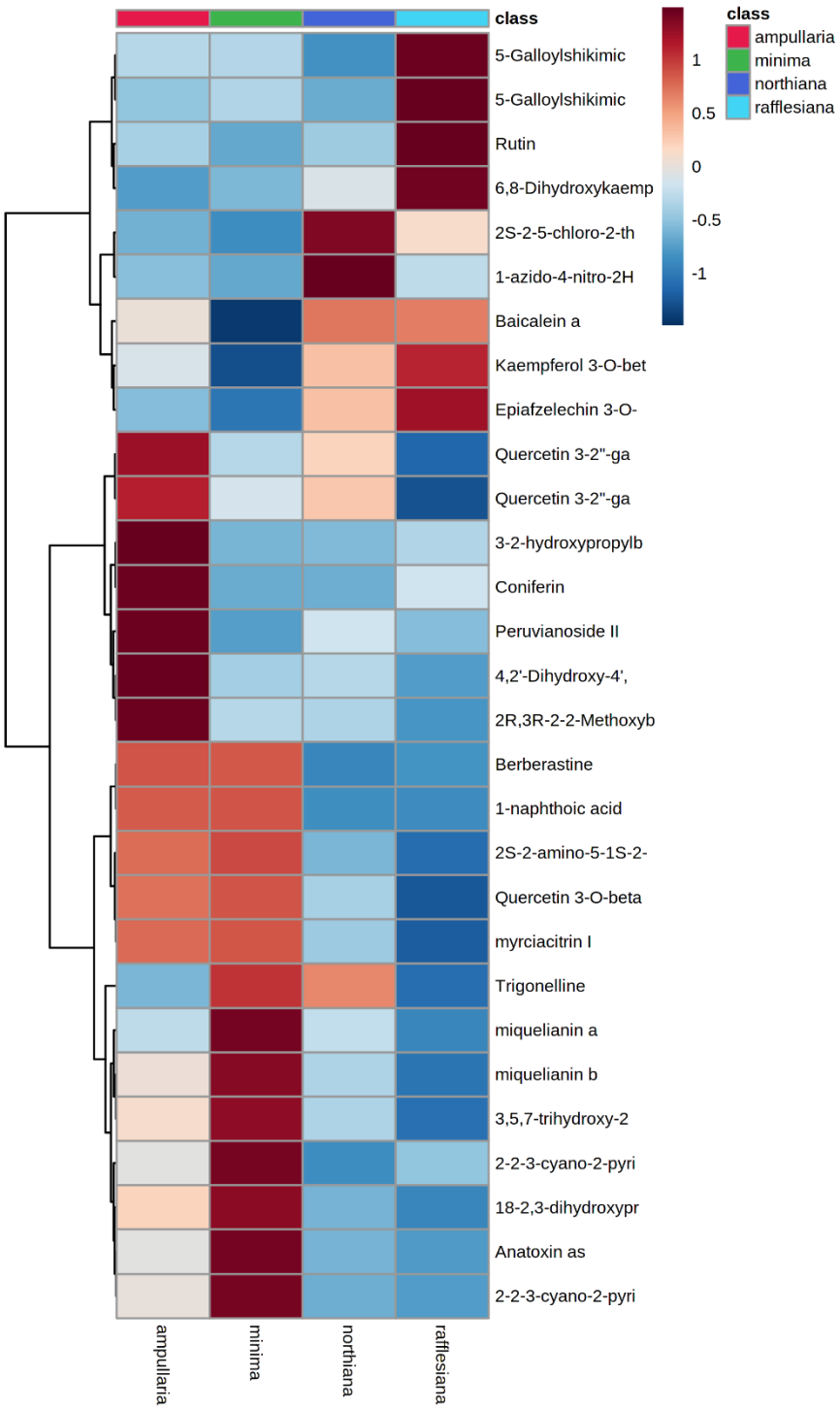


Figure 4.4 Heatmap of 29 identified biomarkers from the 4 *Nepenthes* species based on Euclidean distances and Ward clustering. The concentration of metabolites is represented on a log scale. Note: unknown metabolites were excluded.

4.4 Discussion

Our untargeted metabolomic study of four *Nepenthes* species showed that each species possesses distinctive metabolic fingerprints. Plants closely related are known to exhibit strong similarities in metabolomic profiles, however, the abundances of each metabolite can vary among the species. In our study, we observe similar pattern with all 4 *Nepenthes* species producing the same metabolites but in different abundances. Besides that, the discrimination of the interspecies was possible through biomarker discovery. Our results further highlighted that the abundance of selected metabolites seemed to be correlated to the habitat of the plants.

4.4.1 Infrageneric classification of *Nepenthes* species

Our hierarchical cluster analysis suggests similar metabolic fingerprints for *N. rafflesiana* and *N. northiana*, and the separation of the highlander (*N. minima*) from the lowlanders (*N. ampullaria*, *N. rafflesiana* and *N. northiana*). Similar separation of the three lowland species was also observed in a cluster analysis of *Nepenthes* species based on the pitcher characteristics (presence/absence of waxy layer, presence/absence of viscoelastic fluid, and peristome width) (Clarke & Moran 2016). Besides that, we also observed the separation based on the species distribution. For instance, *N. minima* which endemic to Central Sulawesi (Cheek & Jebb 2016d), is separated from the rest of the species present in the Borneo (Jebb & Cheek 1997). This could be used as one of the potential classification methods to solve the inconsistent grouping of the Bornean taxa observed in a recent phylogenetic study using a high throughput sequencing approach (Murphy et al. 2020).

The infrageneric classification suggested by Danser (1928) placed both *N. rafflesiana* and *N. northiana* under section (sect.) *Insignes* and *N. ampullaria* under sect. *Urceolate*, and Cheek & Jebb (2016) placed *N. minima* under sect. *Regiae*. Although the species *N. rafflesiana* was later removed from sect. *Insignes* and re-placed into the sect. *Pyrophytae* by Clarke et al. (2018), our intraspecies metabolite fingerprints separation congrued with the infrageneric classification suggested by Danser (1928). Further analysis with more *Nepenthes* species is required for better hierarchical clustering (Cheek & Jebb 2016d; Nauheimer et al. 2019; Murphy et al. 2020).

4.4.2 Climate influence on the metabolite abundance

While previous studies demonstrated the influence of climate on their geographical range and the diversity of their prey characteristic, as well as their trapping mechanisms (Clarke & Moran 2016; Moran et al. 2013), our study highlighted that the natural climate is also one of the factors influencing the distinctive metabolite profiles of *Nepenthes*. For example, amino acids are known to be influenced by the altitudinal distribution with higher abundances observed in plants growing at higher elevation. The highlander *N. minima* has generally higher levels of amino acids compared to the three lowland species. Besides that, the highest level of indole-3-acrylic acid and L-tryptophan, which are responsible for lateral root system development, was observed in *N. northiana*, indicating the harsh environment they live in (limestone vegetation) (Cheek & Golos 2019).

4.4.3 Potential biomarkers

Random Forest analysis and the variable importance in projection (VIP) via Partial Least Squares–Discriminant Analysis (PLS-DA) have been used widely in the identification of potential biomarkers in plants (Steinfath et al. 2010; Pujos-Guillot et al. 2013; Fu et al. 2017; Liu et al. 2017; Dos-Santos et al. 2013; Park et al. 2019). Our study discovered over 29 identified metabolites under the groups of flavonoids, organic acids, amino acids, monolignols and alkaloids. Similar groups of metabolites were also used as biomarkers for other plant species, such as *Hypericum perforatum*, potato cultivars, citrus, etc. (Fu et al. 2017; Pujos-Guillot et al. 2013; Steinfath et al. 2010; Park et al. 2019; Liu et al. 2017; Crockett & Khan 2003). Besides that, two identified markers in *Nepenthes*, rutin and tryptophan, were also identified as the biomarker of *Hypericum* sp. (Crockett & Robson 2011) and ginseng berry (Park et al. 2019), respectively. Our study shows the highest abundance of flavonoid rutin in *N. rafflesiana*, while the flavonoid baicalein, which is also a biomarker found in *Scutellaria* spp. (Gao et al. 2008), was recorded with the highest abundance in *N. northiana*. Monolignols coniferin that is involved in lignin biosynthesis, showed the highest abundance in *N. ampullaria*. The previous study by Osunkoya et al. (2008) demonstrated higher lignin percentage in *N. ampullaria* compared to *N. rafflesiana*. Similarly, trigonelline, which displayed the highest abundance in the highlander *N. minima*, was also detected in higher abundance in coffee plants that grew in high altitudes compared to the ones that grew at low altitudes (Sridevi & Giridhar 2013; Avelino et al. 2005; Guyot et al. 1996).

4.4.4 The potential use of *Nepenthes* plant for bioremediation of environmental contaminant diethylene glycol

In the present analysis, we have discovered the potential of *Nepenthes* plant in bioremediation of an environmental contaminant diethylene glycol (Table 5.1). Diethylene glycol is known to be toxic to living organisms, including humans (Marraffa et al. 2008; Schep et al. 2009). It is used as coolant, solvent and the building block in organic synthesis, and can be found in brake fluids (for normal vehicle) and hydraulic fluids (for heavy machinery) (Vale 2007; Marraffa et al. 2008). The sampling sites where the plant samples were collected were located close to the roadside (for *N. ampullaria*) and a land clearing with heavy machinery used such as excavator observed at the sample site (for *N. rafflesiana*). A previous study showed the successful removal of the compound by the aquatic plant *Echinodorus cordifolius* (L.) (Sriprapat et al. 2011). The identification of the diethylene glycol within the leaf extracts indicates the biosorption of the compound by the harvested plant samples, and this accidental discovery merits further studies of the plant species in phytoremediation of the toxic diethylene glycol.

4.5 Conclusion and Final Remarks

Our study discovered a total of 29 important metabolites that could be potentially used as the biomarkers to discriminate *N. ampullaria*, *N. rafflesiana*, *N. northiana* and *N. minima*. We confirm that metabolic fingerprints can be used as an identification tool for the discrimination of *Nepenthes* species. Further studies with other *Nepenthes* species are required to see if the similar approach can be used for other members of the genus as well.

Chapter 5

Antimicrobial activity of methanolic extracts and GC-MS analysis of volatile constituents from *Nepenthes ampullaria* and *Nepenthes rafflesiana*

Abstract

Nepenthes, also known as tropical pitcher plant, has been utilized in traditional medicine to treat wounds, stomachache, and rheumatism. Recent studies discovered the pharmaceutical potential of *Nepenthes ampullaria* and *Nepenthes rafflesiana*. Their extracts have been shown to possess antioxidant, anti-fungal (only in *N. rafflesiana*) and anti-mycobacterial activities (only in *N. ampullaria*). The anti-bacterial properties of *N. rafflesiana* have, however, yet to be demonstrated and, to the best of our knowledge, only one single inhibition study (targeting *Mycobacterium smegmatis*) has been carried out with extracts from *N. ampullaria*. In the present study, we aimed to identify the volatile phytoconstituents of the methanolic leaf extracts of *N. rafflesiana* and *N. ampullaria* using GC-MS analysis, followed by investigation of their anti-bacterial activities against Gram-positive and Gram-negative bacteria. Our results revealed species-specific compounds, and compounds shared between the two species. Their methanolic leaf extracts contained several compounds with previously recorded bioactivities and showed positive inhibition of three Gram-positive bacteria (*Bacillus cereus*, *Bacillus subtilis* and *Staphylococcus epidermidis*) and a Gram-negative bacterium (*Pseudomonas aeruginosa*). While the focus of our study was on anti-bacterial activities, our analyses also revealed that leaves of *Nepenthes* possibly assist the plant in hunting their prey by producing scents known to attract insects. Our present study has also supported the legitimacy of traditional applications of *Nepenthes* by indigenous communities.

5.1 Introduction

Drug resistance among bacterial pathogens is a serious threat to public health and health-care systems worldwide, causing major economic losses and is predicted to become the number one cause of death with estimated 10 million deaths by the year 2050 (O'Neill

2014). Recent reviews highlighted the drug-resistant bacteria *Acinetobacter baumannii*, *Pseudomonas* aeruginosa, *Enterobacteriaceae*, *Enterococcus faecium*, *Staphylococcus aureus* and *Klebsiella* spp. as some of the most threatening drug-resistant bacteria (Tacconelli et al. 2018; Mulani et al. 2019; Yu et al. 2020), with some of them ranked as the first and second most critical bacteria in terms of research for new antibiotics against them (Tacconelli et al. 2018). The ethnobotanical use of plants by indigenous communities if often overlooked by the conventional pharmaceutical industry. However, by understanding and utilizing ethnobotanical knowledge, we can integrate medically potent plant species in the furtherance of the drug discovery process.

Nepenthes, also known as tropical pitcher plant, is one of the most prominent carnivorous plants under the family of *Nepenthaceae*. The plant genus can be found across Madagascar, Northen Australia, New Caledonia and the Malay Archipelago, with the majority of species endemic to Borneo, Sumatra and Philippines (Murphy et al. 2020; McPherson 2009; Kurata et al. 2008; Jebb & Cheek 1997). The unique modification of the leaf, which serves to lure, trap, kill and digest their prey for their nutrition, has given rise to the name 'pitcher plant', (Clarke 1997). In the traditional culture, *Nepenthes* has been used treat several diseases and illnesses. The plant has been used by, Dayak Seberuang comunity to treat cough and stomachache (Setiawan et al. 2015); Jakun community to treat asthma and rheumatism (Sabran et al. 2016); people in Nagaland to treat cuts and injuries (Ramashankar & Sharma 2015); indigenous people of the Nokrek Biosphere Reserve, Meghalaya, India to treat cholera and cataract (Singh et al. 2014); indigenous people of the North-Eastern region of India to treat diabetes and night blindness (Rao 1981; Sudhir 2002). Besides that, it has also been recorded as folk medicine to treat malaria in Malaysia and as astringent (Perry & Metzger 1980; Burkill 1966).

Past studies on the *Nepenthes* plant have revealed the pharmaceutical potential of their extracts such as anti-bacterial, anti-fungal, antioxidant, anti-diabetic, anti-cancer, anti-inflammatory and anti-proliferative agents (Tang et al. 2019a; Shil et al. 2014; De et al. 2019; Tang et al. 2019b; Lien 2019; Ou-Yang et al. 2019; Rosli et al. 2018; Lien 2018; Ismail et al. 2015; Shin et al. 2007a). Several chromatography and mass spectrometry approaches, especially Liquid chromatography-mass spectrometry (LC-MS) and Gas chromatography-mass spectrometry (GC-MS), have been applied to study the metabolites of *Nepenthes* plant. However, previous metabolomic studies focused on olfactory cues in attracting their

mutualistic mammals (Wells et al. 2011), use of scent to mediate prey attraction (Di Giusto et al. 2010), molecular triggers in the prey capture (Raj et al. 2011), ecometabolomics study (Chapter 3; Wong et al. 2020a) and interspecies fingerprints study (Chapter 4; Wong et al. 2020b). Volatile organic compounds (VOC) are secondary metabolites produced by plants and have been reported to possess a wide range of bioactivities (Pandey et al. 2020; Bekinbo et al. 2020; Esmat et al. 2020; Gomathi et al. 2015; Kumar et al. 2012). VOC profiling in combination with bioactivities studies are, however, limited in *Nepenthecea*. To the best of our knowledge, only a single VOC study with potential pharmacological values has been carried out by Huang et al. (2020) on *Nepenthes miranda* in which anti-bacterial activities were observed; hence we hypothesized that it is possible to find more in the other *Nepenthes* species.

In the present study, we used GC-MS as a robust tool to study the volatile compounds from the methanolic leaf extracts of the *N. ampullaria* and *N. rafflesiana*. *Nepenthes ampullaria* and *N. rafflesiana* have been evaluated for their antioxidant, anti-fungal (only in *N. rafflesiana*) and anti-mycobacterial activities (only in *N. ampullaria*) (Rosli et al. 2018; Yolanda et al. 2014; Sanusi et al. 2018). However, the anti-bacterial properties of *N. rafflesiana* have yet to be demonstrated and, to the best of our knowledge, only one single inhibition study (targeting *Mycobacterium smegmatis*) has been carried out with extracts from *N. ampullaria*. Therefore, we aim to verify the ethnobotanical use of *Nepenthes* as a medicine plant via identification of the volatile phytoconstituents with recorded medicinal properties, followed by further investigation of their anti-bacterial activities against Gram-positive and Gram-negative bacteria.

5.2 Methods

5.2.1 Plant Specimen and Preparation of Leaf Extract

Nepenthes plants were collected at Matang area in Kuching, Sarawak, Malaysia. The plants were kept on ice in a polystyrene box during transport to the laboratory. The plants were washed under running tap water, and leaf samples were harvested and freeze dried. The freeze-dried leaf samples were extracted with methanol. The extraction was performed by preparing 500 grams of crushed freeze-dried leaf samples in a 1 litre Schott bottle with enough methanol to completely cover it and left for 24 hours. The extracts were filtered through Whatmann No.

1 filter, concentrated using a rotary evaporator, and freeze dried into solid form for further analyses.

5.2.2 Gas Chromatography-Mass Spectrometry (GC-MS) analysis

Analysis was performed using Shimadzu GC-MS (Model QP-2010, Shimadzu Co., Kyoto, Japan), equipped with Rtx-5MS capillary column (inner diameter of 0.25 mm, length of 30 m, and film thickness of 0.25 μm). The GC-MS condition was modified from the method by Song et al. (2015). Both injector and ion source temperature were set at 200 ^{o}C. The oven temperature was initially programmed at 50 °C (isothermal for 1 min), then increased to 280 °C at 10 °C/min (isothermal for 5 min). The carrier gas helium was used at the flow rate of 1 mL/min and an injector volume of 1 μL, using a 10:1 split ratio. Electron impact at 70 eV was used as the ionization mode. Data was collected in the full scan mode (40 - 400 m/z). The methanolic extracts (1 mg/mL) were prepared, filtered (0.20 μm syringe filter), and injected onto the GC-MS. The GC–MS chromatograms of *N. ampullaria* and *N. rafflesiana* are presented in Supplementary Figures 5 and 6.

5.2.3 Data pre-processing and compound identification

The original GC–MS data were analyzed using the GCMSsolution Software and compared to the database of Wiley library (7th edition). To ensure the reliability of the compound identification, only the candidates with a spectral data match of 80% and above were selected (Luo et al. 2014). The identified compounds were pre-filtered, with only the compounds existed in at least 3 of the selected biological samples were selected. The selected candidates were then further verified via literature search for similar compounds in *Nepenthes* and other plants.

5.2.4 Anti-bacterial activity via disk diffusion assay

Anti-bacterial activity was evaluated against eight bacteria species, including Gram-positive (*Bacillus subtilis*, *Bacillus cereus*, *Streptococcus salivarius* and *Staphylococcus epidermidis*) and Gram-negative bacteria (*Salmonella typhimurium*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*). The antimicrobial activity was studied using the disk diffusion assay following a modified protocol by Murray et al. (1995). The freeze-dried plant extract was re-dissolved in methanol to yield a solution containing 50 mg of extract per mL. The sterilized discs (Whatman grade A filter paper discs of 6 mm diameter) were loaded with

10 µL extracts, with methanol as negative control. The loaded discs, including the negative control, were air dried and placed on the bacteria inoculated agar plate, which were prepared by inoculating the overnight broth bacteria cultures onto nutrient agar plate (20 mL of nutrient agar per petri dish) using sterile cotton swap. The agar plates were incubated at 37 ºC for 24 hours. The inhibition zones were measured, recorded, and analyzed (minus the disk size) in millimeter. All assays were performed in 5 replicates.

5.2.5 Statistical analysis

Statistical analyses including two-way analysis of variance (ANOVA) and tukey's post-hoc test were performed using PAST software (Hammer et al. 2001). The multivariate analyses including hierarchical cluster analysis, Principal Component Analysis (PCA) and heat map were performed using MetaboAnalyst 4.0 (Chong et al. 2019), with the missing values imputed using K-nearest neighbors (Do et al. 2018). The data was log transformed and pareto scaled. The .csv file with the identified metabolite features is provided in the supplementary file. Venn diagram was created using Venny 2.1- developed at Bioinformatics for Genomics and Proteomics (Oliveros 2007). Figure 5 was constructed using ConceptDraw OFFICE 6 (CS Odessa LLC 2019).

5.3 Results

5.3.1 Individual metabolomic profiles of *N. ampullaria* and *N. rafflesiana*

The GC-MS analysis of methanolic extracts of the *N. ampullaria* and *N. rafflesiana* showed distinct differences between the two species ($P < 0.001$; Supplementary Table 8). A dendrogram based on Pearson distances and average clustering showed highly distinct grouping of *N. ampullaria* and *N. rafflesiana* (Figure 5.1a). Principle components 1 and 2 derived from the PLS-DA, showed the total variance among the species at 98.5 % (Figure 5.1b). Our result showed that the metabolites were greatly affected by the species of the plant genus (Figure 5.2).

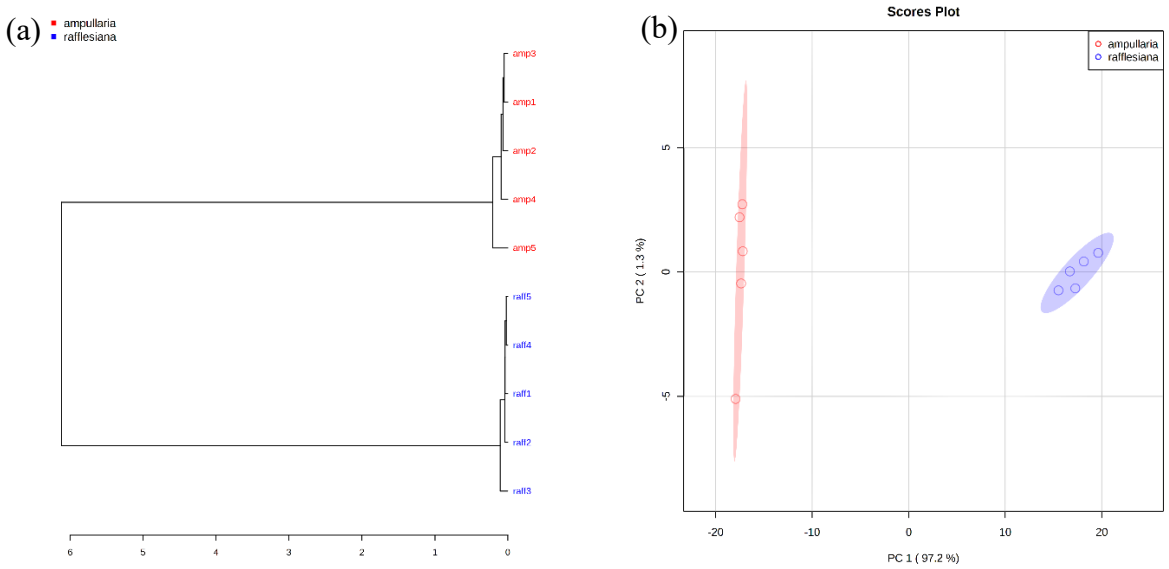


Figure 5.1 Metabolite profiles of the two *Nepenthes* species. (a) Dendrogram shows the relationship among the samples using Pearson distances and average clustering. (b) Principal Component Analysis (PCA) score plot shows the spatial separation between the *Nepenthes* species and the provided growing conditions. Please refer to the supplementary file for the three-dimensional figure. Abbreviations: amp represents *N. ampullaria* and raff represents *N. rafflesiana*. The numbering after the species name represents the biological replicates in Figure 5.1a.

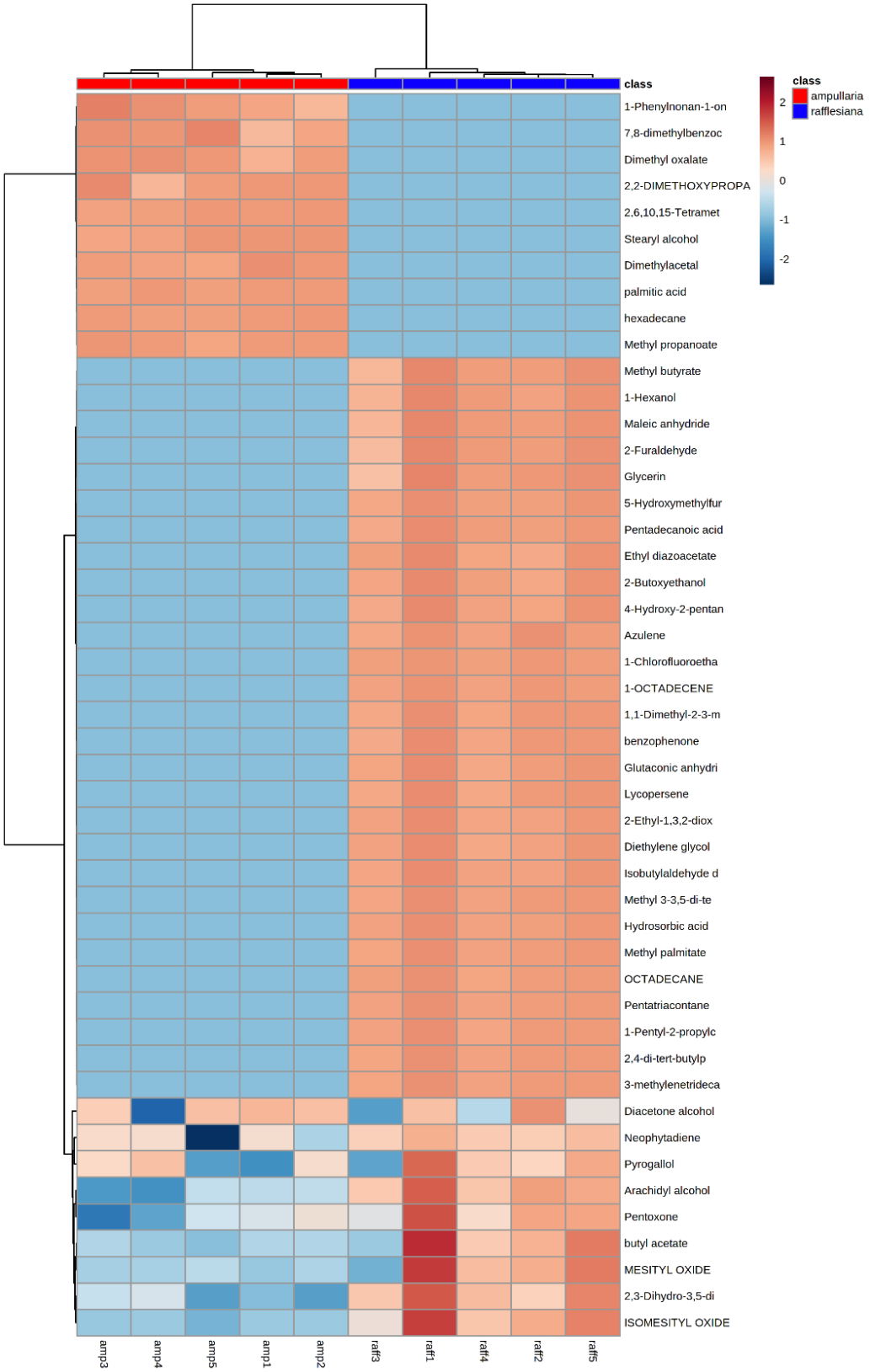


Figure 5.2 Heatmap of 47 identified metabolites from the 2 *Nepenthes* species based on Euclidean distances and Ward clustering. The concentration of metabolites is presented on a log scale. Abbreviations: amp represents *N. ampullaria* and raff represents *N. rafflesiana*. The numbering after the species name represents the biological replicates.

5.3.2 Plant metabolite analysis

In total of 47 compounds were identified using the Wiley 7th edition mass spectra library, with prevailing compounds in *N. ampullaria* being pyrogallol (8.43%), diacetone alcohol (3.24 %), pentoxone (1.59%), dimethyl oxalate (1.07%), stearyl alcohol (1.03%). Compounds in *N. rafflesiana* included pyrogallol (4.16%), 1-chlorofluoroethane (3.92%), lycopersene (2.03%), 5-hydroxymethylfurfural (2.85%) and pentoxone (1.09%) (Table 5.1).

Among the 47 identified compounds, 10 (21.3%) were found only in *N. ampullaria* and 28 (59.6%) only in *N. rafflesiana*. Nine compounds (19.1%) were present in both plant species, which are arachidyl alcohol, butyl acetate, diacetone alcohol, isomesityl oxide, mesityl oxide, neophytadiene, pentoxone, pyrogallol and 2,3-dihydro-3,5-dihydroxy-6-methyl-4h-pyran-4-one (Figure 5.3; Table 5.1).

Table 5.1 Identified compounds of the methanolic leaf extracts of *N. ampullaria* and *N. rafflesiana*.

Chemical name	**Chemical formula**	**Molecular weight**	**Retention time (min)**	**Abundance of compounds in each extract (%) ±Standard error**	
				N. ampullaria	***N. rafflesiana***
1-chlorofluoroethane	C_2H_4ClF	83	1.55	-	3.92 ±0.40
Dimethylacetal	$C_4H_{10}O_2$	90.12	1.905	0.43 ±0.04	-
Methyl propanoate	$C_4H_8O_2$	88.11	2.065	0.20 ±0.02	-
2,2-dimethoxypropane	$C_5H_{12}O_2$	104	2.195	0.67 ±0.09	-
Methyl butyrate	$C_5H_{10}O_2$	102	2.7	-	0.23 ±0.03
Glycerin	$C_3H_8O_3$	92	2.775	-	0.12 ± 0.02
Isomesityl oxide	$C_6H_{10}O$	98	2.985	0.13 ±0.01	0.16 ±0.00
Mesityl oxide	$C_6H_{10}O$	98	3.51	0.48 ±0.02	0.80 ±0.09
Butyl acetate	$C_6H_{12}O_2$	116	3.69	0.12 ±0.01	0.13 ±0.01
4-Hydroxy-2-pentanone	$C_5H_{10}O$	102	3.755	-	0.20 ±0.02
Dimethyl oxalate	$C_4H_6O_4$	118	3.85	1.07 ±0.09	-
Maleic anhydride	$C_4H_2O_3$	98	3.915	-	0.08 ±0.01
2-furaldehyde	$C_5H_4O_2$	96	3.955	-	0.28 ±0.03
Diacetone alcohol	$C_6H_{12}O_2$	116	3.995	3.24 ±0.42	0.53 ±0.14
1-hexanol	$C_6H_{14}O$	102	4.44	-	0.11 ±0.01
2-butoxyethanol	$C_6H_{14}O_2$	118	4.97	-	0.20 ±0.02
Pentoxone	$C_7H_{14}O_2$	130	5.06	1.59 ±0.23	1.09 ±0.09

Diethylene glycol	$C_4H_{10}O_3$	106	5.86	-	0.84 ±0.15
Hydrosorbic acid	$C_6H_{10}O_2$	114	6.21	-	0.44 ±0.04
Glutaconic anhydride	$C_5H_4O_3$	112	6.325	-	0.19 ± 0.01
1-Phenylnonan-1-one	$C_{15}H_{22}O$	218	6.415	0.04 ±0.01	-
Ethyl diazoacetate	$C_4H_6N_2O_2$	114	7.155	-	0.79 ±0.006
2-Ethyl-1,3,2-dioxaborolan-4-one	$C_4H_7BO_3$	114	7.355	-	0.78 ±0.06
Isobutylaldehyde dimethyl acetal	$C_6H_{14}O_2$	118	8.14	-	0.18 ±0.01
2,3-Dihydro-3,5-dihydroxy-6-methyl-4h-pyran-4-one	$C_6H_8O_4$	144	8.725	0.32 ±0.06	0.66 ±0.06
Azulene	$C_{10}H_8$	128	9.465	-	0.91 ±0.17
5-hydroxymethylfurfural	$C_6H_6O_3$	126	9.875	-	2.85 ±0.17
7,8-dimethylbenzocyclooctene	$C_{14}H_{14}$	182	11.125	0.72 ±0.25	-
Pyrogallol	$C_6H_6O_3$	126	11.78	8.43 ±1.19	4.16 ±0.49
Hexadecane	$C_{16}H_{34}$	226	12.37	0.46 ± 0.11	-
2,4-di-tert-butylphenol	$C_{14}H_{22}O$	206	13.74	-	0.31 ±0.02
1,1-Dimethyl-2-(3-methyl-1,3-butadienyl)cyclopropane	$C_{10}H_{16}$	136	14.64	-	0.17 ±0.01
Stearyl alcohol	$C_{18}H_{38}O$	271	14.785	1.03 ±0.03	-
Benzophenone	$C_{13}H_{10}O$	182	15.335	-	0.16 ±0.02
1-Pentyl-2-propylcyclopentane	$C_{13}H_{26}$	182	16.78	-	0.09 ±0.01
1-octadecene	$C_{18}H_{36}$	253	17.05	-	0.78 ±0.06
Octadecane	$C_{18}H_{38}$	255	17.125	-	0.19 ±0.00
2,6,10,15-tetramethylheptadecane	$C_{21}H_{44}$	297	17.125	0.24 ±0.01	-
3-methylenetridecane	$C_{14}H_{28}$	196	17.19	-	0.17 ±0.01
Neophytadiene	$C_{20}H_{38}$	279	17.51	0.31 ±0.05	0.35 ±0.01
Methyl palmitate	$C_{17}H_{34}O_2$	270	18.4	-	0.26 ±0.01
Methyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate	$C_{18}H_{28}O_3$	292	18.485	-	0.26 ±0.01
Palmitic acid	$C_{16}H_{32}O_2$	256	18.72	0.39 ±0.02	-
Pentadecanoic acid	$C_{15}H_{30}O_2$	242	18.72	-	0.57 ±0.01
Arachidyl alcohol	$C_{20}H_{42}O$	299	19.1	0.61 ±0.03	0.62 ±0.07
Pentatriacontane	$C_{35}H_{72}$	493	19.165	-	0.16 ±0.00
Lycopersene	$C_{40}H_{66}$	547	24.165	-	2.03 ±0.15

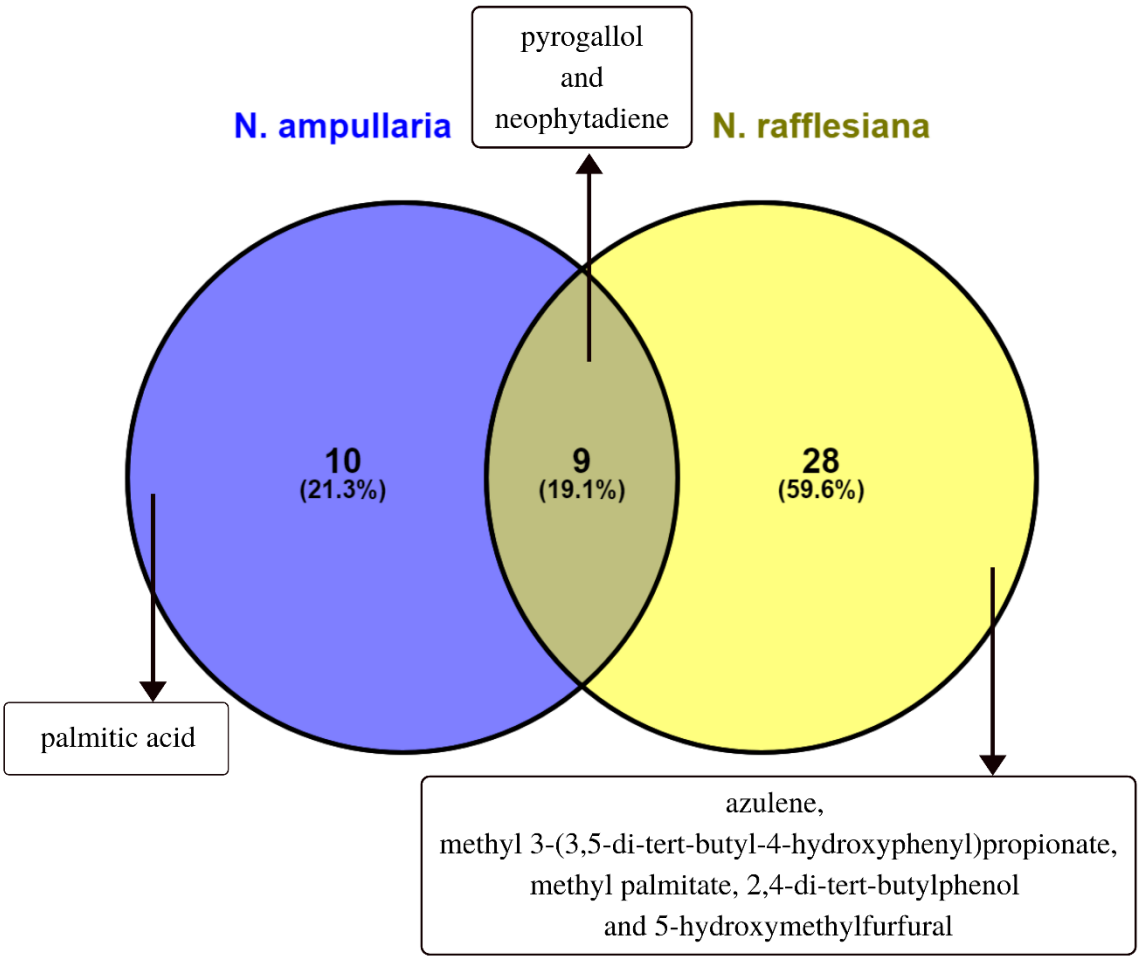


Figure 5.3 Venn diagram based on the identified metabolites overlapping between *N. ampullaria* and *N. rafflesiana*. The important metabolites with previously reported bioactivities were highlighted above.

5.3.3 Anti-bacterial activity of the methanolic *Nepenthes* extracts

Our study showed that there are at least 8 compounds (azulene, methyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate, methyl palmitate, neophytadiene, palmitic acid, pyrogallol, 2,4-di-tert-butylphenol and 5-hydroxymethylfurfural) that have previously been recorded with antioxidant, anti-inflammatory, anti-fungal, anti-cancer, and anti-microbial properties. Table 5.2 provides an overview of compounds and their reported activities.

To investigate the anti-bacterial properties of the plant extracts, disk diffusion assay was carried out with the methanolic extracts against 8 different bacteria species, including Gram positive (*Bacillus subtilis*, *Bacillus cereus*, *Streptococcus salivarius* and *Staphylococcus epidermidis*) and Gram negative bacteria (*Salmonella typhimurium*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*). Our results showed that *B. cereus*, *B.*

subtilis, *P. aeruginosa* and *S. epidermidis* were inhibited. The extracts of *N. ampullaria* showed significantly higher inhibition of growth, indicating more potent antibacterial properties than the extracts of *N. rafflesiana* extract ($P < 0.001$; Supplementary Table 9). The methanolic extracts of *N. ampullaria* had larger inhibition zones against *B. cereus* (14.3 %), *P. aeruginosa* (8.3 %), *S. epidermidis* (9.1 %) and *B. subtilis* (20.0 %) than the extract of *N. rafflesiana*. However, for both species, the trend of inhibition was the same with largest to the smallest inhibition zones observed as follows: *B. subtilis* > *B. cereus* > *P. aeruginosa* > *S. epidermidis*.

Table 5.2 Bioactivities of metabolites identified in the methanolic extracts of *N. ampullaria* and *N. rafflesiana*.

Chemical name	Known Functions	References
Azulene	Antimicrobial effects of azulene induced by light, anti-inflammatory, accelerate skin wound healing.	Afshar et al. (2019) Guarrera et al. (2001) Baptista et al. (2014)
Methyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate	Potential antioxidant	Li et al. (2014)
Methyl palmitate	Anti-inflammatory, anti-fibrotic	Saeed et al. (2012) El-Demerdash (2011) Othman et al. (2015)
Neophytadiene	Anti-bacterial against *Pseudomonas aeruginosa* and *Escherichia coli*, anti-fungal, potential anti-inflammatory, antioxidant, cardioprotective, anti-pyretic, analgesic	Ragasa et al. (2009) Bhardwaj et al. (2020) Raman et al. (2012)
Palmitic acid	Anti-inflammatory, anti-fungal, stimulate the proliferation of bone marrow, cytotoxicity against human colorectal carcinoma cells (HCT-116)	Aparna et al. (2012) Jung et al. (2013) Chen et al. (2010) Ravi & Krishnan (2017)
Pyrogallol	Anti-bacterial against *Chromobacterium violaceum*, *Bacillus subtilis*, *Staphylococcus aureus*, *Enterobacter aerogens*, *Proteus mirabilis*, *Raoultella planticola*, *Vibrio parahaemolyticus*, *Pseudomonas aeruginosa, Pseudomonas* putida, *Pseudomonas* pyocyanea and *Pseudomonas* xerosis, anti-fungal against *Fusarium oxysporum*, *Candida. Albicans*, *Aspergillus flavus* and *Penicillium italicum*, promising anti-lung cancer drug particular for the non-small cell lung cancer	Tinh et al. (2016) Kocaçalışkan et al. (2006) Yang et al. (2009) Çiçek et al. (2019) Inchagova et al. (2019) Ahn et al. (2019) Nicolis et al. (2008) Marrez et al. (2019) Singh & Kumar (2013)

	(NSCLC), potential protective effect on primary neurons, anti-quorum sensing in *Chromobacterium violaceum*, anti-inflammatory	
2,4-di-tert-butylphenol	Potential anticancer activity, antioxidant, anti-fungal against *Candida albicans*, *Fusarium chlamydosporum*, *Fusarium moniliforme*, *Fusarium culmorum*, *Fusarium graminearum*, *Aspergillus carbonarius*, *Umbelatopsis ramanniana*, anti-bacterial against *Staphylococcus aureus*	Song et al. (2018) Belghit et al. (2016) Varsha et al. (2015) Yoon et al. (2006)
5-Hydroxymethylfurfural	Anti-bacterial against *Klebsiella* sp. and *Pseudomonas aeruginosa*, antioxidant, anti-quorum sensing and biofilm potential against *Chromobacterium violaceum*, *Streptococcus mutans*, *Streptococcus pyogenes*, *Staphylococcus epidermidis* and *Staphylococcus aureus*	Kaur et al. (2018) Vijayakumar & Ramanathan (2018)

Table 5.3 Anti-bacterial activities of the methanolic extracts of *N. ampullaria* and *N. rafflesiana*. Values in the table refer to mean of biological replicates (n=5), while ± shows standard error (SE). Letter (s) after the SE, under the same column and same letters indicate significant difference at $P < 0.05$ according to Tukey's post-hoc test (Supplementary Table 9 and 10).

Testing Bacteria	***Nepenthes ampullaria***	***Nepenthes rafflesiana***
Bacillius cereus	8.20 ± 0.8^{a}	6.20 ± 0.7^{a}
Pseudomonas aeruginosa	6.60 ± 0.5	5.20 ± 0.4
Staphylococcus epidermidis	$5.20 \pm 0.2^{a,b}$	$3.40 \pm 0.6^{a,b}$
Bacillius subtilis	9.00 ± 0.8^{b}	6.40 ± 0.8^{b}
Negative control (solvent)	-	-

5.4 Discussion

Nepenthes has been used by indigenous communities to treat several diseases and illnesses, such as cough, stomachache, rheumatism, cuts, injuries, etc. (Singh et al. 2014; Ramashankar & Sharma 2015; Rao 1981; Sudhir 2002; Perry & Metzger 1980; Burkill 1966; Sabran et al. 2016; Setiawan et al. 2015). The plant's extracts have been shown to display several bioactivities (Lien 2019; Ou-Yang et al. 2019; Rosli et al. 2018; Lien 2018; Ismail et al. 2015; Shin et al. 2007a), and contain well-known bioactive compounds such as napthoquinones (Van Thanh et al. 2015; Likhitwitayawuid et al. 1998) and nepenthosides (Van Thanh et al. 2015). There is, however, little information available on the volatile phytochemical composition and anti-bacterial properties of *N. ampullaria* and *N. rafflesiana*.

5.4.1 Comparison of volatile metabolome between *Nepenthes species*

Volatile organic compounds are secondary metabolites that represent adaptive characters, subjected to evolutionary natural selection (Wink 2003). Although the plants that are closely related at the genus or family level show strong similarities in the metabolomic profiles, certain compounds are known to be associated interspecifically (Azuma et al. 1997; French et al. 2018; Knudsen et al. 2006). Similar observation was also obtained from the metabolites of *Nepenthecea*.

Comparison of the volatile metabolome profiles of *N. ampullaria* and *N. rafflesiana* revealed species-specific compounds (10 compounds found only in *N. ampullaria* and 28 compounds only found in *N. rafflesiana*), as well as compounds shared between both species (Figure 5.3). *Nepenthes rafflesiana* has been studied for the scent composition of matured open pitchers. Apart from hexadecane that was also found in the scent of the plant pitcher, we discovered 36 more volatile compounds from *Nepenthes rafflesiana* not detected in the previous study (Di Giusto et al. 2010). Interestingly, furan derivatives were found in both *N. rajah* (furan and 2-furanmethanol) and *N. rafflesiana* (Di Giusto et al. 2010) but not in *N. ampullaria*. The palmitic acid (n-hexadecanoic acid), which is a fatty acid derivative, detected in *N. ampullaria* was recently also discovered in the acetone stem extracts of *N.* 'Miranda' (Huang et al. 2020). Hydrocarbons (hexadecane and octadecane), ketones (4-hydroxy-2-pentanone and 2,3-dihydro-3,5-dihydroxy-6-methyl-4h-pyran-4-one) detected in our study, were also found in the volatiles extracted from secretions of pitcher lids of *N. rajah*. It is possible that these compounds act as olfactory cues in attracting small mammals such as tree shrews and rats to

the pitcher (Wells et al. 2011). Some of the detected compounds such as azulene, benzophenone, butyl acetate, hexadecane, methyl propanoate, octadecane, palmitic acid, pentadecanoic acid, 1-octadecene and 2-butoxyethanol, are also found in flowers of unrelated species (Knudsen et al. 2006), and are used to attract and guide pollinators (Raguso 2001). Among those flower scent related compounds, hexadecane and octadecane were also found in the leaves of *Brasszca napus* (Jakobsen et al. 1994). The high occurrence of scent compounds in the leaves indicate that they could play a role in assisting the pitcher to luring their prey, in order to increase the efficiency in their prey hunting. This is further supported by nectar secreting glands found not only on the peristome or underside the pitcher lid, but also on the undersides of leaves and on tendrils (Exoticplantsplus 2014).

5.4.2 Ethnobotanical use of the *Nepenthes* as medicine plant

Our present study has supported the potential use of *Nepenthes* as a medicinal plant by the indigenous communities. *Nepenthes ampullaria* and *N. rafflesiana* have been utilized by the Dayak Seberuang community in West Kalimantan, Indonesia, as stomachache reliever (Setiawan et al. 2015). We detected neophytadiene, which was recorded with analgesic property. Besides that, our analysis also detected a compound (azulene) with previously recorded skin (wound) healing property which might explain the traditional use of *Nepenthes* plant in cuts and injuries treatment (Ramashankar & Sharma 2015). Moreover, at least 3 anti-inflammatory compounds (pyrogallol, palmitic acid and neophytadiene) were detected in *N. ampullaria* which might support the use of this species by the Jakun community to treat rheumatism (Sabran et al. 2016). We believe that *N. rafflesiana* possesses similar anti-inflammatory properties since the same compounds (pyrogallol, neophytadiene, azulene and methyl palmitate) were found in the plant extracts.

5.4.3 Compounds of biomedical interest

Nepenthes ampullaria and *N. rafflesiana* are known to possess antioxidant and anti-fungal (*N. rafflesiana* only) properties (Rosli et al. 2018; Yolanda et al. 2014) and we found the compounds that may contribute to the reported properties (methyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate, neophytadiene, palmitic acid, 2,4-di-tert-butylphenol and 5-hydroxymethylfurfural; see Table 5.1). Our analysis also identified several compounds with reported anti-cancer, anti-inflammatory and anti-microbial activities. According to Ismail et al. (2015), leaf extracts of *N. bicalcarata* exhibited antioxidant properties, cytotoxicity, as well

as anti-microbial properties. Anti-microbial property has also been observed in the extracts of *N. mirabilis* and *N.* 'Miranda' (Wiart et al. 2004; Lien 2019). Our findings further support the occurrence of several bioactive compounds in *Nepenthes* species.

5.4.4 Anti-bacterial properties of the plant extracts

Our result showed more compounds with anti-bacterial properties in the leaf extract of *N. rafflesiana* compared to that of *N. ampullaria*. For instance, five compounds (azulene, 2,4-di-tert-butylphenol, 5-hydroxymethylfurfural, pyrogallol and neophytadiene) with anti-bacterial properties were detected in *N*. rafflesiana, while only 2 (pyrogallol and neophytadiene) were detected in *N*. *ampullaria*. The anti-bacterial screening, however, showed higher inhibition properties of the leaf extract of *N. ampullaria* compared to *N. rafflesiana*. The inhibition zone of *B. cereus*, *P. aeruginosa*, *S. epidermidis*, *B. subtilis* by *N. ampullaria* methanolic extracts were 14.29 %, 8.33 %, 9.09 % and 20.00 %, respectively, higher than those of the extract of *N. rafflesiana*. This is likely due to the different amounts of bioactive compounds in both plant species. The amount of anti-bacterial compounds presented within the plant extract of *N. ampullaria* was generally higher compared to *N. rafflesiana*. For example, the bioactive compound pyrogallol that is known to inhibit a wide range of bacteria (including *Bacillus*, *Pseudomonas*, and *Staphylococcus* spp.), was found at double the amount in *N*. *ampullaria* (8.43% of all compounds identified) compared to *N*. *rafflesiana* (4.16%). Pyrogallol is a hydroxylated compound whose antimicrobial action occurs through enzymatic inhibition by oxidized compounds (Mason 1987).

While we observed known anti-*E. coli* compounds (neophytadiene) within the plant extracts, we recorded no inhibitory effect on our test strain *E. coli* K12. Similar results have been observed with essential oil extracted from *Alluaudia procera* which also contains neophytadiene but showed no inhibition of *E. coli* (Poma et al. 2019). It could be that the concentration used was not enough to inhibit the growth of *E. coli* K12, thus it would be noteworthy to study the minimum inhibitory concentration of the present extracts.

5.4.5 *Nepenthes* as a research area for novel drug discoveries

The highlight of our findings is the inhibition of *P. aeruginosa* by the methanolic leaf extracts of both *Nepenthes* species. Carbapenem-resistant *P. aeruginosa* is recently ranked as the 2nd most critical-priority bacteria in a priority list for research of new antibiotics for antibiotic-

resistant bacteria by the WHO (Tacconelli et al. 2018). While we did not test on the carbapenem-resistant strain, our observed strong anti-*P. aeruginosa* activity indicates that *Nepenthes* could potentially be the source to search for novel antibiotics to fight against the carbapenem-resistant *P. aeruginosa*. This is further supported by inhibition of *P. aeruginosa* by extracts from other *Nepenthes* species; *N. bicalcarata*, *N. alata* and *N.* 'Miranda' (Ismail et al. 2015; Lien 2019; Elmido et al. 2019). *Nepenthes* exhibits a wide antimicrobial activity as shown in our study and previous studies. *Nepenthes bicalcarata* and *N.* 'Miranda' for example inhibited not only the same bacteria species as the *N. rafflesiana* and *N. ampullaria*, but also *Bacillus spizizenii* (*N. bicalcarata* only), *Escherichia coli*, and *Staphylococcus aureus* (Lien 2019).

5.5 Conclusion

Our study revealed species-specific compounds, and similar compounds shared between the *Nepenthes* species. The methanolic leaf extracts contained several compounds with previously recorded bioactivities and showed positive inhibition of three Gram-positive bacteria (*B. cereus*, *B. subtilis* and *S. epidermidis*) and a Gram-negative bacterium (*P. aeruginosa*). The methanolic leaf extracts of *N. ampullaria* displayed significantly higher antimicrobial activity than the extracts of *N. rafflesiana*. While the focus of our study was on antimicrobial activities, our analyses also revealed that leaves of *Nepenthes* possibly assist the plant in hunting their prey by producing scents known to attract insects. Lastly, our present study supports the potential traditional application of *Nepenthes* as medicinal plant by the indigenous communities for various disease and illness treatments which warrants further investigations.

Chapter 6

Conclusion and Future Perspectives

6.1 Conclusion

The first part of the present study investigated the impacts of heat and cold stress on four Nepenthes species (*N. ampullaria*, *N. rafflesiana*, *N. northiana* and *N. minima*) representing different ecological systems and altitudinal distributions via a MS-based non-targeted ecometabolomic approach. We have successfully identified different metabolic fingerprints and species adaptation strategies based on their different ecological niches. *Nepenthes* responses to thermal stress clearly depend on genotypic parameters, as certain genotypes show unique ways of tolerating the abiotic stress. Our study also demonstrated some universal (shared across all four species studied) responses and adaptation strategies towards heat and cold stress. Importantly, our study revealed the potential of selected lowlanders, *N. ampullaria* and *N. rafflesiana*, to inhabit highland areas, as well as the highlander *N. minima* to inhabit lowland areas. This offers hope for future adaption towards changing climatic conditions.

The second aim was to evaluate the use of metabolomics for the discrimination and infrageneric classification of *N. ampullaria*, *N. rafflesiana*, *N. northiana* and *N. minima*. We have discovered a total of 29 important metabolites that could be potentially used as the biomarkers to discriminate the four *Nepenthes* species. We confirm that metabolic fingerprints can be used as an identification tool for the discrimination of *Nepenthes* species.

Our third aim, GC-MS analysis of the volatile constituents from *N. ampullaria* and *N. rafflesiana* revealed species-specific compounds, and common compounds found in both species. The methanolic leaf extracts of *Nepenthes* possess compounds with previously recorded bioactivities and exhibited inhibition activity against *B. cereus*, *B. subtilis*, *S. epidermidis* and *P. aeruginosa*. While the focus of our study was on the antimicrobial activities, our analyses also revealed that leaves of *Nepenthes* possibly assist the plant in hunting their prey by producing scents known to attract insects. The study further supported the potential

traditional application of *Nepenthes* as a medicinal plant by the indigenous communities for the disease and illness treatments which warrants further investigations.

Lastly, we have fortuitously discovered the mutualistic of the plant-microbes interaction and the potential of *Nepenthes* plant in bioremediation of an environmental contaminant diethylene glycol. There is much to be discovered still about this fascinating carnivorous plant which warrants further investigations.

6.2 Future Work

The carnivorous plant is full of wonders. There are many opportunities to expand the knowledge in hand. Below are several lines of research arising from this work which should be pursued:

- More studies on plant metabolomes such as targeted analysis, focusing on the important plant metabolites such as amino acids, lipid, and phytohormones, and target more plant species to archive better understanding of the adaption of *Nepenthes* species to their habitats.
- The study of amino acid norvaline associated mechanism response in *Nepenthes*, under thermal stress.
- Application of the plant metabolomes as one of the variables in the ecological niche modeling and maxent modeling for the determination of climatically suitable area (habitat) for *Nepenthes* species.
- The study of the interspecific metabolic fingerprints with more *Nepenthes* species to further enhance the hierarchical cluster analysis.
- The application of the same plant species classification and discrimination via intraspecies metabolic fingerprint with the other plant genus.
- Further anti-bacterial testing against the 2nd most critical-priority bacteria in a priority list for research of new antibiotics for antibiotic-resistant bacteria by the WHO- The carbapenem-resistant *Pseudomonas aeruginosa.*
- More biological assays such as antioxidant, anti-cancer, and anti-malarial can be conducted to further determine the potential of traditional application of *Nepenthes* plants.

- The study of mutualism between *Nepenthes* and cyanobacteria via the bacteria isolation, plant inoculation and plant-pathogen research.
- Bioremediation of environmental contaminant (diethylene glycol) research of *Nepenthes* plant.

References

Abd El-Daim, IA, Bejai, S & Meijer, J 2014, 'Improved heat stress tolerance of wheat seedlings by bacterial seed treatment', *Plant and soil*, vol. 379, no. 1-2, pp. 337-350.

Abdelrahman, M, Ishii, T, El-sayed, M & Tran, L-SP 2020, 'Heat sensing and lipid reprogramming as a signaling switch for heat stress responses in wheat', *Plant and Cell Physiology*, vol. 61, no. 8, pp. 1399–1407.

Adam, JH & Wilcock, CC 1995, 'A new natural hybrid of pitcher plant from Sabah, Malaysian Borneo', *Borneo Science*, vol. 1, no. 1, pp. 1-7.

Adam, JH 1997, 'Prey spectra of Bornean Nepenthes species (Nepenthaceae) in relation to their habitat', *Pertanika Journal of Tropical Agricultural Science*, vol. 20, pp. 121-134.

Adam, JH, Omar, R & Wilcock, CC 2002, 'Phytochemical screening of flavonoids in three hybrids of Nepenthes (Nepenthaceae) and their putative parental species from Sarawak and Sabah. Journal of Biological Sciences, vol. 2, no. 9, pp. 623-625.

Adam, JH, Wilcock, CC & Swaine, MD 1992, 'The Ecology and Distribution Of Bornean "Nepenthes"', *Journal of Tropical Forest Science*, vol. 5, no. 1, pp. 13-25.

Adams, MJ, Antoniw, JF & Fauquet, C.M., 2005, 'Molecular criteria for genus and species discrimination within the family Potyviridae', *Archives of Virology*, vol. 150, no. 3, pp. 459-479.

Addo-Bediako, A, Chown, SL & Gaston, KJ 2000, 'Thermal tolerance, climatic variability and latitude', *Proceedings of the Royal Society of London. Series B: Biological Sciences*, vol. 267, no. 1445, pp. 739-745.

Adlassnig, W, Peroutka, M & Lendl, T 2011, 'Traps of carnivorous pitcher plants as a habitat: composition of the fluid, biodiversity and mutualistic activities', *Annals of Botany*, vol. 107, no. 2, pp. 181-194.

Adlassnig, W, Steinhauser, G, Peroutka, M, Musilek, A, Sterba, JH, Lichtscheidl, IK & Bichler, M 2009, 'Expanding the menu for carnivorous plants: uptake of potassium, iron and manganese by carnivorous pitcher plants', *Applied Radiation and Isotopes*, vol. 67, no.12, pp. 2117-2122.

Afshar, M, Zardast, M & Vahdati, F 2019, 'Effects of Azulene on Granulation Tissue Formation and Epithelialization during Skin Wound Healing in BALB/c Mice', *Journal of Mazandaran University of Medical Sciences*, vol. 28, no. 170, pp. 1-10.

Ahn, H, Im, E, Lee, DY, Lee, HJ, Jung, JH, & Kim, SH 2019, 'Antitumor Effect of Pyrogallol via miR-134 Mediated S Phase Arrest and Inhibition of PI3K/AKT/Skp2/cMyc Signaling in Hepatocellular Carcinoma', *International Journal of Molecular Sciences*, vol. *20*, no. 16, pp. 3985.

Airaki, M, Leterrier, M, Mateos, RM, Valderrama, R, Chaki, M, Barroso, JB, Del Río, LA, Palma, JM & Corpas, FJ 2012, 'Metabolism of reactive oxygen species and reactive nitrogen species in pepper (Capsicum annuum L.) plants under low temperature stress', *Plant, Cell & Environment*, vol. 35, no. 2, pp. 281-295.

Akula, R & Ravishankar, GA 2011, 'Influence of abiotic stress signals on secondary metabolites in plants. *Plant Signaling & Behavior*, vol. 6, No. 11, pp. 1720-1731.

Alamsyah, F & Ito, M 2013, 'Phylogenetic analysis of Nepenthaceae, based on internal transcribed spacer nuclear ribosomal DNA sequences', *Acta Phytotaxonomica et Geobotanica*, vol. 64, no. 3, pp. 113-126.

Alcázar, R, Altabella, T, Marco, F, Bortolotti, C, Reymond, M, Koncz, C, Carrasco, P & Tiburcio, AF 2010, 'Polyamines: molecules with regulatory functions in plant abiotic stress tolerance', *Planta*, vol. 231, no.6, pp. 1237-1249.

Alhaithloul, HA, Soliman, MH, Ameta, KL, El-Esawi, MA & Elkelish, A 2020, 'Changes in Ecophysiology, Osmolytes, and Secondary Metabolites of the Medicinal Plants of Mentha piperita and Catharanthus roseus Subjected to Drought and Heat Stress.', *Biomolecules*, vol. 10, no. 1, p. 43.

Allakhverdiev, SI, Kreslavski, VD, Klimov, VV, Los, DA, Carpentier, R & Mohanty, P 2008, 'Heat stress: an overview of molecular responses in photosynthesis', *Photosynthesis Research*, vol. 98, no. 1-3, pp. 541.

Allen, DJ & Ort, DR 2001, 'Impacts of chilling temperatures on photosynthesis in warm-climate plants', *Trends in Plant Science*, vol. 6, no. 1, pp. 36-42.

Allwood, JW, Clarke, A, Goodacre, R & Mur, LA 2010, 'Dual metabolomics: a novel approach to understanding plant–pathogen interactions', *Phytochemistry*, 71, no. 5-6, pp. 590-597.

Alonso, A, Marsal, S & Julià, A 2015, 'Analytical methods in untargeted metabolomics: state of the art in 2015', *Frontiers in Bioengineering and Biotechnology*, vol. 3, p. 23.

Amato, P & Christner, BC 2009, 'Energy Metabolism Response to Low-Temperature and Frozen Conditions in *Psychrobacter cryohalolentis*', *Applied and Environmental Microbiology*, vol. 75, no. 3, pp. 711-718.

An, CI, Fukusaki, EI & Kobayashi, A 2002, 'Aspartic proteinases are expressed in pitchers of the carnivorous plant Nepenthes alata Blanco', *Planta*, vol. 214, no. 5, pp. 661-667.

An, JP, Li, R, Qu, FJ, You, CX, Wang, XF & Hao, YJ 2018, 'R2R3-MYB transcription factor Md MYB 23 is involved in the cold tolerance and proanthocyanidin accumulation in apple', *The Plant Journal*, vol. 96, no. 3, pp. 562-577.

Antunes, AC, Acunha, TDS, Perin, EC, Rombaldi, CV, Galli, V & Chaves, FC 2019, 'Untargeted metabolomics of strawberry (Fragaria x ananassa 'Camarosa') fruit from plants grown under osmotic stress conditions', *Journal of the Science of Food and Agriculture*, vol. 99, no. 15, pp. 6973-6980.

Aparna, V, Dileep, KV, Mandal, PK, Karthe, P, Sadasivan, C & Haridas, M 2012, 'Anti-inflammatory property of n - hexadecanoic acid: structural evidence and kinetic assessment', *Chemical Biology & Drug Design*, vol. 80, no. 3, pp. 434-439.

Araújo, MB, Ferri-Yáñez, F, Bozinovic, F, Marquet, PA, Valladares, F & Chown, SL 2013, 'Heat freezes niche evolution', *Ecology Letters*, vol. 16, no. 9, pp. 1206-1219.

Ashihara, H, Stasolla, C, Fujimura, T & Crozier, A 2018, 'Purine salvage in plants', *Phytochemistry*, vol. 147, pp. 89-124.

Athauda, SB, Matsumoto, K, Rajapakshe, S, Kuribayashi, M, Kojima, M, Kubomura-Yoshida, N, Iwamatsu, A, Shibata, C, Inoue, H & Takahashi, K 2004, 'Enzymic and structural characterization of nepenthesin, a unique member of a novel subfamily of aspartic proteinases', *Biochemical Journal*, vol. 38, no. 1, pp. 295-306.

Atkinson, NJ & Urwin, PE 2012, 'The interaction of plant biotic and abiotic stresses: from genes to the field', *Journal of Experimental Botany*, vol. 63, no. 10, pp. 3523-3543.

Aung, HH, Chia, LS, Goh, NK, Chia, TF, Ahmed, AA, Pare, PW & Mabry, TJ 2002, 'Phenolic constituents from the leaves of the carnivorous plant Nepenthes gracilis', *Fitoterapia*, vol. 73, no. 5, pp. 445-447.

Avelino, J, Barboza, B, Araya, JC, Fonseca, C, Davrieux, F, Guyot, B & Cilas, C 2005, 'Effects of slope exposure, altitude and yield on coffee quality in two altitude terroirs of Costa Rica, Orosi and Santa María de Dota', *Journal of the Science of Food and Agriculture*, vol. 85, no. 11, pp. 1869-1876.

Azuma, H, Toyota, M, Asakawa, Y, Yamaoka, R, Garcia-Franco, JG, Dieringer, G, Thien, LB & Kawano, S 1997, 'Chemical divergence in floral scents of Magnolia and allied genera (Magnoliaceae)', *Plant Species Biology*, vol. 12, no. 2-3, pp. 69-83.

Bai, BB 2014, 'Biological invasions: economic and environmental costs of alien plant, animal, and microbe species. (No. 577.18 B5/2011).

Bala, S, Asthir, B & Bains, NS 2016, 'Syringaldazine Peroxidase Stimulates Lignification by Enhancing Polyamine Catabolism in Wheat during Heat and Drought Stress', *Cereal Research Communications*, vol. 44, no. 4, 2016/12/01, pp. 561-571.

Balangcod, TD & Balangcod, AKD 2011, 'Ethnomedical knowledge of plants and healthcare practices among the Kalanguya tribe in Tinoc, Ifugao, Luzon, Philippines.

Balestrasse, KB, Tomaro, ML, Batlle, A & Noriega, GO 2010, 'The role of 5-aminolevulinic acid in the response to cold stress in soybean plants', *Phytochemistry*, vol. 71, no. 17-18, pp. 2038-2045.

Balfagón, D, Sengupta, S, Gómez-Cadenas, A, Fritschi, FB, Azad, RK, Mittler, R & Zandalinas, SI 2019, 'Jasmonic Acid Is Required for Plant Acclimation to a Combination of High Light and Heat Stress', *Plant Physiology*, vol. 181, no. 4, pp. 1668-1682.

Balmer, D, Flors, V, Glauser, G & Mauch-Mani, B 2013, 'Metabolomics of cereals under biotic stress: current knowledge and techniques', *Frontiers in Plant Science*, vol. 4, p. 82.

Baptista, A, Garcez, AS, Nunez, SC & Ribeiro, MS 2014, 'Antimicrobial efects of azulene induced by light', *Photodiagnosis and Photodynamic Therapy*, vol. 8, no. 2, pp. 179-180.

Barderas, MG, Laborde, CM, Posada, M, de la Cuesta, F, Zubiri, I, Vivanco, F & Alvarez-Llamas, G 2011, 'Metabolomic profiling for identification of novel potential biomarkers in cardiovascular diseases. *BioMed Research International*, 2011.

Barka, EA, Nowak, J & Clément, C 2006, 'Enhancement of chilling resistance of inoculated grapevine plantlets with a plant growth-promoting rhizobacterium, Burkholderia phytofirmans strain PsJN', *Applied and Environmental Microbiology*, vol. 72, no. 11, pp. 7246-7252.

Barthlott, W, Porembski, S, Seine, R & Theisen, I 2007, *The curious world of carnivorous plants,* Timber Press.

Bauer, U, Clemente, CJ, Renner, T & Federle, W 2012, 'Form follows function: morphological diversification and alternative trapping strategies in carnivorous Nepenthes pitcher plants', *Journal of Evolutionary Biology*, vol. 25, no. 1, pp. 90-102.

Bauer, U, Willmes, C & Federle, W 2009, 'Effect of pitcher age on trapping efficiency and natural prey capture in carnivorous Nepenthes rafflesiana plants', *Annals of Botany*, vol. 103, no. 8, pp. 1219-1226.

Bazile, V, Le Moguédec, G, Marshall, DJ & Gaume, L 2015, 'Fluid physico-chemical properties influence capture and diet in Nepenthes pitcher plants', *Annals of Botany*, vol. 115, no. 4, pp. 705-716.

Bekinbo, MT, Amah-Tariah, FS & Dapper, DV 2020, 'Comparative GC-MS determination of bioactive constituents of the methanolic extracts of Curcuma longa rhizome and Spondias mombin leaves', *Journal of Medicinal Plants, 8*, no. 1, pp. 01-06.

Belghit, S, Driche, EH, Bijani, C, Zitouni, A, Sabaou, N, Badji, B & Mathieu, F 2016, 'Activity of 2, 4-Di-tert-butylphenol produced by a strain of Streptomyces mutabilis isolated from a Saharan soil against Candida albicans and other pathogenic fungi', *Journal De Mycologie Medicale*, vol. 26, no. 2, pp. 160-169.

Bhardwaj, M, Sali, VK, Mani, S & Vasanthi, HR 2020, 'Neophytadiene from Turbinaria ornata Suppresses LPS-Induced Inflammatory Response in RAW 264.7 Macrophages and Sprague Dawley Rats', *Inflammation*, pp. 1-14.

Bhore, SJ, Komathi, V & Kandasamy, KI 2013, 'Diversity of endophytic bacteria in medicinally important Nepenthes species', *Journal of natural science, biology, and medicine*, vol. 4, no. 2, pp. 431.

Bickford, D, Lohman, DJ, Sodhi, NS, Ng, PK, Meier, R, Winker, K, Ingram, KK & Das, I 2007, 'Cryptic species as a window on diversity and conservation', *Trends in Ecology & Evolution*, vol. 22, no. 3, pp.148-155.

Biswal, DK, Debnath, M, Konhar, R, Yanthan, S & Tandon, P 2018, 'Phylogeny and biogeography of carnivorous plant family Nepenthaceae with reference to the Indian pitcher plant Nepenthes khasiana reveals an Indian subcontinent origin of Nepenthes colonization in south east Asia during the Miocene epoch', *Frontiers in Ecology and Evolution*, vol. 6, pp.108.

Bita, C & Gerats, T 2013, 'Plant tolerance to high temperature in a changing environment: scientific fundamentals and production of heat stress-tolerant crops', *Frontiers in Plant Science*, vol. 4, pp. 273.

Bittleston, LS 2018, *Commensals of Nepenthes pitchers*. Carnivorous Plants: Physiology, Ecology, and Evolution. Oxford University Press.

Blum, A & Ebercon, A 1981, ‘Cell membrane stability as a measure of drought and heat tolerance in wheat 1’, *Crop Science*, vol. 21, no. 1, pp. 43-47.

Böhm, V, Puspitasari-Nienaber, NL, Ferruzzi, MG & Schwartz, SJ 2002, 'Trolox Equivalent Antioxidant Capacity of Different Geometrical Isomers of α-Carotene, β-Carotene, Lycopene, and Zeaxanthin', *Journal of Agricultural and Food Chemistry*, vol. 50, no. 1, 2002/01/01, pp. 221-226.

Bohn, HF & Federle, W 2004, ‘Insect aquaplaning: Nepenthes pitcher plants capture prey with the peristome, a fully wettable water-lubricated anisotropic surface’, *Proceedings of the National Academy of Sciences*, vol. 101, no. 39, pp. 14138-14143.

Boivin, C, Camut, S, Malpica, CA, Truchet, G & Rosenberg, C 1990, 'Rhizobium meliloti Genes Encoding Catabolism of Trigonelline Are Induced under Symbiotic Conditions', *The Plant Cell*, vol. 2, no. 12, pp. 1157-1170.

Bonhomme, V, Pelloux‐Prayer, H, Jousselin, E, Forterre, Y, Labat, JJ & Gaume, L 2011, 'Slippery or sticky? Functional diversity in the trapping strategy of Nepenthes carnivorous plants', *New Phytologist*, vol. 191, no. 2, pp. 545-554.

Bowne, J, Bacic, A, Tester, M & Roessner, U 2018, ‘Abiotic stress and metabolomics’, *Annual Plant Reviews online*, pp.61-85.

Bronski, MJ, Martinez, CC, Weld, HA & Eisen, MB 2020, ‘Whole genome sequences of 23 species from the Drosophila montium species group (Diptera: Drosophilidae): A resource for testing evolutionary hypotheses’, *G3: Genes, Genomes, Genetics*, vol. 10, no. 5, pp. 1443-1455.

Buch, F, Pauchet, Y, Rott, M & Mithöfer, A 2014, ‘Characterization and heterologous expression of a PR-1 protein from traps of the carnivorous plant Nepenthes mirabilis’, *Phytochemistry*, vol. 100, pp. 43-50.

Buchhaupt, M, Kähne, F, Etschmann, MMW & Schrader, J 2014, 'Chapter 37 - Biotechnological Production of Fatty Aldehydes', in V Ferreira and R Lopez (eds), *Flavour Science*, Academic Press, San Diego, pp. 195-199.

Bunawan, H, Yen, CC, Yaakop, S & Noor, NM 2017, ‘Phylogenetic inferences of Nepenthes species in Peninsular Malaysia revealed by chloroplast (trn L intron) and nuclear (ITS) DNA sequences. BMC research notes’, vol. 10, no. 1, pp. 1-6.

Burkill IH 1966, *A dictionary of the economic products of the Malay Peninsula,* vol. 2. Kuala Lumpur: Ministry of Agriculture and Cooperatives, Malaysia.

Cameron, S, Rubinoff, D & Will, K 2006, ‘Who will actually use DNA barcoding and what will it cost?’, *Systematic biology*, vol. 55, no. 5, pp. 844-847.

Caminer, MA & Ron, SR 2014, ‘Systematics of treefrogs of the Hypsiboas calcaratus and Hypsiboas fasciatus species complex (Anura, Hylidae) with the description of four new species’, *Zookeys*, vol. 370, pp.1-68.

Cannon, J, Lojanapiwatna, V, Raston, C, Sinchai, W & White, A 1980, ‘The quinones of Nepenthes rafflesiana. the crystal structure of 2, 5-dihydroxy-3, 8-dimethoxy-7-methylnaphtho-1, 4-quinone (nepenthone-E) and a synthesis of 2, 5-dihydroxy-3-methoxy-7-

methylnaphtho-1, 4-quinone (nepenthone-C)', *Australian Journal of Chemistry*, vol. 33, no. 5, pp. 1073-1093.

Capó-Bauçà, S, Font-Carrascosa, M, Ribas-Carbó, M, Pavlovič, A & Galmés, J 2020, 'Biochemical and mesophyll diffusional limits to photosynthesis are determined by prey and root nutrient uptake in the carnivorous pitcher plant *Nepenthes* x ventrata. *Annals of Botany*, vol. 126, no. 1, pp. 25–37.

Carlos, G, Dos Santos, FP & Fröehlich, PE 2020, 'Canine metabolomics advances', *Metabolomics*, vol. 16, no. 2, pp. 16.

Carnivorous Plants in the Wilderness 2012, viewed 15 January 2019, <http://www.honda-e.com/A02_World Maps/WorldMap_NepenDetail.htm>

Caspi, R, Altman, T, Dreher, K, Fulcher, CA, Subhraveti, P, Keseler, IM, Kothari, A, Krummenacker, M, Latendresse, M, Mueller, LA, Ong, Q, Paley, S, Pujar, A, Shearer, AG, Travers, M, Weerasinghe, D, Zhang, P & Karp, PD 2011, 'The MetaCyc database of metabolic pathways and enzymes and the BioCyc collection of pathway/genome databases', *Nucleic Acids Research*, vol. 40, no. D1, pp. D742-D753.

Castro-Moretti, FR, Gentzel, IN, Mackey, D & Alonso, AP 2020, 'Metabolomics as an Emerging Tool for the Study of Plant–Pathogen Interactions', *Metabolites*, vol. 10, no. 2, pp. 52.

Catalano, M., 2018. *Nepenthes orbiculata*, una nuova specie da Phang Nga (Thailandia). *AIPC Magazine*, vol. 50, pp. 32-43.

Chan, XY, Hong, KW, Yin, WF & Chan, KG 2016, 'Microbiome and biocatalytic bacteria in monkey cup (Nepenthes pitcher) digestive fluid', *Scientific Reports*, vol. 6, no. 1, pp.1-10.

Chandran, V, Shaji, H & Mathew, L 2020, *Endophytic microbial influence on plant stress responses*. In Microbial Endophytes (pp. 161-193). Woodhead Publishing

Chase, MW, Christenhusz, MJ, Sanders, D & Fay, MF 2009, 'Murderous plants: Victorian Gothic, Darwin and modern insights into vegetable carnivory', *Botanical Journal of the Linnean Society*, vol. 161, no. 4, pp. 329-356.

Cheek M & Jebb M 2001, 'Nepenthaceae', *Flora Malesiana*, vol. 15, pp. 1-157.

Cheek, M & Golos, M 2019, 'Nepenthes (Nepenthaceae) on limestone: new discoveries', vol. 40, 06/06, pp. 6–11.

Cheek, M & Jebb, M 2001, *Flora Malesiana. Series I, Seed plants. Volume 15: Nepenthaceae,* Nationaal Herbarium Nederland, Leiden.

Cheek, M & Jebb, M 2013a, 'The Nepenthes micramphora (Nepenthaceae) group, with two new species from Mindanao, Philippines', *Phytotaxa*, vol. 151, no. 1, pp.25-34.

Cheek, M & Jebb, M 2013b, 'Recircumscription of the Nepenthes alata group (Caryophyllales: Nepenthaceae), in the Philippines, with four new species', *European Journal of Taxonomy*, vol. 69, pp. 1-23.

Cheek, M & Jebb, M 2016a, 'A new section in Nepenthes (Nepenthaceae) and a new species from Sulawesi', *Blumea-Biodiversity, Evolution and Biogeography of Plants*, vol. 61, no. 1, pp. 59-62.

Cheek, M & Jebb, M 2016b, ‘Nepenthes, three new infrageneric names and lectotypifications’, *Planta Carnivora*, vol. 37, no. 2, pp. 34-42.

Cheek, M & Jebb, M 2016c, ‘Nepenthes section Pyrophytae’, *Planta Carnivora*, vol. 38, no. 1, pp. 44-45.

Cheek, M & Jebb, MHP 2016d, ‘Nepenthes minima (Nepenthaceae), a new pyrophytic grassland species from Sulawesi, Indonesia’, *Blumea-Biodiversity, Evolution and Biogeography of Plants*, vol. 61, no. 3, pp. 181-185.

Cheek, M 2015, ‘Nepenthes (Nepenthaceae) of Halmahera, Indonesia’, *Blumea-Biodiversity, Evolution and Biogeography of Plants*, vol. 59, no. 3, pp. 215-225.

Cheek, M, Jebb, M, Mansur, M & Beattie, J 2017, ‘865. NEPENTHES DUBIA: Nepenthaceae’, *Curtis's Botanical Magazine*, vol. 34, no. 2, pp. 111-122.

Cheek, Martin & Golos, Michal 2019, ‘Nepenthes (Nepenthaceae) on limestone: new discoveries’, vol. 40, pp. 6–11.

Chen, DF, Li, X, Xu, Z, Liu, X, Du, SH, Li, H, Zhou, JH, Zeng, HP & Hua, ZC 2010, ‘Hexadecanoic acid from Buzhong Yiqi decoction induced proliferation of bone marrow mesenchymal stem cells’, *Journal of Medicinal Food*, vol. 13, no. 4, pp. 967-975.

Chen, W, Zhu, X, Han, W, Wu, Z & Lai, Q 2016, ‘Morphological, physiological and biochemical responses of gerbera cultivars to heat stress’, *원예과학기술지*, vol. 34, no. 1, pp. 1-14.

Chen, Z & Raji, M 2020, 'Chapter 13 - Role of reactive oxygen species in modulating cross tolerance in plants via flavonoids', in MA Hossain, F Liu, DJ Burritt, M Fujita and B Huang (eds), *Priming-Mediated Stress and Cross-Stress Tolerance in Crop Plants*, Academic Press, pp. 203-214.

Chin, L, Chung, AY & Clarke, C 2014, ‘Interspecific variation in prey capture behavior by co-occurring Nepenthes pitcher plants: Evidence for resource partitioning or sampling-scheme artifacts?’, *Plant Signaling & Behavior*, vol. 9, no. 1, pp. e27930.

Chin, L, Moran, JA & Clarke, C 2010, ‘Trap geometry in three giant montane pitcher plant species from Borneo is a function of tree shrew body size’, *New Phytologist*, vol. 186, no. 2, pp. 461-470.

Chong, J, Wishart, DS & Xia, J 2019, 'Using MetaboAnalyst 4.0 for Comprehensive and Integrative Metabolomics Data Analysis', *Current Protocols in Bioinformatics*, vol. 68, no. 1, p. e86.

Chou, LY, Clarke, CM & Dykes, GA 2014, ‘Bacterial communities associated with the pitcher fluids of three Nepenthes (Nepenthaceae) pitcher plant species growing in the wild’, *Archives of Microbiology*, vol. 196, no. 10, pp. 709-717.

Choudhary, ML, Patel, VB, Siddiqui, MW, Mahdl, SS & Verma, RB eds. 2015, *Climate Dynamics in Horticultural Science*, Two Volume Set. CRC Press.

Christenhusz, MJM & Byng, JW 2016, 'The number of known plants species in the world and its annual increase', *Phytotaxa*, vol. 261, no. 3, pp. 201-217.

Chu, Y, Liu, Y, Yao, T, He, Z, Lu, B & Wang, Y 2019, 'Isolation, identification of a protease-producing endophytic bacteria and its culture conditions', *Journal of Jiangsu University of Science and Technology (Natural Science Edition)*, vol. 1, pp.15.

Çiçek, B, Taghizadehghalehjoughi, A, Şentürk, E, Şentürk, M, Hacımüftüoğlu, A, & Gül, M 2019, 'Investigation of neuron protective and anticancer effects of pyrogallol: in vitro experiment', *Anatomy: International Journal of Experimental & Clinical Anatomy*, vol.13.

Clarke, C & Moran, JA 2016, 'Climate, soils and vicariance - their roles in shaping the diversity and distribution of Nepenthes in Southeast Asia', *Plant and Soil*, vol. 403, no. 1/2, pp. 37-51.

Clarke, C & Wong, KM 1997, *Nepenthes of Borneo*, Natural History Publications in association with Science and Technology Unit, Sabah.

Clarke, C 1997, *Nepenthes of Borneo*, Natural History Publications (Borneo) Sdn. Bhd., Kota Kinabalu.

Clarke, C 2001, *Nepenthes of Sumatra and peninsular Malaysia*, Natural History Publications (Borneo).

Clarke, C, Schlauer, J, Moran, JA & Robinson, A 2018, *Systematics and evolution of Nepenthes*, Oxford University Press.

Clarke, CM, Bauer, U, Lee, CIC, Tuen, AA, Rembold, K & Moran, JA 2009, 'Tree shrew lavatories: a novel nitrogen sequestration strategy in a tropical pitcher plant', *Biology Letters*, vol. 5, no. 5, pp. 632-635.

Cohen, SD, Tarara, JM & Kennedy, JA 2008, 'Assessing the impact of temperature on grape phenolic metabolism', *Analytica Chimica Acta*, vol. 621, no. 1, pp. 57-67.

Coleine, C, Gevi, F, Fanelli, G, Onofri, S, Timperio, AM & Selbmann, L 2020, 'Specific adaptations are selected in opposite sun exposed Antarctic cryptoendolithic communities as revealed by untargeted metabolomics', *PLOS One*, vol. 15, no. 5, pp. e0233805.

Crawford, AJ, McLachlan, DH, Hetherington, AM & Franklin, KA 2012, 'High temperature exposure increases plant cooling capacity', *Current Biology*, vol. 22, no. 10, pp. R396-R397.

Crockett, SL & Khan, IA 2003, 'Challenges of standardization: marker compounds in plant species related and unrelated to top-selling herbs', *Journal of Herbs, Spices & Medicinal Plants*, vol. 10, no. 3, pp. 13-24

Crockett, SL & Robson, NK 2011, 'Taxonomy and chemotaxonomy of the genus Hypericum', *Medicinal and Aromatic Plant Science and Biotechnology*, vol. 5, no. 1, pp. 1.

CS Odessa LLC 2019, *ConceptDraw OFFICE 6* [computer program], CS Odessa LLC, San Jose, CA, USA.

D'amato, P 2013, *The savage garden*, revised: Cultivating carnivorous plants, Ten Speed Press.

Danser, BH 1928, 'The Nepenthaceae of the Netherlands Indies', *Bulletin du Jardin botanique de Buitenzorg*, vol. 10, pp. 249-438.

Darwin, C 1875, *Insectivorous Plants*, John Murray, London.

Daryanto, S, Wang, L & Jacinthe, PA 2016, 'Global synthesis of drought effects on maize and wheat production', *PLOS One*, vol. 11, no. 5, pp. e0156362.

Das, K & Roychoudhury, A 2014, 'Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants', *Frontiers in Environmental Science*, vol. 2, no. 53, 2014-December-02

Davey, MP, Burrell, MM, Woodward, FI & Quick, WP 2008, 'Population-specific metabolic phenotypes of Arabidopsis lyrata ssp. Petraea', *New Phytologist*, vol. 177, no. 2, pp.380-388.

De, U, Son, JY, Jeon, Y, Ha, SY, Park, YJ, Yoon, S, Ha, KT, Choi, WS, Lee, BM, Kim, IS & Kwak, JH 2019, 'Plumbagin from a tropical pitcher plant (*Nepenthes alata* Blanco) induces apoptotic cell death via a p53-dependent pathway in MCF-7 human breast cancer cells', *Food and Chemical Toxicology*, vol. 123, pp. 492-500.

Dettmer, K, Aronov, PA & Hammock, BD 2007, 'Mass spectrometry-based metabolomics', *Mass Spectrometry Reviews*, vol. 26, no. 1, pp.51-78.

Di Giusto, B, Bessière, JM, Guéroult, M, Lim, LB, Marshall, DJ, Hossaert-McKey, M & Gaume, L 2010, 'Flower-scent mimicry masks a deadly trap in the carnivorous plant Nepenthes rafflesiana', *Journal of Ecology*, vol. 98, no. 4, pp. 845-856.

Di Giusto, B, Grosbois, V, Fargeas, E, Marshall, DJ & Gaume, L 2008, 'Contribution of pitcher fragrance and fluid viscosity to high prey diversity in a Nepenthes carnivorous plant from Borneo', *Journal of Biosciences*, vol. 33, no. 1, pp. 121-136.

Ding, X, Jiang, Y, Hao, T, Jin, H, Zhang, H, He, L, Zhou, Q, Huang, D, Hui, D & Yu, J 2016, 'Effects of Heat Shock on Photosynthetic Properties, Antioxidant Enzyme Activity, and Downy Mildew of Cucumber (Cucumis sativus L.)', *PLOS One*, vol. 11, no. 4, p. e0152429.

Djanaguiraman, M, Prasad, PVV & Seppanen, M 2010, 'Selenium protects sorghum leaves from oxidative damage under high temperature stress by enhancing antioxidant defense system', *Plant Physiology and Biochemistry*, vol. 48, no. 12, pp. 999-1007.

Do, KT, Wahl, S, Raffler, J, Molnos, S, Laimighofer, M, Adamski, J, Suhre, K, Strauch, K, Peters, A, Gieger, C, Langenberg, C, Stewart, ID, Theis, FJ, Grallert, H, Kastenmüller, G & Krumsiek, J 2018, 'Characterization of missing values in untargeted MS-based metabolomics data and evaluation of missing data handling strategies', *Metabolomics*, vol. 14, no. 10, 2018/09/20, p. 128.

Dobra, J, Motyka, V, Dobrev, P, Malbeck, J, Prasil, IT, Haisel, D, Gaudinova, A, Havlova, M, Gubis, J & Vankova, R 2010, 'Comparison of hormonal responses to heat, drought and combined stress in tobacco plants with elevated proline content', *Journal of Plant Physiology*, vol. 167, no. 16, pp. 1360-1370.

Dos-Santos, N, Bueso, MC & Fernández-Trujillo, JP 2013, 'Aroma volatiles as biomarkers of textural differences at harvest in non-climacteric near-isogenic lines of melon', *Food Research International*, vol. 54, no. 2, pp. 1801-1812.

Du, H, Liu, H & Xiong, L 2013, 'Endogenous auxin and jasmonic acid levels are differentially modulated by abiotic stresses in rice', *Frontiers in Plant Science*, vol. 4, pp. 397.

Eilenberg, H, Pnini-Cohen, S, Rahamim, Y, Sionov, E, Segal, E, Carmeli, S & Zilberstein, A 2010, 'Induced production of antifungal naphthoquinones in the pitchers of the carnivorous plant Nepenthes khasiana', *Journal of Experimental Botany*, vol. 61, no. 3, pp. 911-922.

El-Demerdash, E. 2011, 'Anti-inflammatory and antifibrotic effects of methyl palmitate', *Toxicology and Applied Pharmacology*, vol. 254, no. 3, pp. 238-244.

Ellison, AM & Adamec, L eds. 2018, *Carnivorous Plants: physiology, ecology, and evolution*, Oxford University Press.

Elmido, CE, Balangcod, KD & Balangcod, TD 2019, 'Inhibition of Pseudomonas aeruginosa by the extracts of indigenous plants from the Cordillera Region, Philippines', *Indian Journal of Traditional Knowledge*, vol. 18, no. 1, pp. 41-46.

Esmat, AU, Mittapally, S & Begum, S 2020, 'GC-MS Analysis of Bioactive Compounds and Phytochemical Evaluation of the Ethanolic Extract of Gomphrena globosa L. Flowers', *Journal of Drug Delivery and Therapeutics*, vol. 10, no. 2, pp. 53-58.

Evans, DP 2009, 'New cultivars: Nepenthes maxima 'Lake Poso'', *Carnivorous Plant Newsletter*, vol. 38, pp. 18–22.

Exoticplantsplus, 2014, viewed from 15 March 2020, <https://www.exoticplantsplus.com/NNectarGlands.htm>

Farooq, M, Bramley, H, Palta, JA & Siddique, KH 2011, 'Heat stress in wheat during reproductive and grain-filling phases', *Critical Reviews in Plant Sciences*, vol. 30, no. 6, pp. 491-507.

Farooq, M, Gogoi, N, Barthakur, S, Baroowa, B, Bharadwaj, N, Alghamdi, SS & Siddique, KHM 2017, 'Drought stress in grain legumes during reproduction and grain filling', *Journal of Agronomy and Crop Science*, vol. 203, no. 2, pp. 81-102.

Feng, Z, Ding, C, Li, W, Wang, D & Cui, D 2020, 'Applications of metabolomics in the research of soybean plant under abiotic stress', *Food Chemistry*, vol. 310, pp. 125914.

Frank, G, Pressman, E, Ophir, R, Althan, L, Shaked, R, Freedman, M, Shen, S & Firon, N 2009, 'Transcriptional profiling of maturing tomato (Solanum lycopersicum L.) microspores reveals the involvement of heat shock proteins, ROS scavengers, hormones, and sugars in the heat stress response', *Journal of Experimental Botany*, vol. 60, no. 13, pp. 3891-3908.

French, KE, Harvey, J & McCullagh, JS 2018, 'Targeted and untargeted metabolic profiling of wild grassland plants identifies antibiotic and anthelmintic compounds targeting pathogen physiology, metabolism and reproduction', *Scientific Reports*, vol. 8, no. 1, pp. 1-10.

Fu, HY, Hu, O, Zhang, YM, Zhang, L, Song, JJ, Lu, P, Zheng, QX, Liu, PP, Chen, QS, Wang, B & Wang, XY, 2017, 'Mass-spectra-based peak alignment for automatic nontargeted metabolic profiling analysis for biomarker screening in plant samples', *Journal of Chromatography A*, vol. 1513, pp. 201-209.

Fujita, M, Fujita, Y, Noutoshi, Y, Takahashi, F, Narusaka, Y, Yamaguchi-Shinozaki, K & Shinozaki, K 2006, 'Crosstalk between abiotic and biotic stress responses: a current view from the points of convergence in the stress signaling networks', *Current Opinion in Plant Biology*, vol. 9, no. 4, pp. 436-442.

Fukushima, K, Fang, X, Alvarez-Ponce, D, Cai, H, Carretero-Paulet, L, Chen, C, Chang, TH, Farr, KM, Fujita, T, Hiwatashi, Y & Hoshi, Y 2017, 'Genome of the pitcher plant Cephalotus reveals genetic changes associated with carnivory', *Nature Ecology & Evolution*, vol. 1, no. 3, pp.1-9.

Galati, G, Gandin, A, Jolivet, Y, Larbat, R & Hehn, A 2019, 'Untargeted Metabolomics Approach Reveals Diverse Responses of Pastinaca Sativa to Ozone and Wounding Stresses', *Metabolites*, vol. 9, no. 7, pp. 153.

Gao, J, Sanchez-Medina, A, Pendry, BA, Hughes, MJ, Webb, GP & Corcoran, O 2008, 'Validation of a HPLC method for flavonoid biomarkers in skullcap (*Scutellaria*) and its use to illustrate wide variability in the quality of commercial tinctures', *Journal of Pharmacy & Pharmaceutical Sciences*, vol. 11, no. 1, pp. 77-87.

Garg, RC 2016, 'Chapter 44 - Fenugreek: Multiple Health Benefits', in RC Gupta (ed.) *Nutraceuticals*, Academic Press, Boston, pp. 599-617.

Gargallo-Garriga, A, Sardans, J, Granda, V, Llusià, J, Peguero, G, Asensio, D, Ogaya, R, Urbina, I, Van Langenhove, L, Verryckt, LT & Chave, J 2020, 'Different "metabolomic niches" of the highly diverse tree species of the French Guiana rainforests', *Scientific Reports*, vol. 10, no. 1, pp.1-10.

Gargallo-Garriga, A, Sardans, J, Pérez-Trujillo, M, Rivas-Ubach, A, Oravec, M, Vecerova, K, Urban, O, Jentsch, A, Kreyling, J, Beierkuhnlein, C & Parella, T 2014, 'Opposite metabolic responses of shoots and roots to drought', *Scientific Reports*, vol. 4, pp. 6829.

Gaume, L & Di Giusto, B 2009, 'Adaptive significance and ontogenetic variability of the waxy zone in Nepenthes rafflesiana', *Annals of Botany*, vol. 104, no. 7, pp. 1281-1291.

Gaume, L & Forterre, Y 2007, 'A viscoelastic deadly trap in Nepenthes pitcher plants', *PLOS One*, vol. 2, pp. e1185.

Gaume, L, Bazile, V, Huguin, M & Bonhomme, V, 2016, 'Different pitcher shapes and trapping syndromes explain resource partitioning in Nepenthes species', *Ecology and Evolution*, vol. 6, no. 5, pp. 1378-1392.

Gaume, L, Gorb, S & Rowe, N 2002, 'Function of epidermal surfaces in the trapping efficiency of Nepenthes alata pitchers', *New Phytologist*, vol. 156, no. 3, pp. 479-489.

Gauthier, L, Tison-Rosebery, J, Morin, S & Mazzella, N 2020, 'Metabolome response to anthropogenic contamination on microalgae: a review', *Metabolomics*, vol. 16, no. 1, p. 8.

Geml, J, Tulloss, RE, Laursen, GA, Sazanova, NA & Taylor, DL 2008, 'Evidence for strong inter-and intracontinental phylogeographic structure in Amanita muscaria, a wind-dispersed ectomycorrhizal basidiomycete', *Molecular Phylogenetics and Evolution*, vol. 48, no. 2, pp. 694-701.

Ghalambor, CK, Huey, RB, Martin, PR, Tewksbury, JJ & Wang, G 2006, 'Are mountain passes higher in the tropics? Janzen's hypothesis revisited', *Integrative and Comparative Biology*, vol. 46, no. 1, pp. 5-17.

Gharibi, S, Tabatabaei, BES, Saeidi, G & Goli, SAH 2016, 'Effect of Drought Stress on Total Phenolic, Lipid Peroxidation, and Antioxidant Activity of Achillea Species', *Applied Biochemistry and Biotechnology*, vol. 178, no. 4, 2016/02/01, pp. 796-809.

Ghosh, R, Roy, K, Subba, A, Mandal, P, Basak, S, Kundu, M & Roy, MN 2020, 'Case to case study for exploring inclusion complexes of an anti-diabetic alkaloid with α and β cyclodextrin molecules for sustained dischargement', *Journal of Molecular Structure*, vol. 1200, pp. 126988.

Gika, HG, Theodoridis, GA, Plumb, RS & Wilson, ID 2014, 'Current practice of liquid chromatography–mass spectrometry in metabolomics and metabonomics', *Journal of Pharmaceutical and Biomedical Analysis*, vol. 87, pp. 12-25.

Gilbert, KJ, Bittleston, LS, Naive, MAK, Kiszewski, AE, Buenavente, PAC, Lohman, DJ & Pierce, NE 2020a, 'Investigation of an Elevational Gradient Reveals Strong Differences Between Bacterial and Eukaryotic Communities Coinhabiting Nepenthes Phytotelmata', *Microbial Ecology*, pp. 1-16.

Gilbert, KJ, Bittleston, LS, Tong, W & Pierce, NE 2020b, 'Tropical pitcher plants (Nepenthes) act as ecological filters by altering properties of their fluid microenvironments', *Scientific Reports*, vol. 10, no. 1, pp. 1-13.

Gilmour SJ, Sebolt AM, Salazar MP, Everard JD & Thomashow MF 2000, 'Overexpression of the Arabidopsis CBF3 transcriptional activator mimics multiple biochemical changes associated with cold acclimation', *Plant Physiology*, vol. 124, pp. 1854–1865.

Goh, H-H, Khairudin, K, Sukiran, NA, Normah, MN & Baharum, SN 2016, 'Metabolite profiling reveals temperature effects on the VOCs and flavonoids of different plant populations', *Plant Biology*, vol. 18, no. S1, pp. 130-139.

Gomathi, D, Kalaiselvi, M, Ravikumar, G, Devaki, K & Uma, C 2015, 'GC-MS analysis of bioactive compounds from the whole plant ethanolic extract of Evolvulus alsinoides (L.) L', *Journal of Food Science and Technology*, vol. 52, no. 2, pp. 1212-1217.

Goodacre, R 2007, 'Metabolomics of a superorganism', *The Journal of Nutrition*, vol. 137, no. 1, pp. 259S-266S.

Goodacre, R, Vaidyanathan, S, Dunn, WB, Harrigan, GG & Kell, DB 2004, 'Metabolomics by numbers: acquiring and understanding global metabolite data', *TRENDS in Biotechnology*, vol. 22, no. 5, pp. 245-252.

Grafe, TU, Schöner, CR, Kerth, G, Junaidi, A & Schöner, MG 2011, 'A novel resource–service mutualism between bats and pitcher plants', *Biology Letters*, vol. 7, no. 3, pp. 436-439.

Gray, GR & Heath, D 2005, 'A global reorganization of the metabolome in Arabidopsis during cold acclimation is revealed by metabolic fingerprinting', *Physiologia Plantarum*, vol. 124, no. 2, pp. 236-248.

Gray, LK, Clarke, C, Wint, GRW & Moran, JA 2017, 'Potential effects of climate change on members of the Palaeotropical pitcher plant family Nepenthaceae', *PLOS One*, vol. 12, no. 8, p. e0183132.

Greenwood, M, Clarke, C, Ch'ien, CL, Gunsalam, A & Clarke, RH 2011, 'A unique resource mutualism between the giant Bornean pitcher plant, Nepenthes rajah, and members of a small mammal community', *PLOS One*, vol. 6, no. 6.

Griffiths, LL, Melvin, SD, Connolly, RM, Pearson, RM & Brown, CJ 2020, 'Metabolomic indicators for low-light stress in seagrass', *Ecological Indicators*, vol. 114, pp. 106316.

Gross, JH 2017, '*Practical Aspects of Electron Ionization*. In Mass Spectrometry (pp. 293-324). Springer, Cham.

Guarrera, M, Turbino, L & Rebora, A 2001, 'The anti - inflammatory activity of azulene', *Journal of the European Academy of Dermatology and Venereology*, vol. 15, no. 5, pp. 486-487.

Guy, C, Kaplan, F, Kopka, J, Selbig, J & Hincha, DK 2008, 'Metabolomics of temperature stress', *Physiologia Plantarum*, vol. 132, no. 2, pp. 220-235.

Guyot, B, Gueule, D, Manez, JC, Perriot, JJ, Giron, J & Villain, L 1996, 'Influence de l'altitude et de l'ombrage sur la qualité des cafés Arabica', *Plantations, Recherche, Développement*, vol. 3, no. 4, pp. 272-283.

Haferkamp, I, Fernie, AR & Neuhaus, HE 2011, 'Adenine nucleotide transport in plants: much more than a mitochondrial issue', *Trends in Plant Science*, vol. 16, no. 9, pp. 507-515.

Hamayun, M, Hussain, A, Iqbal, A, Khan, SA & Lee, IJ 2018, 'Endophytic fungus Aspergillus japonicus mediates host plant growth under normal and heat stress conditions', *BioMed research international*, 2018.

Hammer, Ø, Harper, DA & Ryan, PD 2001, 'PAST: Paleontological statistics software package for education and data analysis', *Palaeontologia Electronica*, vol. 4, no. 1, p. 9.

Harrison, BR, Wang, L, Gajda, E, Hoffman, EV, Chung, BY, Pletcher, SD, Raftery, D & Promislow, DE 2020, 'The metabolome as a link in the genotype-phenotype map for peroxide resistance in the fruit fly, Drosophila melanogaster', *BMC Senomics*, vol. 21, pp. 1-22.

Hasanuzzaman, M, Fujita, M, Oku, H & Islam, MT 2019, *Plant tolerance to environmental stress: Role of phytoprotectants*. CRC Press.

Hassanein, R, El-khawas, S, Ibrahim, S, El-Bassiouny, H, Mostafa, HA & el-Monem, A 2013, 'Improving the thermo tolerance of wheat plant by foliar application of arginine or putrescine', *Pakistan Journal of Botany*, vol. 45, 02/01, pp. 111-118.

Hatano, N & Hamada, T 2008, 'Proteome analysis of pitcher fluid of the carnivorous plant Nepenthes alata', *The Journal of Proteome Research*, vol. 7, no. 2, pp. 809-816.

Hatano, N & Hamada, T 2012, Proteomic analysis of secreted protein induced by a component of prey in pitcher fluid of the carnivorous plant Nepenthes alata', *Journal of Proteomics*, vol. 75, no. 15, pp. 4844-4852.

He, J, Tang, S, Yang, D, Chen, Y, Ling, L, Zou, Y, Zhou, M & Xu, X 2019, 'Chemical and Transcriptomic Analysis of Cuticle Lipids under Cold Stress in *Thellungiella salsuginea*', *International Journal of Molecular Sciences*, 20, no. 18, pp. 4519.

He, M, He, CQ & Ding, NZ 2018, 'Abiotic stresses: general defenses of land plants and chances for engineering multistress tolerance', *Frontiers in Plant Science*, vol. 9, pp. 1771.

Heckathorn, SA, Poeller, GJ, Coleman, JS & Hallberg, RL 1996, 'Nitrogen availability alters patterns of accumulation of heat stress-induced proteins in plants', *Oecologia*, vol. 105, no. 3, 1996/02/01, pp. 413-418.

Higashi, Y & Saito, K 2019, 'Lipidomic studies of membrane glycerolipids in plant leaves under heat stress', *Progress in Lipid Research*, vol. 75, pp. 100990.

Hoffmann, JF, Carvalho, IR, Barbieri, RL, Rombaldi, CV & Chaves, FC 2017, 'Butia spp.(Arecaceae) LC-MS-based metabolomics for species & geographical origin discrimination', *Journal of Agricultural and Food Chemistry*, vol. 65, no. 2, pp. 523-532.

Hollingsworth, PM, Graham, SW & Little, DP 2011, 'Choosing and using a plant DNA barcode', *PLOS One*, vol. 6, no. 5.

Hong, J, Yang, L, Zhang, D & Shi, J 2016, 'Plant metabolomics: an indispensable system biology tool for plant science', *International Journal of Molecular Sciences*, vol. 17, no. 6, pp.767.

Hosseinzadeh, S, Jafarikukhdan, A, Hosseini, A & Armand, R 2015, 'The application of medicinal plants in traditional and modern medicine: a review of Thymus vulgaris', *International Journal of Clinical Medicine*, vol. 6, no. 09, p.635.

Hu, J, Wu, W, Cao, Z, Wen, J, Shu, Q & Fu, S 2016, 'Morphological, physiological and biochemical responses of Camellia oleifera to low-temperature stress', *Pakistan Journal of Botany*, vol. 48, no. 3, pp. 899-905.

Hu, L, Bi, A, Hu, Z, Amombo, E, Li, H & Fu, J 2018, 'Antioxidant Metabolism, Photosystem II, and Fatty Acid Composition of Two Tall Fescue Genotypes With Different Heat Tolerance Under High Temperature Stress', *Frontiers in Plant Science*, vol. 9, no. 1242, 2018-August-22

Hu, Y, Jiang, Y, Han, X, Wang, H, Pan, J & Yu, D 2017, 'Jasmonate regulates leaf senescence and tolerance to cold stress: crosstalk with other phytohormones', *Journal of Experimental Botany*, vol. 68, no. 6, pp. 1361-1369.

Huang, YH, Lien, Y, Chen, JH, Lin, ES & Huang, CY 2020, 'Identification and characterization of dihydropyrimidinase inhibited by plumbagin isolated from Nepenthes miranda extract', *Biochimie*, pp. 171-172.

Hubbard, M, Germida, J & Vujanovic, V 2012, 'Fungal endophytes improve wheat seed germination under heat and drought stress', *Botany*, vol. 90, no. 2, pp. 137-149.

Huseby, S, Degerlund, M, Zingone, A & Hansen, E 2012, 'Metabolic fingerprinting reveals differences between northern and southern strains of the cryptic diatom Chaetoceros socialis', *European Journal of Phycology*, vol. 47, no.4, pp. 480-489.

Hussain, SZ & Maqbool, K 2014, 'GC-MS: Principle, Technique and its application in Food Science', *International Journal of Current Science*, vol. 13, pp. 116-126.

Ichihashi, Y, Kusano, M, Kobayashi, M, Suetsugu, K, Yoshida, S, Wakatake, T, Kumaishi, K, Shibata, A, Saito, K & Shirasu, K 2018, 'Transcriptomic and metabolomic reprogramming from roots to haustoria in the parasitic plant, Thesium Chinense', *Plant and Cell Physiology*, vol. 59, no. 4, pp. 729-738.

Inchagova, KS, Duskaev, GK & Deryabin, DG 2019, 'Quorum Sensing Inhibition in Chromobacterium violaceum by Amikacin Combination with Activated Charcoal or Small Plant-Derived Molecules (Pyrogallol and Coumarin)', *Microbiology*, vol. 88, no.1, pp. 63-71.

Ismail, N, Kamariah, AS, Lim, LBL & Ahmad, N 2015, 'Phytochemical and Pharmacological Evaluation of Methanolic Extracts of the Leaves of *Nepenthes bicalcarata* Hook.

F.', *International Journal of Pharmacognosy and Phytochemical Research,* vol. 7, no. 6, 1127-1138.

Jakobsen, HB, Friis, P, Nielsen, JK, & Olsen, CE 1994, 'Emission of volatiles from flowers and leaves of Brassica napus in situ', *Phytochemistry*, vol. 37, no. 3, pp. 695-699.

Jan, S & Ahmad, P 2019, *Ecometabolomics: Metabolic Fluxes Versus Environmental Stoichiometry*. Academic Press

Jebb, M & Cheek, M 1997, 'A skeletal revision of Nepenthes (Nepenthaceae)', *Blumea journal of plant taxonomy and plant geography*, vol. 42, 01/01, pp. 1-106.

Jian, H, Xie, L, Wang, Y, Cao, Y, Wan, M, Lv, D, Li, J, Lu, K, Xu, X & Liu, L 2020, 'Characterization of cold stress responses in different rapeseed ecotypes based on metabolomics and transcriptomics analyses', *PeerJ*, vol. 8, 2020/03/31, p. e8704.

Jones, OA, Maguire, ML, Griffin, JL, Dias, DA, Spurgeon, DJ & Svendsen, C 2013, 'Metabolomics and its use in ecology', *Austral Ecology*, vol. 38, no. 6, pp. 713-720.

Jung, K, Miyagawa, M, Matsuda, A, Amagai, Y, Oida, K, Okamoto, Y, Takai, M, Nishikawa, S, Jang, H, Ishizaka, S and Ahn, G 2013, 'Antifungal effects of palmitic acid salt and ultrapure soft water on S cedosporium apiospermum', *Journal of applied microbiology*, vol. 115, no. 3, pp. 711-717.

Juniper, B, Robins, RJ, Joel, DM 1989, *The Carnivorous Plants*. Academic Press Ltd., London

Kanokratana, P, Mhuanthong, W, Laothanachareon, T, Tangphatsornruang, S, Eurwilaichitr, L, Kruetreepradit, T, Mayes, S & Champreda, V 2016, 'Comparative study of bacterial communities in Nepenthes pitchers and their correlation to species and fluid acidity', *Microbial ecology*, vol. 72, no. 2, pp. 381-393.

Kaplan, F, Kopka, J, Haskell, DW, Zhao, W, Schiller, KC, Gatzke, N, Sung, DY & Guy, CL 2004, 'Exploring the Temperature-Stress Metabolome of Arabidopsis', *Plant Physiology*, vol. 136, no. 4, pp. 4159-4168.

Kaul, RB 1982, 'Floral and fruit morphology of Nepenthes lowii and N. villosa, montane carnivores of Borneo', *American Journal of Botany*, vol. 69, no. 5, pp. 793-803.

Kaur, R, Kaushal, S & Sharma, P 2018, 'Antimicrobial and antioxidant potential of pomegranate (Punica granatum L.) peel.', *International Journal of Chemical Studies*, vol. 6, no. 5, pp. 3441-3449.

Khan, N, Bano, A, Ali, S & Babar, MA 2020, 'Crosstalk amongst phytohormones from planta and PGPR under biotic and abiotic stresses', *Plant Growth Regulation*, pp.1-15.

Kim, TE, Kim, SK, Han, TJ, Lee, JS and Chang, SC 2002, 'ABA and polyamines act independently in primary leaves of cold - stressed tomato (Lycopersicon esculentum)', *Physiologia Plantarum*, vol. 115, no. 3, pp. 370-376.

Kliebenstein, DJ & Osbourn, A 2012, 'Making new molecules – evolution of pathways for novel metabolites in plants', *Current Opinion in Plant Biology*, vol. 15, no. 4, pp. 415–423.

Knudsen, JT, Eriksson, R, Gershenzon, J & Ståhl, B 2006, 'Diversity and distribution of floral scent', *The Botanical Review*, vol. 72, no. 1, pp. 1.

Kocaçalışkan, I, Talan, I & Terzi, I 2006,' Antimicrobial activity of catechol and pyrogallol as allelochemicals', *Zeitschrift für Naturforschung C*, vol. 61, no. 9/10, 639-642.

Koek, MM, Jellema, RH, van der Greef, J, Tas, AC & Hankemeier, T 2011, 'Quantitative metabolomics based on gas chromatography mass spectrometry: status and perspectives', *Metabolomics*, vol. 7, no. 3, pp. 307-328.

Kosová, K, Prášil, IT, Vítámvás, P, Dobrev, P, Motyka, V, Floková, K, Novák, O, Turečková, V, Rolčik, J, Pešek, B & Trávničková, A 2012, 'Complex phytohormone responses during the cold acclimation of two wheat cultivars differing in cold tolerance, winter Samanta and spring Sandra', *Journal of Plant Physiology*, vol. 169, no. 6, pp. 567-576.

Kotak, S, Larkindale, J, Lee, U, von Koskull-Döring, P, Vierling, E & Scharf, K.D 2007, 'Complexity of the heat stress response in plants', *Current Opinion in Plant Biology*, vol. 10, no. 3, pp. 310-316.

Kráľová, K, Jampílek, J & Ostrovský, I 2012, 'Metabolomics-useful tool for study of plant responses to abiotic stresses', *Ecological Chemistry and Engineering S*, vol. 19, no. 2, pp. 133-161.

Kress, WJ & Erickson, DL 2007, 'A two-locus global DNA barcode for land plants: the coding rbcL gene complements the non-coding trnH-psbA spacer region', *PLOS One*, vol. 2, no. 6.

Kuk, YI, Shin, JS, Burgos, NR, Hwang, TE, Han, O, Cho, BH, Jung, S & Guh, JO 2003, 'Antioxidative Enzymes Offer Protection from Chilling Damage in Rice Plants', *Crop Science*, vol. 43, no. 6, 2003/11/01, pp. 2109-2117.

Kumar, S, Vasudeva, N & Sharma, S 2012, 'GC-MS analysis and screening of antidiabetic, antioxidant and hypolipidemic potential of Cinnamomum tamala oil in streptozotocin induced diabetes mellitus in rats', *Cardiovascular Diabetology*, vol. 11, no. 1, pp. 95.

Kurata, K, Jaffré, T & Setoguchi, H 2008, 'Genetic diversity and geographical structure of the pitcher plant Nepenthes vieillardii in New Caledonia: a chloroplast DNA haplotype analysis', *American Journal of Botany*, vol. 95, no. 12, pp. 1632-1644.

Kurdyukov, S, Faust, A, Trenkamp, S, Bär, S, Franke, R, Efremova, N, Tietjen, K, Schreiber, L, Saedler, H & Yephremov, A 2006, 'Genetic and biochemical evidence for involvement of HOTHEAD in the biosynthesis of long-chain α-,ω-dicarboxylic fatty acids and formation of extracellular matrix', *Planta*, vol. 224, no. 2, pp. 315-329.

Kuspradini, H, Wulandari, I, Putri, AS, Tiya, SY & Kusuma, IW 2018, 'Phytochemical, antioxidant and antimicrobial properties of Litsea angulata extracts', *F1000Research*, vol. 7.

Kuzina, V, Ekstrøm, CT, Andersen, SB, Nielsen, JK, Olsen, CE & Bak, S 2009, 'Identification of defense compounds in *Barbarea vulgaris* against the herbivore *Phyllotreta nemorum* by an ecometabolomic approach', *Plant Physiology*, vol. 151, no. 4, pp. 1977-1990.

Lam, WN, Chong, KY, Anand, GS & Tan, HTW 2017, 'Dipteran larvae and microbes facilitate nutrient sequestration in the *Nepenthes gracilis* pitcher plant host', *Biology Letters*, vol. 13, no. 3, pp. 20160928.

Lam, WN, Chou, YY, Leong, FWS & Tan, HTW 2019, 'Inquiline predator increases nutrient-cycling efficiency of *Nepenthes rafflesiana* pitchers', *Biology Letters*, vol. 15, no. 12, pp. 20190691.

Lam, WN, Ling, J, Lum, TQH & Tan, HTW 2020, 'Ecology and natural history of swimming pitcher mites (*Creutzeria* spp., *Histiostomatidae*) from the traps of Nepenthes pitcher plants', *Journal of Zoology*, vol. 310, no. 1, pp. 1-9.

Lamaoui, M, Jemo, M, Datla, R & Bekkaoui, F 2018, 'Heat and drought stresses in crops and approaches for their mitigation', *Frontiers in Chemistry*, vol. 6, p. 26.

Lee, JM, Tan, WS & Ting, ASY 2014, 'Revealing the antimicrobial and enzymatic potentials of culturable fungal endophytes from tropical pitcher plants (Nepenthes spp.)', *Mycosphere*, vol. 5, No. 2, pp. 364-377.

Lee, L, Zhang, Y, Ozar, B, Sensen, CW & Schriemer, DC 2016, 'Carnivorous nutrition in pitcher plants (Nepenthes spp.) via an unusual complement of endogenous enzymes', *Journal of Proteome Research*, vol. 15, no. 9, pp. 3108-3117.

Leiss, K, Choi, Y & Klinkhamer, P 2016, *Application of eco-metabolomics in biological science*. In AIP Conference Proceedings (Vol. 1744, No. 1, p. 020033), AIP Publishing LLC.

Levitt, J 1972, *Responses of Plants to Environmental Stresses*, Academic Press New York, pp. 697.

Li, B, Cong, W, Wang, B, Du, H, Zhang, AH & Meng, XC 2020, 'Metabolomics Reveals Ecological Significance of Secondary Metabolites in *Scutellaria baicalensis* under drought stress', *Research Square*.

Li, CR, Hou, XH, Xu, YY, Gao, W, Li, P & Yang, H 2020, 'Manual annotation combined with untargeted metabolomics for chemical characterization and discrimination of two major crataegus species based on liquid chromatography quadrupole time-of-flight mass spectrometry', *Journal of Chromatography A*, vol. 1612, pp. 460628.

Li, H, Li, X, Liu, S, Zhu, X, Song, F and Liu, F., 2020. *Induction of cross tolerance by cold priming and acclimation in plants: Physiological, biochemical and molecular mechanisms*. In Priming-Mediated Stress and Cross-Stress Tolerance in Crop Plants (pp. 183-201). Academic Press.

Li, W, Li, S, Li, J, Ye, G, Zhu, L, Wang, X, An, W, Yang, L & Liu, Y 2012, 'Isolation and identification of the protease producing endophyte bacteria from Nepenthes mirabilis', *Acta Botanica Boreali-Occidentalia Sinica*, vol. 32, no. 12, pp. 2551-2555.

Li, X, Wang, ZG, Chen, HH & Liu, SG 2014, 'The antioxidant methyl 3-(3, 5-di-tert-butyl-4-hydroxyphenyl) propionate', *Acta Crystallographica Section C: Structural Chemistry*, vol. 70, no. 11, pp. 1050-1053.

Liede-Schumann, S, Grimm, GW, Nürk, NM, Potts, AJ, Meve, U & Hartmann, HE 2020, 'Phylogenetic relationships in the southern African genus Drosanthemum (Ruschioideae, Aizoaceae)', *PeerJ*, vol. 8, pp. e8999.

Lien, Y 2018, '豬籠草 Nepenthes miranda 可能的藥性應用', *中山醫學大學生物醫學科學學系學位論文*, pp. 1-111.

Lien, Y 2019, 'Pharmacological activities of Nepenthes miranda extracts', *The FASEB Journal*, vol. 33(1_supplement), pp. 471-12.

Lii, SBW, Wong, C & Mueller, M 2017, ‘Ability of endophytic fungi isolated from Nepenthes ampullaria to degrade polyurethane’, *Malaysian Journal of Microbiology*, vol. 13, no. 3, pp. 172-179.

Likhitwitayawuid, K, Kaewamatawong, R, Ruangrungsi, N & Krungkrai, J 1998, ‘Antimalarial naphthoquinones from Nepenthes thorelii’, *Planta Medica*, vol. 64, no. 03, pp. 237-241.

Ling, YS, Lim, LR, Yong, YS, Tamin, O & Puah, PY 2018, 'MS-based metabolomics revealing Bornean Sinularia sp. extract dysregulated lipids triggering programmed cell death in Hepatocellular carcinoma', *Natural Product Research*, vol. 34, no. 12, 2020/06/17, pp. 1796-1803.

Liu, J, Liu, Y, Wang, Y, Abozeid, A, Zu, YG & Tang, ZH 2017, ‘The integration of GC–MS and LC–MS to assay the metabolomics profiling in Panax ginseng and Panax quinquefolius reveals a tissue-and species-specific connectivity of primary metabolites and ginsenosides accumulation’, *Journal of Pharmaceutical and Biomedical analysi*s, vol. 135, pp. 176-185.

Liu, X & Huang, B 2000, ‘Carbohydrate accumulation in relation to heat stress tolerance in two creeping bentgrass cultivars’, *Journal of the American Society for Horticultural Science*, vol. 125, no. 4, pp. 442-447.

Liu, X, Ma, D, Zhang, Z, Wang, S, Du, S, Deng, X & Yin, L 2019, ‘Plant lipid remodeling in response to abiotic stresses’, *Environmental and Experimental Botany*, vol. 165, pp. 174-184.

Liu, X, Zhou, Y, Xiao, J & Bao, F 2018, 'Effects of Chilling on the Structure, Function and Development of Chloroplasts', *Frontiers in Plant Science*, vol. 9, no. 1715.

Liu, Y, Liu, J, Liu, M, Liu, Y, Strappe, P, Sun, H & Zhou, Z 2020, ‘Comparative non-targeted metabolomic analysis reveals insights into the mechanism of rice yellowing’, *Food Chemistry*, vol. 308, pp. 125621.

López-Gresa, MP, Maltese, F, Bellés, JM, Conejero, V, Kim, HK, Choi, YH & Verpoorte, R 2010, ‘Metabolic response of tomato leaves upon different plant–pathogen interactions’, *Phytochemical Analysis: An International Journal of Plant Chemical and Biochemical Techniques*, vol. 21, no. 1, pp. 89-94.

Lu, L, Huang, M, Huang, Y, Corvini, PFX, Ji, R & Zhao, L 2020, ‘Mn 3 O 4 nanozymes boost endogenous antioxidant metabolites in cucumber (Cucumis sativus) plant and enhance resistance to salinity stress’, *Environmental Science: Nano*.

Luo, Q, Sun, L, Hu, X, & Zhou, R 2014, ‘The variation of root exudates from the hyperaccumulator Sedum alfredii under cadmium stress: metabonomics analysis’, *PLOS One*, vol. 9, no. 12, pp. e115581.

Ma, D, Gandra, SV, Manoharlal, R, La Hovary, C.& XIE, D 2019, ‘Untargeted Metabolomics of Nicotiana tabacum Grown in United States and India Characterizes Association of Plant Metabolomes with Natural Climate and Geography’, *Frontiers in Plant Science*, vol. 10, pp. 1370.

Makita, C, Chimuka, L, Steenkamp, P, Cukrowska, E & Madala, E 2016, ‘Comparative analyses of flavonoid content in Moringa oleifera and Moringa ovalifolia with the aid of UHPLC-qTOF-MS fingerprinting’, *South African Journal of Botany*, vol. 105, pp. 116-122.

Mallet, J, Beltrán, M, Neukirchen, W & Linares, M 2007, 'Natural hybridization in heliconiine butterflies: the species boundary as a continuum', *BMC Evolutionary Biology*, vol. 7, no. 1, pp. 28.

Mandre, M 2002, 'Relationships between Lignin and Nutrients in Picea Abies L. under Alkaline Air Pollution', *Water, Air, and Soil Pollution*, vol. 133, no. 1, 2002/01/01, pp. 363-379.

Mano, Y & Nemoto, K 2012, 'The pathway of auxin biosynthesis in plants', *Journal of Experimental Botany*, vol. 63, no. 8, pp. 2853-2872.

Mansell, G & Suarez, W 2016, 'Nepenthes nebularum, a new species from Mindanao, Philippines', *Carnivorous Plant Newsletter*, vol. 45, no. 4, pp. 132–139.

Marraffa, JM, Holland, MG, Stork, CM, Hoy, CD & Hodgman, MJ 2008, 'Diethylene glycol: widely used solvent presents serious poisoning potential', *The Journal of Emergency Medicine*, vol. 35, no. 4, pp. 401-406.

Marrez, DA, Abdelhamid, AE & Darwesh, OM 2019, 'Eco-friendly cellulose acetate green synthesized silver nano-composite as antibacterial packaging system for food safety', *Food Packaging and Shelf Life*, vol. 20, pp. 100302.

Martinez, V, Mestre, TC, Rubio, F, Girones-Vilaplana, A, Moreno, DA, Mittler, R & Rivero, RM 2016, 'Accumulation of flavonols over hydroxycinnamic acids favors oxidative damage protection under abiotic stress', *Frontiers in Plant Science*, vol. 7, pp. 838.

Martinez-Murcia, AJ, Monera, A, Saavedra, MJ, Oncina, R, Lopez-Alvarez, M, Lara, E & Figueras, MJ 2011, 'Multilocus phylogenetic analysis of the genus Aeromonas', *Systematic and Applied Microbiology*, vol. 34, no.3, pp.189-199

Mason, TL 1987, 'Inactivation of red beet β-glucan synthase by native & oxidized phenolic compounds', *Phytochemistry*, vol. 26, no. 8, pp. 2197-2202.

Matich, EK, Soria, NC, Aga, DS & Atilla-Gokcumen, GE 2019, 'Applications of metabolomics in assessing ecological effects of emerging contaminants and pollutants on plants', *Journal of Hazardous Materials*, vol. 373, pp. 527-535.

Maycock, C, Majapun, R, Khoo, E, Pereira, J, Sugau, J & Burslem, D 2011, *The potential impacts of climate change on the distribution of Nepenthes and dipterocarps of the Trus Madi Forest Reserve*, Conservation of biodiversity in Trus Madi Forest Reserve.

McPherson, S 2009, *Pitcher plants of the Old World. v. 2 v. 2,* Redfern Natural History Productions, Poole.

McPherson, S, Robinson, A & Robinson, A 2011, *New Nepenthes,* Redfern Natural History Productions.

Meimberg, H & Heubl, G 2006, 'Introduction of a nuclear marker for phylogenetic analysis of Nepenthaceae', *Plant Biology*, vol. 8, no. 6, pp.831-840.

Meimberg, H, Thalhammer, S, Brachmann, A & Heubl, G 2006, 'Comparative analysis of a translocated copy of the trnK intron in carnivorous family Nepenthaceae', *Molecular Phylogenetics and Evolution*, vol. 39, no. 2, pp. 478-490.

Meimberg, H, Wistuba, A, Dittrich, P & Heubl, G 2001, 'Molecular phylogeny of Nepenthaceae based on cladistic analysis of plastid trnK intron sequence data', *Plant Biology*, vol. 3, no. 2, pp. 164-175.

Melandri, G, AbdElgawad, H, Riewe, D, Hageman, JA, Asard, H, Beemster, GT, Kadam, N, Jagadish, K, Altmann, T, Ruyter-Spira, C & Bouwmeester, H 2020, 'Biomarkers for grain yield stability in rice under drought stress', *Journal of Experimental Botany*, vol. 71, no. 2, pp. 669-683.

Mendoza, N & Silva, EME 2018, 'Introduction to phytochemicals: secondary metabolites from plants with active principles for pharmacological importance', *Phytochemicals: Source of Antioxidants and Role in Disease Prevention*, pp. 25.

Merbach, MA, Merbach, DJ, Maschwitz, U, Booth, WE, Fiala, B & Zizka, G 2002, 'Mass march of termites into the deadly trap', *Nature*, vol. 415, no. 6867, pp. 36-37.

Merbach, MA, Zizka, G, Fiala, B, Maschwitz, U & Booth, WE 2001, 'Patterns of nectar secretion in five Nepenthes species from Brunei Darussalam, Northwest Borneo, and implications for ant-plant relationships', *Flora*, vol. 196, no. 2, pp. 153-160.

Merbach, MA, Zizka, G, Fiala, B, Merbach, D, Booth, WE & Maschwitz, U 2007, 'Why a carnivorous plant cooperates with an ant-selective defense against pitcher-destroying weevils in the myrmecophytic pitcher plant Nepenthes bicalcarata Hook. F', *Ecotropica*, vol. 13, pp. 45-56.

Merckx, VS, Hendriks, KP, Beentjes, KK, Mennes, CB, Becking, LE, Peijnenburg, KT, Afendy, A, Arumugam, N, de Boer, H, Biun, A & Buang, MM 2015, 'Evolution of endemism on a young tropical mountain', *Nature*, vol. 524, no. 7565, pp. 347-350.

Metcalf, JS & Bruno, M 2016, 'Anatoxin-a(S)', in *Handbook of Cyanobacterial Monitoring and Cyanotoxin Analysis*, pp. 155-159.

Mishra, S, Bhardwaj, M, Mehrotra, S, Chowdhary, AA & Srivastava, V 2020, 'The Contribution of Phytohormones in Plant Thermotolerance', *Heat Stress Tolerance in Plants: Physiological, Molecular and Genetic Perspectives*, pp. 213-238.

Miura, K & Tada, Y 2014, 'Regulation of water, salinity, and cold stress responses by salicylic acid', *Frontiers in Plant Science*, vol. 5, pp. 4.

Molyneux, RJ, Lee, ST, Gardner, DR, Panter, KE & James, LF 2007, 'Phytochemicals: the good, the bad and the ugly?', *Phytochemistry*, vol. 68, no. 22/24, pp. 2973-2985.

Monroy, AF, Sangwan, V & Dhindsa, RS 1998, 'Low temperature signal transduction during cold acclimation: protein phosphatase 2A as an early target for cold-inactivation', *The Plant Journal*, vol. 13, no. 5, pp. 653-660.

Moran, JA 1996, 'Pitcher dimorphism, prey composition and the mechanisms of prey attraction in the pitcher plant Nepenthes rafflesiana in Borneo', *Journal of Ecology*, pp. 515-525.

Moran, JA, Booth, WE & Charles, JK 1999, 'Aspects of Pitcher Morphology and Spectral Characteristics of Six Bornean Nepenthes Pitcher Plant Species: Implications for Prey Capture', *Annals of Botany*, vol. 83, no. 5, pp. 521-528.

Moran, JA, Clarke, C & Gowen, BE 2012, 'The use of light in prey capture by the tropical pitcher plant *Nepenthes aristolochioides'*, *Plant Signaling & Behavior*, vol. 7, no. 8, pp. 957-960.

Moran, JA, Clarke, CM & Hawkins, BJ 2003, 'From carnivore to detritivore? Isotopic evidence for leaf litter utilization by the tropical pitcher plant Nepenthes ampullaria', *International Journal of Plant Sciences*, vol. 164, no. 4, pp. 635-639.

Moran, JA, Gray, LK, Clarke, C & Chin, L 2013, 'Capture mechanism in Palaeotropical pitcher plants (Nepenthaceae) is constrained by climate', *Annals of Botany*, vol. 112, no. 7, pp. 1279-1291.

Moran, JA, Hawkins, BJ, Gowen, BE & Robbins, SL 2010, 'Ion fluxes across the pitcher walls of three Bornean *Nepenthes* pitcher plant species: flux rates and gland distribution patterns reflect nitrogen sequestration strategies', *Journal of Experimental Botany*, vol. 61, no. 5, pp. 1365-1374.

Moran, JA, Merbach, MA, Livingston, NJ, Clarke, CM & Booth, WE 2001, 'Termite Prey Specialization in the Pitcher Plant *Nepenthes albomarginata* —Evidence from Stable Isotope Analysis', *Annals of Botany*, vol. 88, no. 2, pp. 307-311.

Morrison, BA & Shain, DH 2008, 'An AMP nucleosidase gene knockout in *Escherichia coli* elevates intracellular ATP levels and increases cold tolerance', *Biology Letters*, vol. 4, no. 1, pp. 53-56.

Mueller, L & Boehm, V 2011, 'Antioxidant Activity of β-Carotene Compounds in Different in Vitro Assays', *Molecules*, vol. 16, no. 2, pp. 1055-1069.

Mulani, MS, Kamble, EE, Kumkar, SN, Tawre, MS & Pardesi, KR 2019, 'Emerging strategies to combat ESKAPE pathogens in the era of antimicrobial resistance: a review', *Frontiers in Microbiology,* vol. 10, no. 539.

Müller, L, Goupy, P, Fröhlich, K, Dangles, O, Caris-Veyrat, C & Böhm, V 2011, 'Comparative Study on Antioxidant Activity of Lycopene (Z)-Isomers in Different Assays', *Journal of Agricultural and Food Chemistry*, vol. 59, no. 9, 2011/05/11, pp. 4504-4511.

Mullins, JT 2000, Molecular systematics of *Nepenthaceae* (Doctoral dissertation, University of Reading)

Murphy, B, Forest, F, Barraclough, T, Rosindell, J, Bellot, S, Cowan, R, Golos, M, Jebb, M & Cheek, M 2020, 'A phylogenomic analysis of *Nepenthes* (*Nepenthaceae*)', *Molecular Phylogenetics and Evolution*, vol. 144, pp. 106668.

Murray, PR, Baron, EJ, Pfaller, MA, Tenover, FC, Yolk, RH 1995, *Manual of Clinical Microbiology*, 6th ed. American Society for Microbiology, Washington, D.C.

Musatenko, LI, Vedenicheva, NP, Vasyuk, VA, Generalova, VN, Martyn, GI & Sytnik, KM 2003, 'Phytohormones in seedlings of maize hybrids differing in their tolerance to high temperatures', *Russian Journal of Plant Physiology*, vol. 50, no. 4, pp. 444-448.

Nagler, M, Nägele, T, Gilli, C, Fragner, L, Korte, A, Platzer, A, Farlow, A, Nordborg, M & Weckwerth, W 2018, 'Eco-metabolomics and metabolic modeling: Making the leap from model systems in the lab to native populations in the field', *Frontiers in Plant Science*, vol. 9, pp. 1556.

Nakayama, S & Amagase, S 1968, 'Acid Protease in Nepenthes', *Proceedings of the Japan Academy*, vol. 44, no. 5, pp. 358-362.

Napolitano, MJ & Shain, DH 2005, 'Distinctions in adenylate metabolism among organisms inhabiting temperature extremes', *Extremophiles*, vol. 9, no. 2, pp. 93-98.

Napolitano, MJ, Nagele, RG & Shain, DH 2004, 'The ice worm, Mesenchytraeus solifugus, elevates adenylate levels at low physiological temperature', *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, vol. 137, no. 1, 2004/01/01/, pp. 227-235.

Naseem, F & Kayang, H 2018, 'Fungal endophytes associated with nepenthes khasiana hook. f., An endemic plant of meghalaya, India', *International Journal of Current Research in Life Sciences*, vol. 7, no. 04, pp. 1907-1912.

Nauheimer, L, Cui, L, Clarke, C, Crayn, DM, Bourke, G & Nargar, K 2019, 'Genome skimming provides well resolved plastid and nuclear phylogenies, showing patterns of deep reticulate evolution in the tropical carnivorous plant genus *Nepenthes* (*Caryophyllales*)', *Australian Systematic Botany*, vol. 32, no. 3, pp. 243-254.

Nayyar, H, Bains, TS, Kumar, S & Kaur, G 2005, 'Chilling effects during seed filling on accumulation of seed reserves and yield of chickpea', *Journal of the Science of Food and Agriculture*', vol. 85, no. 11, pp. 1925-1930.

Negi, NP, Shrivastava, DC, Sharma, V & Sarin, NB 2015, 'Overexpression of CuZnSOD from Arachis hypogaea alleviates salinity and drought stress in tobacco', *Plant Cell Reports*, vol. 34, no. 7, pp. 1109-1126.

Nicholson, JK & Lindon, JC 2008, 'Metabonomics', *Nature*, vol. 455, no. 7216, pp. 1054-1056.

Nicolis, E, Lampronti, I, Dechecchi, MC, Borgatti, M, Tamanini, A, Bianchi, N, Bezzerri, V, Mancini, I, Giri, MG, Rizzotti, P & Gambari, R 2008, 'Pyrogallol, an active compound from the medicinal plant Emblica officinalis, regulates expression of pro-inflammatory genes in bronchial epithelial cells', *International Immunopharmacology*, vol. 8, no. 12, pp. 1672-1680.

Nolan, MJ & Cribb, TH, 2005, 'The use and implications of ribosomal DNA sequencing for the discrimination of digenean species', *Advances in Parasitology*, vol. 60, pp. 101-163.

Normand, S, Treier, UA, Randin, C, Vittoz, P, Guisan, A & Svenning, JC 2009, 'Importance of abiotic stress as a range-limit determinant for European plants: insights from species responses to climatic gradients', *Global Ecology and Biogeography*, vol. 18, no. 4, pp. 437-449.

Núñez, N, Vidal-Casanella, O, Sentellas, S, Saurina, J & Núñez, O 2020, 'Non-Targeted Ultra-High Performance Liquid Chromatography-High-Resolution Mass Spectrometry (UHPLC-HRMS) Fingerprints for the Chemometric Characterization and Classification of Turmeric and Curry Samples', *Separations*, no. 7, no. 2, pp. 32.

O'Neill, J 2014, *Antimicrobial resistance: tackling a crisis for the health and wealth of nations. Review on antimicrobial resistance*, Review on Antimicrobial Resistance, London, United Kingdom.

Okumoto, S, Funck, D, Trovato, M & Forlani, G 2016, 'Editorial: Amino Acids of the Glutamate Family: Functions beyond Primary Metabolism', *Frontiers in Plant Science*, vol. 7, no. 318.

Oliveira, DM, Mota, TR, Salatta, FV, Sinzker, RC, Končitíková, R, Kopečný, D, Simister, R, Silva, M, Goeminne, G, Morreel, K & Rencoret, J 2020, 'Cell wall remodeling under salt stress: Insights into changes in polysaccharides, feruloylation, lignification, and phenolic metabolism in maize', *Plant, Cell & Environment*.

Oliveros, JC 2007, 'VENNY. An interactive tool for comparing lists with Venn Diagrams. https://bioinfogp.cnb.csic.es/tools/venny/index.html',

Osunkoya, OO, Daud, SD & Wimmer, FL 2008, 'Longevity, lignin content and construction cost of the assimilatory organs of *Nepenthes* species', *Annals of Botany*, vol. 102, no. 5, pp. 845-853.

Othman, AR, Abdullah, N, Ahmad, S, Ismail, IS & Zakaria, MP 2015, 'Elucidation of in-vitro anti-inflammatory bioactive compounds isolated from *Jatropha curcas* L. plant root', *BMC Complementary and Alternative Medicine*, vol. 15, no. 1, pp. 11.

Ou-Yang, F, Tsai, I, Tang, JY, Yen, CY, Cheng, YB, Farooqi, AA, Chen, SR, Yu, SY, Kao, JK & Chang, HW 2019, 'Antiproliferation for Breast Cancer Cells by Ethyl Acetate Extract of *Nepenthes thorellii* x (*ventricosa* x *maxima*)', *International Journal of Molecular Sciences*, vol. 20, no. 13, pp. 3238.

Owen Jr, TP & Lennon, KA 1999, 'Structure and development of the pitchers from the carnivorous plant *Nepenthes alata* (*Nepenthaceae*)', *American Journal of Botany*, vol. 86, no. 10, pp. 1382-1390.

Owen Jr., TP, Lennon, KA, Santo, MJ & Anderson, AN 1999, 'Pathways for Nutrient Transport in the Pitchers of the Carnivorous Plant *Nepenthes alata*', *Annals of Botany*, vol. 84, no. 4, pp. 459-466.

Pabbathi, NPP, Nathani, NM, Gadhvi, IR & Chandrashekar, M 2020, *Environmental Metabolomics: With the Perspective of Marine Toxicology Assessment*. In Environmental Biotechnology Vol. 1 (pp. 197-225). Springer, Cham.

Pandey, DK, Chaudhary, R, Dey, A, Nandy, S, Banik, RM, Malik, T & Dwivedi, P 2020, *Current Knowledge of Cinnamomum Species: A Review on the Bioactive Components, Pharmacological Properties, Analytical and Biotechnological Studies*. In Bioactive Natural products in Drug Discovery (pp. 127-164). Springer, Singapore.

Pareek, A, Khurana, A, K Sharma, A & Kumar, R 2017, 'An overview of signaling regulons during cold stress tolerance in plants', *Current Genomics*, vol. 18, no. 6, pp. 498-511.

Park, SE, Seo, SH, Kim, EJ, Park, DH, Park, KM, Cho, SS & Son, HS 2019, 'Metabolomic Approach for Discrimination of Cultivation Age and Ripening Stage in Ginseng Berry Using Gas Chromatography-Mass Spectrometry', *Molecules*, vol. 24, no. 21, pp. 3837.

Paul, MJ & Foyer, CH 2001, 'Sink regulation of photosynthesis', *Journal of Experimental Botany*, vol. 52, no. 360, pp. 1383-1400.

Pauling, L, Robinson, AB, Teranishi, R & Cary, P 1971, 'Quantitative analysis of urine vapor and breath by gas-liquid partition chromatography', *Proceedings of the National Academy of Sciences*, vol. 68, no. 10, pp. 2374-2376.

Paupière, MJ, Müller, F, Li, H, Rieu, I, Tikunov, YM, Visser, RG & Bovy, AG 2017, 'Untargeted metabolomic analysis of tomato pollen development and heat stress response', *Plant Reproduction*, vol. 30, no. 2, pp. 81-94.

Pavlovič, A 2012, 'Adaptive radiation with regard to nutrient sequestration strategies in the carnivorous plants of the genus Nepenthes', *Plant Signaling & Behavior*, vol. 7, no. 2, pp. 295-297.

Pavlovič, A, Slováková, Ľ & Šantrůček, J 2011, 'Nutritional benefit from leaf litter utilization in the pitcher plant Nepenthes ampullaria', *Plant, Cell & Environment*, vol. 34, no. 11, pp. 1865-1873.

Penuelas, J & Sardans, J 2009, 'Ecological metabolomics', *Chemistry and Ecology*, vol. 25, no. 4, pp. 305-309.

Perras, M & Sarhan, F 1984, 'Energy state of spring and winter wheat during cold hardening. Soluble sugars and adenine nucleotides', *Physiologia Plantarum*, vol. 60, no. 2, pp. 129-132.

Perry LM & Metzger, J 1980, *Medicinal plants of East and Southeast Asia: Attributed Properties and Uses*. Cambridge: The MIT Press.

Peters, K, Worrich, A, Weinhold, A, Alka, O, Balcke, G, Birkemeyer, C, Bruelheide, H, Calf, OW, Dietz, S, Dührkop, K & Gaquerel, E 2018, 'Current challenges in plant eco-metabolomics', *International Journal of Molecular Sciences*, vol. 19, no. 5, pp. 1385.

Phillipps, A & Lamb, A 1988, 'Pitcher-plants of east Malaysia and Brunei', *Nature Malaysiana*, vol. 13, no. 4, pp. 8-27.

Pluskal, T, Castillo, S, Villar-Briones, A & Orešič, M 2010, 'MZmine 2: Modular framework for processing, visualizing, and analyzing mass spectrometry-based molecular profile data', *BMC Bioinformatics*, vol. 11, no. 1, p. 395.

Poma, P, Labbozzetta, M, Zito, P, Alduina, R, Ramarosandratana, AV, Bruno, M, Rosselli, S, Sajeva, M & Notarbartolo, M 2019, 'Essential oil composition of Alluaudia procera and in vitro biological activity on two drug-resistant models', *Molecules*, vol. 24, no. 16, p.2871.

Pujos-Guillot, E, Hubert, J, Martin, JF, Lyan, B, Quintana, M, Claude, S, Chabanas, B, Rothwell, JA, Bennetau-Pelissero, C, Scalbert, A & Comte, B 2013, 'Mass spectrometry-based metabolomics for the discovery of biomarkers of fruit and vegetable intake: citrus fruit as a case study', *Journal of Proteome Research*, vol. 12, no. 4, pp. 1645-1659.

Qi, H, Siu, SO, Chen, Y, Han, Y, Chu, IK, Tong, Y, Lau, ASY & Rong, J 2010, 'Senkyunolides reduce hydrogen peroxide-induced oxidative damage in human liver HepG2 cells via induction of heme oxygenase-1', *Chemico-Biological Interactions*, vol. 183, no. 3, 2010/02/12/, pp. 380-389.

Ragasa, CY, Tsai, PW & Shen, CC 2009, 'Antimicrobial terpenoids from Erigeron sumatrensis', *NRCP Research Journal*, vol. 10, no. 1, pp. 27-32.

Raguso, RA 2001, *Floral scent, olfaction, & scent driven foraging behavior*, Pp. 83-105 in L. Chittka & J.D. Thomson (eds.), Cognitive ecology of pollination; Animal behavior and floral evolution. Cambridge University Press, Cambridge.

Rahman, A 2013, 'Auxin: a regulator of cold stress response', *Physiologia Plantarum*, vol. 147, no. 1, pp. 28-35.

Raj, G, Kurup, R, Hussain, AA & Baby, S 2011, 'Distribution of naphthoquinones, plumbagin, droserone, and 5-O-methyl droserone in chitin-induced and uninduced Nepenthes khasiana: molecular events in prey capture', *Journal of Experimental Botany*, vol. 62, no. 15, pp. 5429-5436.

Rajaniemi, P, Komárek, J, Willame, R, Hrouzek, P, Kaštovská, K, Hoffmann, L & Sivonen, K 2005, 'Taxonomic consequences from the combined molecular and phenotype evaluation of selected Anabaena and Aphanizomenon strains', *Algological Studies*, vol. 117, no. 1, pp. 371-391.

Raman, BV, Samuel, LA, Saradhi, MP, Rao, BN, Krishna, NV, Sudhakar, M & Radhakrishnan, TM 2012, 'Antibacterial, antioxidant activity and GC-MS analysis of Eupatorium odoratum', *Asian Journal of Pharmaceutical and Clinical Research*, vol. 5, no. 2, pp. 99-106.

Ramashankar, Deb S & Sharma, BK 2015, 'Traditional healing practices in North East India', *Indian Journal of History of Science*, vol. 50, no. 2, pp. 324–332.

Rampino, P, Mita, G, Pataleo, S, De Pascali, M, Di Fonzo, N & Perrotta, C 2009, 'Acquisition of thermotolerance and HSP gene expression in durum wheat (Triticum durum Desf.) cultivars', *Environmental and Experimental Botany*, vol. 66, no. 2, pp. 257-264.

Rao, RR 1981, 'Ethnobotanical studies on the flora of Meghalaya-Some interesting reports of herbal medicines', *Glimpses of Indian Ethnobotany*, pp. 137-148.

Ravi, L & Krishnan, K 2017, 'Cytotoxic potential of N-hexadecanoic acid extracted from Kigelia pinnata leaves', *Asian Journal of Cell Biology*, vol. 12, pp. 20-27.

Renaut, J, Hausman, JF & Wisniewski, ME 2006, 'Proteomics and low‐temperature studies: bridging the gap between gene expression and metabolism', *Physiologia Plantarum*, vol. 126, no. 1, pp. 97-109.

Renaut, J, Lutts, S, Hoffmann, L & Hausman, JF 2004, 'Responses of poplar to chilling temperatures: proteomic and physiological aspects', *Plant Biology*, vol. 7, no. 01, pp. 81-90.

Renner, T & Specht, CD 2011, 'A sticky situation: assessing adaptations for plant carnivory in the Caryophyllales by means of stochastic character mapping', *International Journal of Plant Sciences*, vol. 172, no. 7, pp. 889-901.

Rezasoltani, S, Bashirzadeh, DA, Mojarad, EN, Aghdaei, HA, Norouzinia, M & Shahrokh, S 2020, 'Signature of Gut Microbiome by Conventional and Advanced Analysis Techniques: Advantages and Disadvantages', *Middle East Journal of Digestive Diseases*, vol. 12, no. 1, p. 5.

Rivas-Ubach, A, Peñuelas, J, Hódar, JA, Oravec, M, Paša-Tolić, L, Urban, O & Sardans, J 2019, 'We Are What We Eat: A Stoichiometric and Ecometabolomic Study of Caterpillars

Feeding on Two Pine Subspecies of Pinus sylvestris,' *International journal of Molecular Sciences*, vol. 20, no. 1, p. 59.

Rivas-Ubach, A, Pérez-Trujillo, M, Sardans, J, Gargallo-Garriga, A, Parella, T & Penuelas, J 2013, 'Ecometabolomics: optimized NMR-based method', *Methods in Ecology and Evolution*, vol. 4, no. 5, pp.464-473.

Rivas-Ubach, A, Sardans, J, Pérez-Trujillo, M, Estiarte, M & Peñuelas, J 2012, 'Strong relationship between elemental stoichiometry and metabolome in plants', *Proceedings of the National Academy of Sciences*, vol. 109, no. 11, pp. 4181-4186.

Rivero, RM, Ruiz, JM, Garcıa, PC, Lopez-Lefebre, LR, Sánchez, E & Romero, L 2001, 'Resistance to cold and heat stress: accumulation of phenolic compounds in tomato and watermelon plants', *Plant Science*, vol. 160, no. 2, pp. 315-321.

Rizzo, WB 2014, 'Fatty aldehyde and fatty alcohol metabolism: review and importance for epidermal structure and function', *Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids*, vol. 1841, no. 3, pp. 377-389.

Roger, F, Marchandin, H, Jumas-Bilak, E, Kodjo, A, Lamy, B & colBVH Study Group 2012, 'Multilocus genetics to reconstruct aeromonad evolution', *BMC Microbiology*, vol. 12, no. 1, p. 62.

Rosli, MAF, Azizan, KA & Goh, H 2018, 'Antioxidant Activity of Pitcher Extracts from Three *Nepenthes* Species', *Sains Malaysiana*, vol. 47, no. 12, pp. 3069-3075.

Rossoni, AW, Schönknecht, G, Lee, HJ, Rupp, RL, Flachbart, S, Mettler-Altmann, T, Weber, AP & Eisenhut, M 2019, 'Cold Acclimation of the Thermoacidophilic Red Alga Galdieria sulphuraria: Changes in Gene Expression and Involvement of Horizontally Acquired Genes', *Plant and Cell Physiology*, vol. 60, no. 3, pp. 702-712.

Rottloff, S, Miguel, S, Biteau, F, Nisse, E, Hammann, P, Kuhn, L, Chicher, J, Bazile, V, Gaume, L, Mignard, B & Hehn, A 2016, 'Proteome analysis of digestive fluids in Nepenthes pitchers', *Annals of botan*y, vol. 117, no. 3, pp. 479-495.

Ruan, J, Zhou, Y, Zhou, M, Yan, J, Khurshid, M, Weng, W, Cheng, J & Zhang, K 2019, 'Jasmonic Acid Signaling Pathway in Plants', *International Journal of Molecular Sciences*, vol. 20, no. 10, p. 2479.

Ruhsam, M, Rai, HS, Mathews, S, Ross, TG, Graham, SW, Raubeson, LA, Mei, W, Thomas, PI, Gardner, MF, Ennos, RA & Hollingsworth, PM 2015, 'Does complete plastid genome sequencing improve species discrimination and phylogenetic resolution in Araucaria?', *Molecular Ecology Resources*, vol. 15, no. 5, pp. 1067-1078.

Ruttkies, C, Schymanski, EL, Wolf, S, Hollender, J & Neumann, S 2016, 'MetFrag relaunched: incorporating strategies beyond in silico fragmentation', *Journal of Cheminformatics*, vol. 8, no. 1, 2016/01/29, p. 3.

Sabran, SF, Mohamed, M, Bakar, A & Fadzelly, M 2016, *Ethnomedical knowledge of plants used for the treatment of tuberculosis in Johor, Malaysia*, Evidence-Based Complementary and Alternative Medicine, 2016.

Sadiq, Y, Zaid, A & Khan, MMA 2020, *Adaptive Physiological Responses of Plants under Abiotic Stresses: Role of Phytohormones*, In Plant Ecophysiology and Adaptation under Climate Change: Mechanisms and Perspectives I (pp. 797-824). Springer, Singapore.

Saeed, NM, El-Demerdash, E, Abdel-Rahman, HM, Algandaby, MM, Al-Abbasi, FA & Abdel-Naim, AB 2012, 'Anti-inflammatory activity of methyl palmitate and ethyl palmitate in different experimental rat models', *Toxicology and Applied Pharmacology*, vol. 264, no. 1, pp. 84-93.

Saganová, M, Bokor, B, Stolárik, T & Pavlovič, A 2018, 'Regulation of enzyme activities in carnivorous pitcher plants of the genus Nepenthes', *Planta*, vol. 248, no. 2, pp. 451-464.

Saini, R, Adhikary, A, Nayyar, H & Kumar, S 2019, 'Cross-priming accentuates key biochemical and molecular indicators of defense and improves cold tolerance in chickpea (Cicer arietinum L.)', *Acta Physiologiae Plantarum*, vol. 41, no. 11, pp. 181.

Sakamoto, A & Murata, N 2002, 'The role of glycine betaine in the protection of plants from stress: clues from transgenic plants', *Plant, Cell & Environment*, vol. 25, no. 2, pp.163-171.

Samanta, A, Das, G & Das, S 2011, 'Roles of flavonoids in Plants', *International Journal of Pharmaceutical Science and Technology*, vol. 6, 01/01, pp. 12-35.

Sanusi, S, Abu Bakar, MF, Mohamed, M, Sabran, SF & Mainasara, M 2017, 'Ethnobotanical, phytochemical, and pharmacological properties of nepenthes species: A review', *Asian Journal of Pharmaceutical and Clinical Research*, vol. 10, 11/01, pp. 16-19.

Sanusi, SB, Bakar, MFA, Mohamed, M & Sabran, SF 2018, 'Preliminary Phytochemical and Antimycobacterial Investigation of Some Selected Medicinal Plants of Endau Rompin, Johor, Malaysia', *Journal of Science and Technology*, vol. 10, no. 2, pp. 30-37.

Sarabia, LD, Hill, CB, Boughton, BA & Roessner, U 2018, 'Advances of metabolite profiling of plants in challenging environments', *Annual Plant Reviews Online*, pp. 1-45.

Sardans, J, Penuelas, J & Rivas-Ubach, A 2011, 'Ecological metabolomics: overview of current developments and future challenges', *Chemoecology*, vol. 21, no. 4, pp. 191-225.

Schep, LJ, Slaughter, RJ, Temple, WA & Beasley, DMG 2009, 'Diethylene glycol poisoning', *Clinical Toxicology*, vol. 47, no. 6, pp. 525-535.

Schulze, W, Frommer, WB & Ward, JM 1999, 'Transporters for ammonium, amino acids and peptides are expressed in pitchers of the carnivorous plant Nepenthes', *The Plant Journal*, vol. 17, no. 6, pp. 637-646.

Schwallier, R, de Boer, HJ, Visser, N, van Vugt, RR & Gravendeel, B 2015, 'Traps as treats: a traditional sticky rice snack persisting in rapidly changing Asian kitchens', *Journal of Ethnobiology and Ethnomedicine*, vol. 11, no. 1, pp. 24.

Schwallier, R, Raes, N, de Boer, HJ, Vos, RA, van Vugt, RR & Gravendeel, B 2016, 'Phylogenetic analysis of niche divergence reveals distinct evolutionary histories and climate change implications for tropical carnivorous pitcher plants', *Diversity and Distributions*, vol. 22, no. 1, pp. 97-110.

Seine, R, Porembski, S & Barthlott, W 1996, 'A neglected habitat of carnivorous plants: Inselbergs', *Feddes Repertorium*, vol. 106, no. 5-8, pp. 555-562.

Senevirathna, RWKM, Seneviratne, VN & Rajapakse, S 2019, 'Chitinases from pitcher fluid of Nepenthes distillatoria', *Ceylon Journal of Science*, vol. 48, no. 3, pp. 243-249.

Setiawan, H, Hakim, L & Batoro, J 2015, 'Ethnobotany of Nepenthes spp. in Dayak Seberuang People, West Kalimantan, Indonesia', *Journal of Biodiversity and Environmental Sciences*, vol. 7, no. 6, pp. 275-284.

Sevenster, JG 1996, 'Aggregation and coexistence. I. Theory and analysis', *Journal of Animal Ecology*, pp. 297-307.

Sharma, A, Kumar, V, Shahzad, B, Ramakrishnan, M, Sidhu, GPS, Bali, AS, Handa, N, Kapoor, D, Yadav, P, Khanna, K & Bakshi, P 2019, 'Photosynthetic response of plants under different abiotic stresses: a review', *Journal of Plant Growth Regulation*, pp. 1-23.

Sharma, M & Laxmi, A 2016, 'Jasmonates: Emerging Players in Controlling Temperature Stress Tolerance', *Frontiers in Plant Science*, vol. 6, no. 1129, 2016-January-06

Sharma, P, Jha, AB, Dubey, RS & Pessarakli, M 2012, 'Reactive Oxygen Species, Oxidative Damage, and Antioxidative Defense Mechanism in Plants under Stressful Conditions', *Journal of Botany*, vol. 2012, 2012/04/24, p. 217037.

Shi, H, Ye, T, Song, B, Qi, X & Chan, Z 2015, 'Comparative physiological and metabolomic responses of four Brachypodium distachyon varieties contrasting in drought stress resistance', *Acta Physiologiae Plantarum*, vol. 37, no. 6, 2015/06/04, p. 122.

Shi, L, Wang, R, Liu, T, Wu, J, Zhang, H, Liu, Z, Liu, S & Liu, Z 2020, 'A rapid protocol to distinguish between Citri Exocarpium Rubrum and Citri Reticulatae Pericarpium based on the characteristic fingerprint and UHPLC-Q-TOF MS methods', *Food & Function*, vol. 11, no. 4, pp. 3719-3729.

Shil, D, Mohanty, JP, Das, T, Bhuyan, NR, Uriah, T & Saleem, TM 2010, 'Protective Role of Pitcher of Nepenthes khasiana Hook against Dexametha-zone Induced Hyperlipidemia and Insulin Resistance in Rats', *International Journal of Research in Pharmaceutical Sciences*, vol. 1, no. 2.

Shil, D, Mohanty, JP, Saleem, TM, Debnath, J & Uriah, T 2014, 'Anti-diabetic activity of leaf extract of Nepenthes khasiana hook on dexamethasone induced diabetic rats', *Journal of Pharmaceutical, Chemical and Biological Sciences*, vol. 1, no. 1, pp. 6-11.

Shin, KS, Lee, S & Cha, BJ, 2007a, 'Suppression of phytopathogenic fungi by hexane extract of Nepenthes ventricosa x maxima leaf', *Fitoterapia*, vol. 78, no. 7/8, pp. 585-586.

Shin, KS, Lee, SK & Cha, BJ 2007b, 'Antifungal activity of plumbagin purified from leaves of Nepenthes ventricosa x maxima against phytopathogenic fungi', *The Plant Pathology Journal*, vol. 23, no. 2, pp. 113-115.

Shinozaki, K & Dennis, ES 2003, 'Cell signalling and gene regulation: global analyses of signal transduction and gene expression profiles', *Current Opinion in Plant Biology*, vol. 6, no. 5, pp. 405-409.

Shivas, RG and Brown, JF 1989, 'Yeasts associated with fluid in pitchers of Nepenthes', *Mycological Research*, vol. 93, no. 1, pp. 96-100.

Shulaev, V, Cortes, D, Miller, G & Mittler, R 2008, 'Metabolomics for plant stress response', *Physiologia Plantarum*, vol. 132, no. 2, pp. 199-208.

Sickel, W, Grafe, TU, Meuche, I Steffan-Dewenter, I & Keller, A 2016, 'Bacterial diversity and community structure in two Bornean Nepenthes species with differences in nitrogen acquisition strategies', *Microbial Ecology*, vol. 71, no. 4, pp. 938-953.

Sinaga, E, Tobing, IS & Pravita, RV 2016, *Pemanfaatan Tumbuhan Obat oleh Suku Dayak Iban di Desa Meliau Kalimantan Barat*, Global Science Publishing House, Jakarta.

Singh, B, Borthakur, SK & Phukan, SJ 2014, 'A survey of ethnomedicinal plants utilized by the indigenous people of Garo Hills with special reference to the Nokrek Biosphere Reserve (Meghalaya), India', *Journal of Herbs, Spices & Medicinal plants*, vol. 20, no. 1, pp. 1-30.

Singh, G & Kumar, P 2013, 'Extraction, gas chromatography–mass spectrometry analysis and screening of fruits of Terminalia chebula Retz. for its antimicrobial potential', *Pharmacognosy Research*, vol. 5, no. 3, 162.

Slack, A 2000, *Carnivorous plants*, MIT Press.

Sobczyk, EA & Kacperska-Palacz, A 1978, 'Adenine Nucleotide Changes during Cold Acclimation of Winter Rape Plants', *Plant Physiology*, vol. 62, no. 6, pp. 875-878.

Song, T, Xu, H, Sun, N, Jiang, L, Tian, P, Yong, Y, Yang, W, Cai, H & Cui, G, 2017, 'Metabolomic analysis of alfalfa (Medicago sativa L.) root-symbiotic rhizobia responses under alkali stress', *Frontiers in Plant Science*, vol. 8, pp. 1208.

Song, YW, Lim, Y, & Cho, SK 2018, '2, 4-Di-tert-butylphenol, a potential HDAC6 inhibitor, induces senescence and mitotic catastrophe in human gastric adenocarcinoma AGS cells', *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research*, vol. 1865, no. 5, pp. 675-683.

Song, YW, Shrestha, S, Gyawali, R, Lee, DS & Cho, SK 2015, 'Citrus unshiu leaf extract containing phytol as a major compound induces autophagic cell death in human gastric adenocarcinoma AGS cells', *Journal of the Korean Society for Applied Biological Chemistry*, vol. 58, no. 2, pp. 257-265.

Souri, Z, Karimi, N, Farooq, MA & Akhtar, J 2020, 'Chapter 16 - Phytohormonal signaling under abiotic stress', in DK Tripathi, V Pratap Singh, DK Chauhan, S Sharma, SM Prasad, NK Dubey and N Ramawat (eds), *Plant Life Under Changing Environment*, Academic Press, pp. 397-466.

Sridevi, V & Giridhar, P 2013, 'Influence of altitude variation on trigonelline content during ontogeny of Coffea canephora fruit', *Journal of Food Studies*, vol. 2, no. 1, pp. 62-72.

Staswick, PE & Tiryaki, I 2004, 'The Oxylipin Signal Jasmonic Acid Is Activated by an Enzyme That Conjugates It to Isoleucine in Arabidopsis', *The Plant Cell*, vol. 16, no. 8, pp. 2117-2127.

Steinfath, M, Strehmel, N, Peters, R, Schauer, N, Groth, D, Hummel, J, Steup, M, Selbig, J, Kopka, J, Geigenberger, P & Van Dongen, JT, 2010, 'Discovering plant metabolic biomarkers for phenotype prediction using an untargeted approach', *Plant Biotechnology Journal*, vol. 8, no. 8, pp. 900-911.

Stephenson, P & Hogan, J 2006, ‘Cloning and characterization of a ribonuclease, a cysteine proteinase, and an aspartic proteinase from pitchers of the carnivorous plant Nepenthes ventricosa Blanco’, *International Journal of Plant Sciences*, vol. 167, no. 2, pp. 239-248.

Stuart, BL, Inger, RF & Voris, HK 2006, ‘High level of cryptic species diversity revealed by sympatric lineages of Southeast Asian forest frogs’, *Biology Letters*, vol. 2, no. 3, pp. 470-474.

Sudhir, K 2002, *The medicinal plants of North-East India*. Scientific Publishers, India.

Sweeney, BW, Battle, JM, Jackson, JK & Dapkey, T 2011, ‘Can DNA barcodes of stream macroinvertebrates improve descriptions of community structure and water quality?’, *Journal of the North American Benthological Society*, vol. 30, no. 1, pp. 195-216.

Tacconelli, E, Carrara, E, Savoldi, A, Harbarth, S, Mendelson, M, Monnet, DL, Pulcini, C, Kahlmeter, G, Kluytmans, J, Carmeli, Y and Ouellette, M 2018, ‘Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis’, *The Lancet Infectious Diseases*, vol. 18, no. 3, pp. 318-327.

Takahashi, H, Kosaka, M, Watanabe, Y, Nakade, K & Fukuyama, Y 2003, 'Synthesis and neuroprotective activity of bergenin derivatives with antioxidant activity', *Bioorganic & Medicinal Chemistry*, vol. 11, no. 8, pp. 1781-1788.

Takahashi, K, Athauda, SB, Matsumoto, K, Rajapakshe, S, Kuribayashi, M, Kojima, M, Kubomura-Yoshida, N, Iwamatsu, A, Shibata, C & Inoue, H 2005, ‘Nepenthesin, a unique member of a novel subfamily of aspartic proteinases: enzymatic and structural characteristics’, *Current Protein and Peptide Science*, vol. 6, no. 6, pp. 513-525.

Takeuchi, Y, Chaffron, S, Salcher, MM, Shimizu-Inatsugi, R, Kobayashi, MJ, Diway, B, von Mering, C, Pernthaler, J & Shimizu, KK, 2015, ‘Bacterial diversity and composition in the fluid of pitcher plants of the genus Nepenthes’, *Systematic and Applied Microbiology*, vol. 38, no. 5, pp. 330-339.

Tang, JY, Li, LJ, Ou-Yang, F, Wang, CL, Shu, CW, Wu, KH, Wang, HR, Yen, CH, Cheng, YB & Chang, HW 2019a, ‘Ethyl Acetate Extract of *Nepenthes ventricosa* x *maxima* Exerts Preferential Killing to Oral Cancer Cells’, *DNA and Cell Biology*, vol. 38, no. 8, pp. 763-772.

Tang, JY, Peng, SY, Cheng, YB, Wang, CL, Farooqi, AA, Yu, TJ, Hou, MF, Wang, SC, Yen, CH, Chan, LP & Ou‐Yang, F 2019b, Ethyl acetate extract of *Nepenthes adrianii* x *clipeata* induces antiproliferation, apoptosis, and DNA damage against oral cancer cells through oxidative stress. *Environmental Toxicology*, 34(8), pp.891-901.

Tangmouo, JG, Ho, R, Lannang, AM, Komguem, J, Lontsi, AT, Lontsi, D & Hostettmann, K 2009, 'Norbergenin derivatives from the stem bark of Diospyros sanza-minika (Ebenaceae) and their radical scavenging activity', *Phytochemistry Letters*, vol. 2, no. 4, 2009/11/19/, pp. 192-195.

Terzi, R, Saruhan Güler, N, çalişkan, N & Kadioğlu, A 2013, 'Lignification response for rolled leaves of Ctenanthe setosa under long-term drought stress', *Turkish Journal of Biology*, vol. 37, pp. 614-619.

Thakur, P & Nayyar, H 2013, *Facing the cold stress by plants in the changing environment: sensing, signaling, and defending mechanisms.* In Plant Acclimation to Environmental Stress (pp. 29-69). Springer, New York, NY.

Thakur, P, Kumar, S, Malik, JA, Berger, JD & Nayyar, H 2010, 'Cold stress effects on reproductive development in grain crops: an overview', *Environmental and Experimental Botany*, vol. 67, no. 3, pp. 429-443.

Thao, NP, Luyen, BTT, Koo, JE, Kim, S, Koh, YS, Thanh, NV, Cuong, NX, Kiem, PV, Minh, CV & Kim, YH 2016, 'In vitro anti-inflammatory components isolated from the carnivorous plant Nepenthes mirabilis (Lour.) Rafarin', *Pharmaceutical Biology*, vol. 54, no. 4, pp. 588-594.

Thornham, DG, Smith, JM, Ulmar Grafe, T & Federle, W 2012, 'Setting the trap: cleaning behaviour of Camponotus schmitzi ants increases long-term capture efficiency of their pitcher plant host, Nepenthes bicalcarata', Functional Ecology, vol. 26, no. 1, pp.11-19.

Thornhill, AH, Harper, IS & Hallam, ND 2008, 'The development of the digestive glands and enzymes in the pitchers of three Nepenthes species: N. alata, N. tobaica, and N. ventricosa (Nepenthaceae)', *International Journal of Plant Sciences*, vol. 169, no. 5, pp. 615-624.

Thorogood, CJ, Bauer, U & Hiscock, SJ 2018, 'Convergent and divergent evolution in carnivorous pitcher plant traps', *New Phytologist*, vol. 217, no. 3, pp. 1035-1041.

Timperio, AM, Egidi, MG & Zolla, L 2008, 'Proteomics applied on plant abiotic stresses: role of heat shock proteins (HSP)', *Journal of Proteomics*, vol. 71, no. 4, pp. 391-411.

Tinh, TH, Nuidate, T, Vuddhakul, V & Rodkhum, C 2016, 'Antibacterial activity of pyrogallol, a polyphenol compound against vibrio parahaemolyticus isolated from the central region of thailand', *Procedia Chem*, vol. 18, pp. 162-168.

Tortosa, M, Cartea, ME, Rodríguez, VM & Velasco, P 2018, 'Unraveling the metabolic response of Brassica oleracea exposed to Xanthomonas campestris pv. Campestris*', Journal of the Science of Food and Agricultur*e, vol. 98, no. 10, pp. 3675-3683.

Turan, M, Gulluce, M & Şahin, F 2012, 'Effects of plant-growth-promoting rhizobacteria on yield, growth, and some physiological characteristics of wheat and barley plants', *Communications in Soil Science and Plant Analysis*, vol. 43, no. 12, pp. 1658-1673.

Turner, MF, Heuberger, AL, Kirkwood, JS, Collins, CC, Wolfrum, EJ, Broeckling, CD, Prenni, JE & Jahn, CE 2016, 'Non-targeted metabolomics in diverse sorghum breeding lines indicates primary and secondary metabolite profiles are associated with plant biomass accumulation and photosynthesis', *Frontiers in Plant Science*, vol. 7, pp. 953.

Uriah, T, Patil, MB & Kumar, S 2015, 'In Vitro Antioxidant and Hepatoprotective Potential of Nepenthes Khasiana Hook. F against Ethanol-Induced Liver Injury in Rats', *Journal of Pharmaceutical Research*, vol. 14, no. 4, pp. 81-89.

Valitova, J, Renkova, A, Mukhitova, F, Dmitrieva, S, Beckett, RP & Minibayeva, FV 2019, 'Membrane sterols and genes of sterol biosynthesis are involved in the response of Triticum aestivum seedlings to cold stress', *Plant Physiology and Biochemistry*, vol. 142, pp. 452-459.

Van Dam, NM & van der Meijden, E 2018, 'A role for metabolomics in plant ecology', *Annual Plant Reviews Online*, pp. 87-107.

Van Thanh, N, Thao, NP, Huong, PTT, Lee, SH, Jang, HD, Cuong, NX, Nam, NH, Van Kiem, P, Van Minh, C & Kim, YH 2015a, 'Two new naphthalene glucosides and other bioactive

compounds from the carnivorous plant Nepenthes mirabilis', *Archives of Pharmacal Research*, vol. 38, no. 10, pp. 1774-1782.

Van Thanh, N, Thao, NP, Huong, PTT, Lee, SH, Jang, HD, Cuong, NX, Nam, NH, Van Kiem, P, Kim, YH & Van Minh, C 2015b, 'Naphthoquinone and flavonoid constituents from the carnivorous plant Nepenthes mirabilis and their anti-osteoporotic and antioxidant activities,' *Phytochemistry Letters*, vol. 11, pp. 254-259.

VanWallendael, A, Soltani, A, Emery, NC, Peixoto, MM, Olsen, J & Lowry, DB, 2019, 'A molecular view of plant local adaptation: incorporating stress-response networks', *Annual Review of Plant Biology*, vol. 70, pp. 559-583.

Varsha, KK, Devendra, L, Shilpa, G, Priya, S, Pandey, A, & Nampoothiri, K M 2015, '2, 4-Di-tert-butyl phenol as the antifungal, antioxidant bioactive purified from a newly isolated Lactococcus sp', *International Journal of Food Microbiology*, vol. 211, pp. 44-50.

Velu, G, Palanichamy, V & Rajan, AP 2018, *Phytochemical and Pharmacological Importance of Plant Secondary Metabolites in Modern Medicine*. In Bioorganic Phase in Natural Food: An Overview (pp. 135-156). Springer, Cham

Vijayakumar, K & Ramanathan, T 2018, 'Antiquorum sensing and biofilm potential of 5-Hydroxymethylfurfural against Gram positive pathogens', *Microbial Pathogenesis*, vol. 125, pp. 48-50.

Vinaixa, M, Schymanski, EL, Neumann, S, Navarro, M, Salek, RM & Yanes, O, 2016, 'Mass spectral databases for LC\MS-and GC\MS-based metabolomics: State of the field and future prospects', *TrAC Trends in Analytical Chemistry*, vol. 78, pp. 23-35.

Vorkas, PA, Isaac, G, Anwar, MA, Davies, AH, Want, EJ, Nicholson, JK & Holmes, E 2015, 'Untargeted UPLC-MS profiling pipeline to expand tissue metabolome coverage: application to cardiovascular disease', *Analytical Chemistry*, vol. 87, no. 8, pp. 4184-4193.

Wahid, A 2007, 'Physiological implications of metabolite biosynthesis for net assimilation and heat-stress tolerance of sugarcane (Saccharum officinarum) sprouts', *Journal of Plant Research*, vol. 120, no. 2, pp. 219-228.

Wahid, A, Gelani, S, Ashraf, M & Foolad, MR 2007, 'Heat tolerance in plants: An overview', *Environmental and Experimental Botany*, vol. 61, no. 3, 2007/12/01/, pp. 199-223.

Wan, AS, Aexel, RT, Ramsey, RB & Nicholas, HJ 1972, 'Sterols and triterpenes of the pitcher plant', *Phytochemistry*, vol. 11, no. 1, pp. 456-461.

Wang, B, Wang, G & Zhu, S 2020, 'DNA Damage Inducible Protein 1 is Involved in Cold Adaption of Harvested Cucumber Fruit', *Frontiers in Plant Science*, vol. 10, no. 1723.

Wang, L, Zhang, S, Li, S, Yan, S & Dong, S 2020, 'Inner surface of Nepenthes slippery zone: ratchet effect of lunate cells causes anisotropic superhydrophobicity', *Royal Society Open Science*, vol. 7, no. 3, pp. 200066.

Wani, SH & Herath, V eds. 2018, *Cold Tolerance in Plants: Physiological, Molecular and Genetic Perspectives*. Springer.

Wani, SH & Kumar, V eds. 2020, *Heat Stress Tolerance in Plants: Physiological, Molecular and Genetic Perspectives*. John Wiley & Sons.

Weckwerth, W 2003, 'Metabolomics in Systems Biology', *Annual Review of Plant Biology*, vol. 54, no. 1, pp. 669-689.

Wei, S, Yang, X, Huo, G, Ge, G, Liu, H, Luo, L, Hu, J, Huang, D & Long, P 2020, 'Distinct Metabolome Changes during Seed Germination of Lettuce (Lactuca sativa L.) in Response to Thermal Stress as Revealed by Untargeted Metabolomics Analysis', *International Journal of Molecular Sciences*, vol. 21, no. 4, p. 1481.

Wells, K, Lakim, MB, Schulz, S & Ayasse, M 2011, 'Pitchers of Nepenthes rajah collect faecal droppings from both diurnal and nocturnal small mammals and emit fruity odour', *Journal of Tropical Ecology*, vol. 27, no. 4, pp. 347-353.

Wiart, C, Mogana, S, Khalifah, S, Mahan, M, Ismail, S, Buckle, M, Narayana, AK & Sulaiman, M 2004, 'Antimicrobial screening of plants used for traditional medicine in the state of Perak, Peninsular Malaysia', *Fitoterapia*, vol. 75, no. 1, pp. 68-73.

Willett, DS, Filgueiras, CC, Benda, ND, Zhang, J & Kenworthy, KE 2020, 'Sting nematodes modify metabolomic profiles of host plants', *Scientific Reports*, vol. 10, no. 1, pp. 1-10.

Wilson, EO 1999, *The diversity of life*, WW Norton & Company.

Wilson, GW & Venter, S 2016, 'Nepenthes parvula (Nepenthaceae), a new species from Cape York, Queensland, Australia', *Phytotaxa*, vol. 277, no. 2, pp. 199-204.

Wink, M 2003, 'Evolution of secondary metabolites from an ecological and molecular phylogenetic perspective', *Phytochemistry*, vol. 64, no. 1, pp. 3-19.

Wong, C, Tan, LT, Mujahid, A, Lihan, S, Wee, JLS, Ting, LF & Müller, M 2018, 'Biosorption of copper by endophytic fungi isolated from *Nepenthes ampullaria'*, *Letters in Applied Microbiology*, vol. 67, no. 4, pp.384-391.

Wu, S, Tohge, T, Cuadros-Inostroza, Á, Tong, H, Tenenboim, H, Kooke, R, Méret, M, Keurentjes, JB, Nikoloski, Z, Fernie, AR & Willmitzer, L 2018, 'Mapping the Arabidopsis metabolic landscape by untargeted metabolomics at different environmental conditions', *Molecular Plant*, vol. 11, no. 1, pp. 118-134.

Xalxo, R, Yadu, B, Chandra, J, Chandrakar, V & Keshavkant, S 2020, 'Alteration in Carbohydrate Metabolism Modulates Thermotolerance of Plant under Heat Stress', *Heat Stress Tolerance in Plants: Physiological, Molecular and Genetic Perspectives*, pp.77-115.

Xu, J-G, Hu, Q-P & Liu, Y 2012, 'Antioxidant and DNA-Protective Activities of Chlorogenic Acid Isomers', *Journal of Agricultural and Food Chemistry*, vol. 60, no. 46, 2012/11/21, pp. 11625-11630.

Yadav, A, Singh, J, Ranjan, K, Kumar, P, Khanna, S, Gupta, M, Kumar, V, Wani, SH & Sirohi, A 2020, 'Heat Shock Proteins: Master Players for Heat‐stress Tolerance in Plants during Climate Change', *Heat Stress Tolerance in Plants: Physiological, Molecular and Genetic Perspectives*, pp.189-211.

Yang, CJ, Wang, CS, Hung, JY, Huang, HW, Chia, YC, Wang, PH, Weng, CF and Huang, MS 2009, 'Pyrogallol induces G2-M arrest in human lung cancer cells and inhibits tumor growth in an animal model', *Lung Cancer*, vol. 66, no. 2, pp. 162-168.

Yang, X, Wei, S, Liu, B, Guo, D, Zheng, B, Feng, L, Liu, Y, Tomás-Barberán, FA, Luo, L & Huang, D 2018, 'A novel integrated non-targeted metabolomic analysis reveals significant

metabolite variations between different lettuce (Lactuca sativa. L) varieties', *Horticulture Research*, vol. 5, no. 1, pp. 1-14.

Yilamujiang, A, Zhu, A, Ligabue-Braun, R, Bartram, S, Witte, CP, Hedrich, R, Hasabe, M, Schöner, CR, Schöner, MG, Kerth, G and Carlini, CR 2017, 'Coprophagous features in carnivorous *Nepenthes* plants: a task for ureases', *Scientific Reports*, vol. 7, no. 1, pp. 1-9.

Yolanda, H, Makahinda, IM, Aprilia, M, Sanjaya, N, Gunawan, H & Dewi, R 2014, '*Nepenthes rafflesiana* pitcher liquid has antifungal activity against *Candida* spp.', *Universa Medicina*, vol. 33, no. 2, pp. 83-90.

Yoon, MA, Jeong, TS, Park, DS, Xu, MZ, Oh, HW, Song, KB, Lee, WS and Park, HY, 2006, 'Antioxidant effects of quinoline alkaloids and 2, 4-di-tert-butylphenol isolated from *Scolopendra subspinipes'*, *Biological and Pharmaceutical Bulletin*, vol. 29, no. 4, pp. 735-739.

Yoon, Y, Seo, DH, Shin, H, Kim, HJ, Kim, CM & Jang, G 2020, 'The Role of Stress-Responsive Transcription Factors in Modulating Abiotic Stress Tolerance in Plants', *Agronomy*, vol. 10, no. 6, p. 788.

Yu, M, Lamattina, L, Spoel, SH & Loake, GJ 2014, 'Nitric oxide function in plant biology: a redox cue in deconvolution', *New Phytologist*, vol. 202, no. 4, pp. 1142-1156.

Yu, W, Wang, L, Zhao, R, Sheng, J, Zhang, S, Li, R & Shen, L 2019, 'Knockout of SlMAPK3 enhances tolerance to heat stress involving ROS homeostasis in tomato plants', *BMC plant biology*, vol. 19, no. 1, pp. 1-13.

Yu, Z, Tang, J, Khare, T & Kumar, V 2020, 'The alarming antimicrobial resistance in ESKAPEE pathogens: Can essential oils come to the rescue?', *Fitoterapia*, vol. 40, pp. 104433.

Yuan, Y, Liu, Y, Luo, Y, Huang, L, Chen, S, Yang, Z & Qin, S 2011, 'High temperature effects on flavones accumulation and antioxidant system in Scutellaria baicalensis Georgi cells', *African Journal of Biotechnology*, vol. 10, 06/13, pp. 5182-5192.

Zakaria, WNAW, Aizat, WM, Goh, HH & Noor, NM 2019, 'Protein replenishment in pitcher fluids of Nepenthes× ventrata revealed by quantitative proteomics (SWATH-MS) informed by transcriptomics', *Journal of Plant Research*, vol.132, no. 5, pp. 681-694.

Zhang, A, Sun, H, Wang, P, Han, Y & Wang, X 2012, 'Modern analytical techniques in metabolomics analysis', *Analyst*, vol. 137, no. 2, pp. 293-300.

Zhang, H, Dong, JL & Wang, J 2019, 'Research progress in membrane lipid metabolism and molecular mechanism in peanut cold tolerance', *Frontiers in Plant Science*, vol. 10, pp. 838.

Zhang, Y, Ma, XM, Wang, XC, Liu, JH, Huang, BY, Guo, XY, Xiong, SP & La, GX 2017, 'UPLC-QTOF analysis reveals metabolomic changes in the flag leaf of wheat (Triticum aestivum L.) under low-nitrogen stress', *Plant Physiology and Biochemistry*, vol. 111, pp. 30-38.

Zhao, J, Missihoun, TD & Bartels, D 2017, 'The role of Arabidopsis aldehyde dehydrogenase genes in response to high temperature and stress combinations', *Journal of Experimental Botany*, vol. 68, no. 15, pp. 4295-4308.

Zhao, M, Jin, J, Gao, T, Zhang, N, Jing, T, Wang, J, Ban, Q, Schwab, W & Song, C 2019, ‘Glucosyltransferase CsUGT78A14 Regulates Flavonols Accumulation and Reactive Oxygen Species Scavenging in Response to Cold Stress in Camellia sinensis’, *Frontiers in Plant Science*, vol. 10, pp. 1675.

Zhao, Y 2012, 'Auxin Biosynthesis: A Simple Two-Step Pathway Converts Tryptophan to Indole-3-Acetic Acid in Plants', *Molecular Plant*, vol. 5, no. 2, pp. 334-338.

Zhu, X, Feng, Y, Liang, G, Liu, N & Zhu, JK, 2013, ‘Aequorin-based luminescence imaging reveals stimulus-and tissue-specific Ca2+ dynamics in Arabidopsis plants’, *Molecular Plant*, vol. 6, no. 2, pp. 444-455.

Appendices

Supplementary Table 1 Identification of the 89 significantly altered metabolic features.

Compound Groups	No.	RT (min)	Adduct	Mass	Tentative Identification	Formula	Monoisotopic Mass	PPM (error)	Database ID
Flavanoids	19	2.17	[M + NA]+	471.0905	Quercetin 3-O-rhamnoside	$C_{21}H_{20}O_{11}$	448.101	0.62	C01750
	135	2.14	[M + H]+	449.1091	Kaempferol-3-glucoside	$C_{21}H_{20}O_{11}$	448.101	1.83	C12249
	149	1.94	[M + H]+	291.085	5-Deoxyleucocyanidin	$C_{15}H_{14}O_{6}$	290.079	-4.31	C09736
	165	1.98	[M + H]+	617.1169	Quercetin 3-(6"-galloylglucoside)	$C_{28}H_{24}O_{16}$	616.106	5.81	CID 44259190
	172	1.91	[M + NH4]+	316.2121	minaprine	$C_{17}H_{22}N_{4}O$	298.1794	-3.79	CHEBI:51038
	277	2.02	[M + H]+	443.0981	Catechin 5-O-gallate	$C_{22}H_{18}O_{10}$	442.09	1.85	CID 15689618
	312	2.12	[M + H]+	303.0503	Quercetin	$C_{15}H_{10}O_{7}$	302.0427	1.06	C00389
	394	2.2	[M + NA]+	485.1431	4,2'-Dihydroxy-4',6'-dimethoxychalcone 4-glucoside	$C_{23}H_{26}O_{10}$	462.153	1.88	CID 13870531
	406	2.21	[M + NA]+	609.0862	Quercetin 3-(2"-galloyl-alpha-L-arabinopyranoside)	$C_{27}H_{22}O_{15}$	586.096	1.74	CID 44259256
	425	2.16	[M + H]+	587.1048	Quercetin 3-(2"-galloyl-alpha-L-arabinopyranoside)	$C_{27}H_{22}O_{15}$	586.096	2.51	CID 44259256
	409	1.92	[M + NH4]+	512.1772	Peruvianoside II	$C_{23}H_{26}O_{12}$	494.142	2.75	CID 42608014
	464	1.76	[M + H]+	447.0904	Baicalin	$C_{21}H_{18}O_{11}$	446.0849	-3.95	C10025
	541	12.77	[M + H]+	271.0604	Baicalein (a)	$C_{15}H_{10}O_{5}$	270.0528	1.11	C10023
	548	2.02	[M + H]+	271.0604	Baicalein (b)	$C_{15}H_{10}O_{5}$	270.0528	1.11	C10023
	591	1.82	[M + H]+	271.0604	Baicalein (c)	$C_{15}H_{10}O_{5}$	270.0528	1.11	C10023
	542	12.77	[M + NA]+	441.0802	Kaempferol 3-O-beta-D-xyloside (a)	$C_{20}H_{18}O_{10}$	418.09	2.39	C20727
	543	2.09	[M + NA]+	441.0802	Kaempferol 3-O-beta-D-xyloside (b)	$C_{20}H_{18}O_{10}$	418.09	2.39	C20727
	550	2.01	[M + NA]+	633.1449	Rutin	$C_{27}H_{30}O_{16}$	610.1534	3.67	C05625
	553	1.89	[M + NA]+	649.1396	6,8-Dihydroxykaempferol 3-rutinoside	$C_{27}H_{30}O_{17}$	626.148	3.75	CID 44260049

	588	2.34	[M + H]+	883.1735	Epiafzelechin 3-O-gallate-(4beta->6)-epigallocatechin 3-O-gallate	$C_{44}H_{34}O_{20}$	882.164	2.55	CID 14521010
	128	2.24	[M + NA]+	455.0961	Afzelin	$C_{21}H_{20}O_{10}$	432.106	2.11	C16911
	473	1.98	[M + H]+	479.0828	Miquelianin (a)	$C_{21}H_{18}O_{13}$	478.075	1.09	CHEBI:66395
	500	2	[M + NA]+	501.0653	Miquelianin (b)	$C_{21}H_{18}O_{13}$	478.0747	2.89	CHEBI:66395
	192	2.19	[M + H]+	287.0556	Luteolin	$C_{15}H_{10}O_6$	286.0477	2.17	C01514
	278	2.05	[M + H]+	273.0763	Butin (a)	$C_{15}H_{12}O_5$	272.068	3.68	C09614
	281	1.82	[M + H]+	273.0763	Butin (b)	$C_{15}H_{12}O_5$	272.068	3.68	C09614
	427	2.04	[M + NA]+	501.1375	myrciacitrin I	$C_{23}H_{26}O_{11}$	478.1475	1.51	CHEBI:66417
	193	2.27	[M + H]+	623.1018	3,5,7-trihydroxy-2-[3,4,5-trihydroxy-2-[2,3,4-trihydroxy-6-(3,5,7-trihydroxychroman-2-yl)phenyl]phenyl]chromen-4-one (a)	$C_{30}H_{22}O_{15}$	622.096	-2.38	CID 57691813
	280	2.13	[M + H]+	623.1018	3,5,7-trihydroxy-2-[3,4,5-trihydroxy-2-[2,3,4-trihydroxy-6-(3,5,7-trihydroxychroman-2-yl)phenyl]phenyl]chromen-4-one (b)	$C_{30}H_{22}O_{15}$	622.096	-2.38	CID 57691813
	488	1.97	[M + NA]+	745.1761	(2S)-2-(3,4-dihydroxyphenyl)-8-[(2S,4R)-2-(3,4-dihydroxyphenyl)-7-hydroxy-5-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy-3,4-dihydro-2H-chromen-4-yl]-5,7-dihydroxy-2,3-dihydrochromen-4-one	$C_{36}H_{34}O_{16}$	722.185	2.62	CID 6325552
Alkaloids	15	0.47	[M + H]+	138.0549	Trigonelline	$C_7H_7NO_2$	137.0477	2.80	C01004
	209	2.44	[M + H]+	253.1074	Anatoxin a(s)	$C_7H_{17}N_4O_4P$	252.0987	5.63	C19998
	577	2	[M + NA]+	375.1056	Berberastine	$C_{20}H_{18}NO_5$	352.118	-4.35	CHEBI:3065
Amino acids	158	0.5	[M + H]+	175.118	L-Arginine	$C_6H_{14}N_4O_2$	174.1117	-5.74	C02385
	162	1.53	[M + H]+	205.0977	L-Tryptophan	$C_{11}H_{12}N_2O_2$	204.09	2.06	C00078
	228	0.47	[M + H]+	132.1018	Isoleucine	$C_6H_{13}NO_2$	131.095	-3.66	C00407
	242	0.47	[M + H]+	118.0835	L-Norvaline	$C_5H_{11}NO_2$	117.079	-23.74	C01826
Fatty Acyls	155	3.63	[M + H]+	199.1694	7-Dodecenoic acid	$C_{12}H_{22}O_2$	198.162	0.61	CID 5282732
	200	4.28	[M + H]+	313.2747	18-oxononadecanoic acid	$C_{19}H_{36}O_3$	312.266	4.39	CID 5312917
	299	4.92	[M + H]+	267.2693	Olealdehyde (a)	$C_{18}H_{34}O$	266.261	3.72	CID17029
	300	4.67	[M + H]+	267.2693	Olealdehyde (b)	$C_{18}H_{34}O$	266.261	3.72	CID17029
	329	0.93	[M + H]+	202.1805	11-aminoundecanoic acid	$C_{11}H_{23}NO_2$	201.173	1.09	CID 17083

	360	7.93	[M + H]+	596.6016	2-[(2-Hydroxyethyl)(octadecyl)amino]ethyl hexadecanoate	$C_{38}H_{77}NO_3$	595.59	7.25	CID 71326110
Indoles	486	0.75	[M + H]+	188.0708	Indole-3-acrylic acid (a)	$C_{11}H_9NO_2$	187.063	2.78	CID 5375048
	494	1.53	[M + H]+	188.0708	Indole-3-acrylic acid (b)	$C_{11}H_9NO_2$	187.063	2.78	CID 5375048
peptides	18	2.52	[M + NA]+	568.2972	Pro-Gln-Arg-Phe amide trifluoroacetate salt	$C_{25}H_{39}N_9O_5$	545.307	1.80	CID 3382578
	47	2.45	[M + K]+	257.0577	L-Alanyl-D-glutamate	$C_8H_{14}N_2O_5$	218.0903	19.44	C20957
Organic acids	285	2.04	[M + H]+	191.0707	1-naphthoic acid	$C_{11}H_{10}O_3$	190.063	2.21	C15102
	547	2.03	[M + H]+	123.0441	Benzoic acid (a)	$C_7H_6O_2$	122.037	-1.47	C00180
	580	1.84	[M + H]+	123.0441	Benzoic acid (b)	$C_7H_6O_2$	122.037	-1.47	C00180
	545	1.35	[M + H]+	327.0687	5-Galloylshikimic acid (a)	$C_{14}H_{14}O_9$	326.064	-7.91	CID 460897
	585	0.7	[M + H]+	327.0687	5-Galloylshikimic acid (b)	$C_{14}H_{14}O_9$	326.064	-7.91	CID 460897
	433	2.18	[M + H]+	285.0612	(2R,3R)-2-(2-Methoxybenzoyloxy)-3-hydroxysuccinic acid	$C_{12}H_{12}O_8$	284.053	3.06	CID 70014677
	440	1.79	[M + H]+	181.0863	3-(2-hydroxypropyl)benzoic acid	$C_{10}H_{12}O_3$	180.079	0.28	CID 67568076
	97	7.76	[M + H]+	593.4588	2-triacontanoylbenzenesulfonic acid	$C_{36}H_{64}O_4S$	592.453	-2.50	CID 23652140
inorganic acid	44	12.83	[M + H]+	258.917	Triphosphoric Acid	$H_5O_{10}P_3$	257.91	-1.09	C00536
Trihydroxybenzoic acids	462	1.78	[M + H]+	315.0713	Norbergenin	$C_{13}H_{14}O_9$	314.064	0.06	CID 73192
purine base	467	1.33	[M + H]+	268.1045	Adenosine (a)	$C_{10}H_{13}N_5O_4$	267.097	0.82	C00212
	586	0.7	[M + H]+	268.1045	Adenosine (b)	$C_{10}H_{13}N_5O_4$	267.097	0.82	C00212
	141	0.68	[M + H]+	136.0615	adenine (a)	$C_5H_5N_5$	135.054	1.63	C00147
	310	1.33	[M + H]+	136.0617	adenine (b)	$C_5H_5N_5$	135.054	1.63	C00147
monolignols	309	1.76	[M + NA]+	395.1316	Syringin	$C_{17}H_{24}O_9$	372.142	0.89	C01533
	372	1.78	[M + NA]+	365.1212	Coniferin	$C_{16}H_{22}O_8$	342.1315	1.46	C00761
Phenolic acid	367	2.9	[M + H]+	280.2639	Crucigasterin E	$C_{18}H_{33}NO$	279.256	2.22	CID 46937368
Sugars	495	1.78	[M + NA]+	507.0752	4-O,6-O-Digalloyl-D-glucopyranose	$C_{20}H_{20}O_{14}$	484.085	2.02	CID 101630404
lumazine derivative	100	1.51	[M + K]+	369.0804	reduced 6-(hydroxymethyl)-8-(1-D-ribityl)lumazine	$C_{20}H_{16}O_7$	330.1176	-1.03	CHEBI:70986

2-benzofuran-1(3H)-one derivative	268	3.42	[M + H]+	205.086	3-Butylidene-7-hydroxyphthalide	$C_{12}H_{12}O_3$	204.0786	0.34	C09921
Others	3	2.9	[M + H]+	415.212	2-[4-(4-methoxyphenyl)-3-methyl-pyrazolo[3,4-b]pyridin-1-yl]-N-(1-phenylpropyl)acetamide	$C_{25}H_{26}N_4O_2$	414.206	-3.10	CID 19506043
	20	0.42	[M + H]+	261.0582	8-methoxy-4,5-dihydrobenzo[g]benzothiophene-2-carboxylic acid	$C_{14}H_{12}O_3S$	260.051	-0.35	CID 81605969
	29	0.41	[M + H]+	203.0529	N-(3-Nitramido-4,5-dihydropyridazin-4-yl)nitramide	$C_4H_6N_6O_4$	202.045	3.07	CID 419333
	34	8.09	[M + H]+	753.4297	4-[[4-(benzenesulfonyl)-5-oxido-1,2,5-oxadiazol-5-ium-3-yl]oxy]butan-2-yl (4aS,6aR,6aS,6bR,8aR,10S,12aR,14bS)-10-hydroxy-2,2,6a,6b,9,9,12a-heptamethyl-1,3,4,5,6,6a,7,8,8a,10,11,12,13,14b-tetradecahydropicene-4a-carboxylate	$C_{42}H_{60}N_2O_8S$	752.407	20.49	CID 24949892
	40	2.15	[M + H]+	494.1245	(2S)-2-amino-5-[[(1S)-2-(carboxymethylamino)-1-[(8-hydroxy-3-methyl-1,4-dioxo-2-naphthyl)sulfanylmethyl]-2-oxo-ethyl]amino]-5-oxo-pentanoic acid	$C_{21}H_{23}N_3O_9S$	493.116	2.47	CID 52942238
	41	3.21	[M + H]+	291.2536	5-isopropyl-N6-methyl-N4-[2-(4-methylcyclohexyl)ethyl]pyrimidine-4,6-diamine	$C_{17}H_{30}N_4$	290.247	-2.34	CID 80822319
	49	12.83	[M + NA]+	356.8936	5-[(3,5-dichloro-4-pyridyl)sulfanyl]-4-nitro-thiophene-2-carbaldehyde	$C_{10}H_4Cl_2N_2O_3S_2$	333.904	1.14	CID 87562543
	50	2.44	[M + H]+	359.1494	2-[2-[(3-cyano-2-pyridyl)oxy]anilino]-N-(p-tolyl)acetamide (a)	$C_{21}H_{18}N_4O_2$	358.143	-2.46	CID 60537784
	51	3.24	[M + NH4]+	335.2798	N-[1-(3-Aminophenyl)ethylideneamino]undecanamide	$C_{19}H_{31}N_3O$	317.247	-3.34	CID 3616475
	81	2.44	[M + NA]+	381.1314	2-[2-[(3-cyano-2-pyridyl)oxy]anilino]-N-(p-tolyl)acetamide (b)	$C_{21}H_{18}N_4O_2$	358.143	-2.29	CID 60537784
	102	0.42	[M + H]+	247.0425	1-(3-hydroxy-2-thienyl)-3-phenyl-propane-1,3-dione	$C_{13}H_{10}O_3S$	246.035	0.89	CID 13004301
	121	0.44	[M + H]+	229.0317	9-hydroxy-2-thioxo-3H-phenalen-1-one	$C_{13}H_8O_2S$	228.025	-2.41	CID 88740582
	258	3.13	[M + NA]+	391.1649	Phthalic acid, 2-chloroethyl decyl ester	$C_{20}H_{29}ClO_4$	368.175	1.85	CID 6424038
	290	3.05	[M + NA]+	555.2936	2-(2,6-dihydroxyphenyl)-6-(1-hydroxy-3-oxo-octadecyl)-2-methyl-6H-furo[3,4-d][1,3]dioxol-4-one	$C_{30}H_{44}O_8$	532.304	0.71	CID 25069128
	297	4.92	[M + H]+	359.3165	18-(2,3-dihydroxypropoxy)octadecan-2-one (a)	$C_{21}H_{42}O_4$	358.308	3.32	CID 21475594
	298	4.67	[M + H]+	359.3165	18-(2,3-dihydroxypropoxy)octadecan-2-one (b)	$C_{21}H_{42}O_4$	358.308	3.32	CID 21475594
	345	7.79	[M + H]+	579.5385	N"-[3-[3-[[4-(5-tert-butyl-2-methyl-phenyl)-1-[3-(5-tert-butyl-2-methyl-	$C_{38}H_{66}N_4$	578.529	3.84	CID 90674863

				phenyl)propyl]butyl]amino]propylamino]propyl]propane-1,3-diamine				
347	8.03	[M + H]+	647.5638	2-[2-[4-(4,6-dimethyldecan-3-yloxy)-3-methyl-5-pent-4-ynyloxolan-2-yl]oxy-3,4,6-trimethylcyclohexyl]oxy-6-ethyl-3,4,5-trimethyloxane	$C_{41}H_{74}O_5$	646.554	3.90	CID 90993583
350	7.79	[M + H]+	619.5316	2-({16-[3-(Benzyloxy)-2-(octyloxy)propoxy]hexadecyl}oxy)tetrahydro-2H-pyran	$C_{39}H_{70}O_5$	618.5223	3.27	CSID:8992942
354	7.53	[M + H]+	551.5068	(Z)-19-methoxycarbonyltritriacont-9-enoic acid	$C_{35}H_{66}O_4$	550.496	6.39	CID 87313417
460	0.41	[M + H]+	207.0357	(2S)-2-(5-chloro-2-thienyl)-1-hydrazino-propan-2-ol	$C_7H_{11}ClN_2OS$	206.028	2.04	CID 82077398
461	0.42	[M + H]+	185.0534	1-azido-4-nitro-2H-triazin-5-amine	$C_3H_4N_8O_2$	184.046	0.65	CID 22213878

Supplementary Table 2 ANOVA results for comparison of metabolites regulation between *Nepenthes* species (n = 12) and metabolite in response to the environmental conditions.

	Sum of squares	df	Mean square	F	p (same)
Sample:	2054.44	11	186.768	63.61	$6.267E^{-135}$
metabolite:	41796.8	124	337.071	114.8	< 0.001
Interaction:	27132.6	1364	19.8919	6.775	< 0.001
Within:	17615.6	6000	2.93594		
Total:	88599.4	7499			

Supplementary Table 3 Tukey's post-hoc results for comparison of each *Nepenthes* species under the provided environmental condition. HL – Highland condition; IL – Intermediate condition; LL – Lowland condition.

	amp HL	amp IL	amp LL	min HL	min IL	min LL	north HL	north IL	north LL	raff HL	raff IL	raff LL
amp HL		0.4547	$1.2E^{-12}$	$1.32E^{-12}$	$1.33E^{-12}$	$1.31E^{-12}$	0.9368	0.7910	0.7212	0.7792	0.0638	0.0115
amp IL	0.4547		$1.32E^{-12}$	$1.33E^{-12}$	$2.25E^{-10}$	$2.43E^{-12}$	0.9997	1	1	0.0016	$2.32E^{-06}$	$1.1E^{-07}$
amp LL	$1.2E^{-12}$	$1.32E^{-12}$		0.9579	0.1780	0.6256	$1.32E^{-12}$	$1.32E^{-12}$	$1.32E^{-12}$	$1.2E^{-12}$	$1.2E^{-12}$	$1.2E^{-12}$
min HL	$1.32E^{-12}$	$1.33E^{-12}$	0.9579		0.9682	1	$1.34E^{-12}$	$1.29E^{-12}$	$1.3E^{-12}$	$1.2E^{-12}$	$1.2E^{-12}$	$1.2E^{-12}$
min IL	$1.33E^{-12}$	$2.25E^{-10}$	0.1780	0.9682		0.9999	$1.79E^{-12}$	$7.88E^{-12}$	$1.65E^{-11}$	$1.24E^{-12}$	$1.2E^{-12}$	$1.2E^{-12}$
min LL	$1.31E^{-12}$	$2.43E^{-12}$	0.6256	1	0.9999		$1.32E^{-12}$	$1.36E^{-12}$	$1.41E^{-12}$	$1.2E^{-12}$	$1.2E^{-12}$	$1.2E^{-12}$
north HL	0.9368	0.9997	$1.32E^{-12}$	$1.34E^{-12}$	$1.79E^{-12}$	$1.32E^{-12}$		1	1	0.03492	0.0002	$1.2E^{-05}$
north IL	0.7910	1	$1.32E^{-12}$	$1.29E^{-12}$	$7.88E^{-12}$	$1.36E^{-12}$	1		1	0.01104	$3.13E^{-05}$	$1.91E^{-06}$
north LL	0.7212	1	$1.32E^{-12}$	$1.3E^{-12}$	$1.65E^{-11}$	$1.41E^{-12}$	1	1		0.00728	$1.76E^{-05}$	$1.01E^{-06}$
raff HL	0.7792	0.0016	$1.2E^{-12}$	$1.2E^{-12}$	$1.24E^{-12}$	$1.2E^{-12}$	0.03492	0.01104	0.00728		0.9766	0.7973
raff IL	0.0638	$2.32E^{-06}$	$1.2E^{-12}$	$1.2E^{-12}$	$1.2E^{-12}$	$1.2E^{-12}$	0.0002	$3.13E^{-05}$	1.76E-05	0.9766		1
raff LL	0.0115	$1.1E^{-07}$	$1.2E^{-12}$	$1.2E^{-12}$	$1.2E^{-12}$	$1.2E^{-12}$	$1.2E^{-05}$	$1.91E^{-06}$	1.01E-06	0.7973	1	

Supplementary Table 4 Similarly, and individually expressed thermal stressed metabolites among the 4 *Nepenthes* species in response to the provided environmental conditions. Abbreviation: (H) represents the highest expression recorded on highland condition and (L) represents the highest expression recorded on lowland condition.

12 common elements in "minima", "ampullaria", "northiana" and "rafflesiana"	(H)	Epiafzelechin 3-O-gallate-(4beta->6)-epigallocatechin 3-O-gallate
	(H)	4-[[4-(benzenesulfonyl)-5-oxido-1,2,5-oxadiazol-5-ium-3-yl]oxy]butan-2-yl (4aS,6aR,6aS,6bR,8aR,10S,12aR,14bS)-10-hydroxy-2,2,6a,6b,9,9,12a-heptamethyl-1,3,4,5,6,6a,7,8,8a,10,11,12,13,14b-tetradecahydropicene-4a-carboxylate
	(H)	Berberastine
	(H)	1-naphthoic acid
	(H)	adenine (a)
	(H)	adenine (b)
	(L)	L-Tryptophan
	(L)	18-oxononadecanoic acid
	(L)	Olealdehyde (a)
	(L)	Olealdehyde (b)
	(L)	Indole-3-acrylic acid (a)
	(L)	2-[2-[(3-cyano-2-pyridyl)oxy]anilino]-N-(p-tolyl)acetamide (a)
8 common elements in "minima", "ampullaria" and "northiana"	(L)	Anatoxin a(s)
	(L)	2-[(2-Hydroxyethyl)(octadecyl)amino]ethyl hexadecanoate
	(L)	2-[2-[(3-cyano-2-pyridyl)oxy]anilino]-N-(p-tolyl)acetamide (b)
	(L)	18-(2,3-dihydroxypropoxy)octadecan-2-one (b)
	(L)	N"-[3-[3-[[4-(5-tert-butyl-2-methyl-phenyl)-1-[3-(5-tert-butyl-2-methyl-phenyl)propyl]butyl]amino]propylamino]propyl]propane-1,3-diamine
	(L)	2-[2-[4-(4,6-dimethyldecan-3-yloxy)-3-methyl-5-pent-4-ynyloxolan-2-yl]oxy-3,4,6-trimethylcyclohexyl]oxy-6-ethyl-3,4,5-trimethyloxane
	(L)	2-({16-[3-(Benzyloxy)-2-(octyloxy)propoxy]hexadecyl}oxy)tetrahydro-2H-pyran
	(L)	(Z)-19-methoxycarbonyltritriacont-9-enoic acid
7 common elements in "ampullaria", "northiana" and "rafflesiana"	(H)	Kaempferol 3-O-beta-D-xyloside(b)
	(H)	Pro-Gln-Arg-Phe amide trifluoroacetate salt
	(H)	Adenosine (a)
	(H)	Adenosine (b)
	(H)	(2S)-2-amino-5-[[(1S)-2-(carboxymethylamino)-1-[(8-hydroxy-3-methyl-1,4-dioxo-2-naphthyl)sulfanylmethyl]-2-oxo-ethyl]amino]-5-oxo-pentanoic acid
	(H)	5-isopropyl-N6-methyl-N4-[2-(4-methylcyclohexyl)ethyl]pyrimidine-4,6-diamine

	(H)	Phthalic_acid,2-chloroethyl-decyl-ester
5 common elements in "minima", "ampullaria" and "rafflesiana"	(H)	5-Deoxyleucocyanidin
	(L)	Kaempferol 3-O-beta-D-xyloside(a)
	(L)	Indole-3-acrylic acid (b)
	(L)	5-Galloylshikimic acid (b)
	(H)	Triphosphoric_Acid
2 common elements in "minima", "northiana" and "rafflesiana"	(L)	Kaempferol 3-O-glucoside
	(L)	(2S)-2-(3,4-dihydroxyphenyl)-8-[(2S,4R)-2-(3,4-dihydroxyphenyl)-7-hydroxy-5-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy-3,4-dihydro-2H-chromen-4-yl]-5,7-dihydroxy-2,3-dihydrochromen-4-one
6 common elements in "ampullaria" and "northiana"	(H)	Baicalein (b)
	(L)	Baicalein (c)
	(L)	2-[4-(4-methoxyphenyl)-3-methyl-pyrazolo[3,4-b]pyridin-1-yl]-N-(1-phenylpropyl)acetamide
	(L)	N-(3-Nitramido-4,5-dihydropyridazin-4-yl)nitramide
	(L)	1-(3-hydroxy-2-thienyl)-3-phenyl-propane-1,3-dione
	(L)	2-(2,6-dihydroxyphenyl)-6-(1-hydroxy-3-oxo-octadecyl)-2-methyl-6H-furo[3,4-d][1,3]dioxol-4-one
7 common elements in "ampullaria" and "rafflesiana"	(H)	Rutin
	(H)	L-Alanyl-D-glutamate
	(H)	3-(2-hydroxypropyl)benzoic acid
	(H)	Norbergenin
	(H)	3-Butylidene-7-hydroxyphthalide
	(H)	5-[(3,5-dichloro-4-pyridyl)sulfanyl]-4-nitro-thiophene-2-carbaldehyde
	(H)	N-[1-(3-Aminophenyl)ethylideneamino]undecanamide
8 common elements in "minima" and "rafflesiana"	(H)	Quercetin 3-(2"-galloyl-alpha-L-arabinopyranoside) (a)
	(H)	Baicalin
	(L)	Baicalein (b)
	(H)	Luteolin
	(L)	myrciacitrin I
	(L)	L-Arginine
	(H)	2-(2,6-dihydroxyphenyl)-6-(1-hydroxy-3-oxo-octadecyl)-2-methyl-6H-furo[3,4-d][1,3]dioxol-4-one
	(L)	(2S)-2-(5-chloro-2-thienyl)-1-hydrazino-propan-2-ol
4 common elements in "minima" and "northiana"	(H)	Quercetin 3-(2"-galloyl-alpha-L-arabinopyranoside) (b)
	(L)	4-O,6-O-Digalloyl-D-glucopyranose
	(L)	9-hydroxy-2-thioxo-3H-phenalen-1-one

	(L)	8-methoxy-4,5-dihydrobenzo[g]benzothiophene-2-carboxylic acid
9 common elements in "minima" and "ampullaria"	(H)	4,2'-Dihydroxy-4',6'-dimethoxychalcone 4-glucoside
	(H)	Peruvianoside II
	(L)	Baicalein (a)
	(L)	Norvaline
	(L)	11-aminoundecanoic acid
	(H)	3,5,7-trihydroxy-2-[3,4,5-trihydroxy-2-[2,3,4-trihydroxy-6-(3,5,7-trihydroxychroman-2-yl)phenyl]phenyl]chromen-4-one (b)
	(H)	Benzoic acid (a)
	(H)	Benzoic acid (b)
	(L)	18-(2,3-dihydroxypropoxy)octadecan-2-one (a)
8 common elements in "northiana" and "rafflesiana"	(H)	minaprine
	(H)	Baicalein (a)
	(L)	Butin (b)
	(H)	7-Dodecenoic acid
	(H)	11-aminoundecanoic acid
	(L)	Benzoic acid (b)
	(L)	Syringin
	(H)	Crucigasterin E
41 elements included exclusively in "minima"	(H)	Catechin 5-O-gallate
	(H)	Baicalein (c)
	(H)	Afzelin
	(H)	Butin (a)
	(H)	Butin (b)
	(H)	(2R,3R)-2-(2-Methoxybenzoyloxy)-3-hydroxysuccinic acid
	(H)	N-(3-Nitramido-4,5-dihydropyridazin-4-yl)nitramide
	(L)	Kaempferol 3-O-beta-D-xyloside(b)
	(L)	2-triacontanoylbenzenesulfonic acid
	(L)	Trigonelline
	(L)	Isoleucine
	(L)	(2S)-2-amino-5-[[(1S)-2-(carboxymethylamino)-1-[(8-hydroxy-3-methyl-1,4-dioxo-2-naphthyl)sulfanylmethyl]-2-oxo-ethyl]amino]-5-oxo-pentanoic acid
	1	Quercetin-3-O-rhamnoside
	1	Quercetin 3-(6"-galloylglucoside)
	1	minaprine
	1	Quercetin
	1	Rutin
	1	6,8-Dihydroxykaempferol 3-rutinoside

	1	miquelianin (a)
	1	miquelianin (b)
	1	7-Dodecenoic acid
	1	Pro-Gln-Arg-Phe amide trifluoroacetate salt
	1	L-Alanyl-D-glutamate
	1	5-Galloylshikimic acid (a)
	1	3-(2-hydroxypropyl)benzoic acid
	1	Norbergenin
	1	Adenosine (a)
	1	Adenosine (b)
	1	Syringin
	1	Coniferin
	1	Crucigasterin E
	1	reduced 6-(hydroxymethyl)-8-(1-D-ribityl)lumazine
	1	3-Butylidene-7-hydroxyphthalide
	1	2-[4-(4-methoxyphenyl)-3-methyl-pyrazolo[3,4-b]pyridin-1-yl]-N-(1-phenylpropyl)acetamide
	1	5-isopropyl-N6-methyl-N4-[2-(4-methylcyclohexyl)ethyl]pyrimidine-4,6-diamine
	1	5-[(3,5-dichloro-4-pyridyl)sulfanyl]-4-nitro-thiophene-2-carbaldehyde
	1	N-[1-(3-Aminophenyl)ethylideneamino]undecanamide
	1	1-(3-hydroxy-2-thienyl)-3-phenyl-propane-1,3-dione
	1	Phthalic_acid,2-chloroethyl-decyl-ester
	1	3,5,7-trihydroxy-2-[3,4,5-trihydroxy-2-[2,3,4-trihydroxy-6-(3,5,7-trihydroxychroman-2-yl)phenyl]phenyl]chromen-4-one (a)
	1	1-azido-4-nitro-2H-triazin-5-amine
35 elements included exclusively in "ampullaria"	(H)	9-hydroxy-2-thioxo-3H-phenalen-1-one
	(H)	(2S)-2-(5-chloro-2-thienyl)-1-hydrazino-propan-2-ol
	(H)	8-methoxy-4,5-dihydrobenzo[g]benzothiophene-2-carboxylic acid
	(H)	Trigonelline
	(H)	Isoleucine
	(H)	Coniferin
	(L)	Crucigasterin E
	(L)	reduced 6-(hydroxymethyl)-8-(1-D-ribityl)lumazine
	(L)	7-Dodecenoic acid
	(L)	minaprine
	2	Quercetin-3-O-rhamnoside
	2	Kaempferol 3-O-glucoside

	2	Quercetin 3-(6"-galloylglucoside)
	2	Catechin 5-O-gallate
	2	Quercetin
	2	Quercetin 3-(2"-galloyl-alpha-L-arabinopyranoside) (a)
	2	Quercetin 3-(2"-galloyl-alpha-L-arabinopyranoside) (b)
	2	Baicalin
	2	6,8-Dihydroxykaempferol 3-rutinoside
	2	Afzelin
	2	miquelianin (a)
	2	miquelianin (b)
	2	Luteolin
	2	Butin (a)
	2	Butin (b)
	2	myrciacitrin I
	2	(2S)-2-(3,4-dihydroxyphenyl)-8-[(2S,4R)-2-(3,4-dihydroxyphenyl)-7-hydroxy-5-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy-3,4-dihydro-2H-chromen-4-yl]-5,7-dihydroxy-2,3-dihydrochromen-4-one
	2	L-Arginine
	2	5-Galloylshikimic acid (a)
	2	(2R,3R)-2-(2-Methoxybenzoyloxy)-3-hydroxysuccinic acid
	2	3,5,7-trihydroxy-2-[3,4,5-trihydroxy-2-[2,3,4-trihydroxy-6-(3,5,7-trihydroxychroman-2-yl)phenyl]phenyl]chromen-4-one (a)
	2	2-triacontanoylbenzenesulfonic acid
	2	Syringin
	2	4-O,6-O-Digalloyl-D-glucopyranose
	2	1-azido-4-nitro-2H-triazin-5-amine
42 elements included exclusively in "northiana"	(H)	5-Galloylshikimic acid (a)
	(H)	Kaempferol 3-O-beta-D-xyloside(a)
	(H)	myrciacitrin I
	(H)	Norvaline
	(L)	Rutin
	(L)	6,8-Dihydroxykaempferol 3-rutinoside
	(L)	Butin (a)
	(L)	Benzoic acid (a)
	(L)	Quercetin 3-(6"-galloylglucoside)
	(L)	Quercetin
	(L)	(2R,3R)-2-(2-Methoxybenzoyloxy)-3-hydroxysuccinic acid
	(L)	3-(2-hydroxypropyl)benzoic acid

	(L)	Triphosphoric_Acid
	(L)	Norbergenin
	(L)	5-[(3,5-dichloro-4-pyridyl)sulfanyl]-4-nitro-thiophene-2-carbaldehyde
	(L)	N-[1-(3-Aminophenyl)ethylideneamino]undecanamide
	3	Quercetin-3-O-rhamnoside
	3	5-Deoxyleucocyanidin
	3	Catechin 5-O-gallate
	3	4,2'-Dihydroxy-4',6'-dimethoxychalcone 4-glucoside
	3	Quercetin 3-(2"-galloyl-alpha-L-arabinopyranoside) (a)
	3	Peruvianoside II
	3	Baicalin
	3	Afzelin
	3	miquelianin (a)
	3	miquelianin (b)
	3	Luteolin
	3	3,5,7-trihydroxy-2-[3,4,5-trihydroxy-2-[2,3,4-trihydroxy-6-(3,5,7-trihydroxychroman-2-yl)phenyl]phenyl]chromen-4-one
	3	3,5,7-trihydroxy-2-[3,4,5-trihydroxy-2-[2,3,4-trihydroxy-6-(3,5,7-trihydroxychroman-2-yl)phenyl]phenyl]chromen-4-one (a)
	3	Trigonelline
	3	L-Arginine
	3	Isoleucine
	3	Indole-3-acrylic acid (b)
	3	L-Alanyl-D-glutamate
	3	5-Galloylshikimic acid (b)
	3	2-triacontanoylbenzenesulfonic acid
	3	Coniferin
	3	reduced 6-(hydroxymethyl)-8-(1-D-ribityl)lumazine
	3	3-Butylidene-7-hydroxyphthalide
	3	18-(2,3-dihydroxypropoxy)octadecan-2-one (a)
	3	(2S)-2-(5-chloro-2-thienyl)-1-hydrazino-propan-2-ol
	3	1-azido-4-nitro-2H-triazin-5-amine
40 elements included exclusively in "rafflesiana"	(H)	Quercetin 3-(6"-galloylglucoside)
	(H)	2-[(2-Hydroxyethyl)(octadecyl)amino]ethyl hexadecanoate
	(H)	18-(2,3-dihydroxypropoxy)octadecan-2-one (a)
	(H)	N"-[3-[3-[[4-(5-tert-butyl-2-methyl-phenyl)-1-[3-(5-tert-butyl-2-methyl-phenyl)propyl]butyl]amino]propylamino]propyl]propane-1,3-diamine

(H)	2-[2-[4-(4,6-dimethyldecan-3-yloxy)-3-methyl-5-pent-4-ynyloxolan-2-yl]oxy-3,4,6-trimethylcyclohexyl]oxy-6-ethyl-3,4,5-trimethyloxane
(H)	2-({16-[3-(Benzyloxy)-2-(octyloxy)propoxy]hexadecyl}oxy)tetrahydro-2H-pyran
(H)	(Z)-19-methoxycarbonyltritriacont-9-enoic acid
(H)	2-triacontanoylbenzenesulfonic acid
(H)	4-O,6-O-Digalloyl-D-glucopyranose
(H)	reduced 6-(hydroxymethyl)-8-(1-D-ribityl)lumazine
(H)	2-[4-(4-methoxyphenyl)-3-methyl-pyrazolo[3,4-b]pyridin-1-yl]-N-(1-phenylpropyl)acetamide
(L)	1-azido-4-nitro-2H-triazin-5-amine
(L)	3,5,7-trihydroxy-2-[3,4,5-trihydroxy-2-[2,3,4-trihydroxy-6-(3,5,7-trihydroxychroman-2-yl)phenyl]phenyl]chromen-4-one (a)
(L)	5-Galloylshikimic acid (a)
(L)	miquelianin (a)
(L)	miquelianin (b)
(L)	4,2'-Dihydroxy-4',6'-dimethoxychalcone 4-glucoside
(L)	Quercetin 3-(2"-galloyl-alpha-L-arabinopyranoside) (b)
(L)	Peruvianoside II
4	Quercetin-3-O-rhamnoside
4	Catechin 5-O-gallate
4	Quercetin
4	Baicalein (c)
4	6,8-Dihydroxykaempferol 3-rutinoside
4	Afzelin
4	Butin (a)
4	Trigonelline
4	Anatoxin a(s)
4	Isoleucine
4	Norvaline
4	Benzoic acid (a)
4	(2R,3R)-2-(2-Methoxybenzoyloxy)-3-hydroxysuccinic acid
4	Coniferin
4	N-(3-Nitramido-4,5-dihydropyridazin-4-yl)nitramide
4	2-[2-[(3-cyano-2-pyridyl)oxy]anilino]-N-(p-tolyl)acetamide (b)
4	1-(3-hydroxy-2-thienyl)-3-phenyl-propane-1,3-dione
4	9-hydroxy-2-thioxo-3H-phenalen-1-one
4	18-(2,3-dihydroxypropoxy)octadecan-2-one (b)

	4	3,5,7-trihydroxy-2-[3,4,5-trihydroxy-2-[2,3,4-trihydroxy-6-(3,5,7-trihydroxychroman-2-yl)phenyl]phenyl]chromen-4-one (b)
	4	8-methoxy-4,5-dihydrobenzo[g]benzothiophene-2-carboxylic acid

Supplementary Table 5 Identification of 86 significantly altered metabolic features.

Compound Groups	No.	RT (min)	Adduct	Mass	Tentative identification	Formula	Monoisotopic Mass	PPM (error)	Database ID
Flavonoids	19	2.17	[M + NA]+	471.0905	Quercetin 3-O-rhamnoside	$C_{21}H_{20}O_{11}$	448.101	0.62	C01750
	37	1.89	[M + H]+	731.1623	procyanidin B2 3'-O-gallate	$C_{37}H_{30}O_{16}$	730.1534	-2.26	CHEBI:75647
	45	2.10	[M+NA]+	487.0854	Myricitrin	$C_{21}H_{20}O_{12}$	464.0955	-1.38	C10108
	135	2.14	[M + H]+	449.1091	Kaempferol 3-O-glucoside	$C_{21}H_{20}O_{11}$	448.101	1.83	C12249
	145	2.08	[M + H]+	465.1033	Hyperin	$C_{21}H_{20}O_{12}$	464.0955	-1.12	C10073
	549	0.44	[M + H]+	449.1091	Kaempferol 3-O-glucoside	$C_{21}H_{20}O_{11}$	448.1006	2.72	C12249
	149	1.94	[M + H]+	291.0850	5-Deoxyleucocyanidin	$C_{15}H_{14}O_{6}$	290.079	-4.31	C09736
	165	1.98	[M + H]+	617.1169	Quercetin 3-(6"-galloylglucoside)	$C_{28}H_{24}O_{16}$	616.106	5.81	CID 44259190
	277	2.02	[M + H]+	443.0981	Catechin 5-O-gallate	$C_{22}H_{18}O_{10}$	442.09	1.85	CID 15689618
	312	2.12	[M + H]+	303.0503	Quercetin	$C_{15}H_{10}O_{7}$	302.0427	1.06	C00389
	394	2.20	[M + NA]+	485.1431	4,2'-Dihydroxy-4',6'-dimethoxychalcone 4-glucoside	$C_{23}H_{26}O_{10}$	462.153	1.88	CID 13870531
	460	0.41	[M + H]+	207.0357	(2S)-2-(5-chloro-2-thienyl)-1-hydrazino-propan-2-ol	$C_{7}H_{11}ClN_{2}OS$	206.028	2.04	CID 82077398
	581	2.04	[M + H]+	443.0981	(-)-epicatechin-5-gallate	$C_{22}H_{18}O_{10}$	442.09	-1.81	CHEBI:65848
	425	2.16	[M + H]+	587.1048	Quercetin 3-(2"-galloyl-alpha-L-arabinopyranoside)	$C_{27}H_{22}O_{15}$	586.096	2.51	CID 44259256
	409	1.92	[M + NH4]+	512.1772	Peruvianoside II	$C_{23}H_{26}O_{12}$	494.142	2.75	CID 42608014
	464	1.76	[M + H]+	447.0904	Baicalin	$C_{21}H_{18}O_{11}$	446.0849	-3.95	C10025
	541	12.77	[M + H]+	271.0604	Baicalein	$C_{15}H_{10}O_{5}$	270.0528	1.11	C10023
	548	2.02	[M + H]+	271.0604	Baicalein	$C_{15}H_{10}O_{5}$	270.0528	1.11	C10023
	591	1.82	[M + H]+	271.0604	Baicalein	$C_{15}H_{10}O_{5}$	270.0528	1.11	C10023
	183	2.20	[M + NA]+	441.0802	Kaempferol 3-O-beta-D-xyloside	$C_{20}H_{18}O_{10}$	418.09	2.39	C20727
	542	12.77	[M + NA]+	441.0802	Kaempferol 3-O-beta-D-xyloside	$C_{20}H_{18}O_{10}$	418.09	2.39	C20727
	543	2.09	[M + NA]+	441.0802	Kaempferol 3-O-beta-D-xyloside	$C_{20}H_{18}O_{10}$	418.09	2.39	C20727
	550	2.01	[M + NA]+	633.1449	Rutin	$C_{27}H_{30}O_{16}$	610.1534	3.67	C05625
	553	1.89	[M + NA]+	649.1396	6,8-Dihydroxykaempferol 3-rutinoside	$C_{27}H_{30}O_{17}$	626.148	3.75	CID 44260049
	588	2.34	[M + H]+	883.1735	Epiafzelechin 3-O-gallate-(4beta->6)-epigallocatechin 3-O-gallate	$C_{44}H_{34}O_{20}$	882.164	2.55	CID 14521010
	128	2.24	[M + NA]+	455.0961	Afzelin	$C_{21}H_{20}O_{10}$	432.106	2.11	C16911
	473	1.98	[M + H]+	479.0828	Miquelianin	$C_{21}H_{18}O_{13}$	478.075	1.09	CHEBI:66395

	500	2.00	[M + NA]+	501.0653	miquelianin	$C_{21}H_{18}O_{13}$	478.0747	2.89	CHEBI:66395
	406	2.21	[M + NA]+	609.0862	Quercetin 3-(2"-galloyl-alpha-L-arabinopyranoside)	$C_{27}H_{22}O_{15}$	586.096	1.74	CID 44259256
	192	2.19	[M + H]+	287.0556	Luteolin	$C_{15}H_{10}O_6$	286.0477	2.17	C01514
	129	2.17	[M + NA]+	457.0755	Quercetin 3-O-beta-D-xylopyranoside	$C_{20}H_{18}O_{11}$	434.085	2.95	CID 5320861
	278	2.05	[M + H]+	273.0763	Butin	$C_{15}H_{12}O_5$	272.068	3.68	C09614
	281	1.82	[M + H]+	273.0763	Butin	$C_{15}H_{12}O_5$	272.068	3.68	C09614
	427	2.04	[M + NA]+	501.1375	myrciacitrin I	$C_{23}H_{26}O_{11}$	478.1475	1.51	CHEBI:66417
	193	2.27	[M + H]+	623.1018	3,5,7-trihydroxy-2-[3,4,5-trihydroxy-2-[2,3,4-trihydroxy-6-(3,5,7-trihydroxychroman-2-yl)phenyl]phenyl]chromen-4-one	$C_{30}H_{22}O_{15}$	622.096	-2.38	CID 57691813
	280	2.13	[M + H]+	623.1018	3,5,7-trihydroxy-2-[3,4,5-trihydroxy-2-[2,3,4-trihydroxy-6-(3,5,7-trihydroxychroman-2-yl)phenyl]phenyl]chromen-4-one	$C_{30}H_{22}O_{15}$	622.096	-2.38	CID 57691813
	488	1.97	[M + NA]+	745.1761	(2S)-2-(3,4-dihydroxyphenyl)-8-[(2S,4R)-2-(3,4-dihydroxyphenyl)-7-hydroxy-5-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy-3,4-dihydro-2H-chromen-4-yl]-5,7-dihydroxy-2,3-dihydrochromen-4-one	$C_{36}H_{34}O_{16}$	722.185	2.62	CID 6325552
Alkaloids	15	0.47	[M + H]+	138.0549	Trigonelline	$C_7H_7NO_2$	137.0477	2.80	C01004
	54	8	[M + NH4]+	832.2419	Ulithiacyclamide G	$C_{35}H_{42}N_8O_7S_4$	814.20593	-2.63	100927669
	209	2.44	[M + H]+	253.1074	Anatoxin a(s)	$C_7H_{17}N_4O_4P$	252.0987	5.63	C19998
	577	2	[M + NA]+	375.1056	Berberastine	$C_{20}H_{18}NO_5$	352.118	-4.35	CHEBI:3065
amino acids	158	0.5	[M + H]+	175.1180	L-Arginine	$C_6H_{14}N_4O_2$	174.1117	-5.74	C02385
	162	1.53	[M + H]+	205.0977	L-Tryptophan	$C_{11}H_{12}N_2O_2$	204.09	2.06	C00078
	228	0.47	[M + H]+	132.1018	Isoleucine	$C_6H_{13}NO_2$	131.095	-3.66	C00407
	242	0.47	[M + H]+	118.0835	Norvaline	$C_5H_{11}NO_2$	117.079	-23.74	CID 439575
Fatty Acyls	98	5.24	[M+NA]+	321.3159	18-methylnonadecan-1-ol	$C_{20}H_{42}O$	298.3236	-10.49	CHEBI:84914
	200	4.28	[M + H]+	313.2747	18-oxononadecanoic acid	$C_{19}H_{36}O_3$	312.266	4.39	CID 5312917
	299	4.92	[M + H]+	267.2693	Olealdehyde	$C_{18}H_{34}O$	266.261	3.72	CID17029
	300	4.67	[M + H]+	267.2693	Olealdehyde	$C_{18}H_{34}O$	266.261	3.72	CID17029
Indoles	486	0.75	[M + H]+	188.0708	Indole-3-acrylic acid	$C_{11}H_9NO_2$	187.063	1.90	CID 5375048
	494	1.53	[M + H]+	188.0708	Indole-3-acrylic acid	$C_{11}H_9NO_2$	187.063	1.90	CID 5375048
Organic acids	285	2.04	[M + H]+	191.0707	1-naphthoic acid	$C_{11}H_{10}O_3$	190.063	2.21	C15102
	547	2.03	[M + H]+	123.0441	Benzoic acid	$C_7H_6O_2$	122.037	-1.47	C00180

	580	1.84	[M + H]+	123.0441	Benzoic acid	$C_7H_6O_2$	122.037	-1.47	C00180
	545	1.35	[M + H]+	327.0697	5-Galloylshikimic acid	$C_{14}H_{14}O_9$	326.064	-7.91	CID 460897
	585	0.7	[M + H]+	327.0697	5-Galloylshikimic acid	$C_{14}H_{14}O_9$	326.064	7.90	CID 460897
	433	2.18	[M + H]+	285.0612	(2R,3R)-2-(2-Methoxybenzoyloxy)-3-hydroxysuccinic acid	$C_{12}H_{12}O_8$	284.053	3.06	CID 70014677
	440	1.79	[M + H]+	181.0863	3-(2-hydroxypropyl)benzoic acid	$C_{10}H_{12}O_3$	180.079	0.28	CID 67568076
	97	7.76	[M + H]+	593.4588	2-triacontanoylbenzenesulfonic acid	$C_{36}H_{64}O_4S$	592.453	-2.50	CID 23652140
inorganic acid	31	12.82	[M + H]+	178.9506	diphosphoric acid	$H_4O_7P_2$	177.9432	-0.51	CHEBI:29888
	44	12.83	[M + H]+	258.9170	Triphosphoric Acid	$H_5O_{10}P_3$	257.91	-1.09	C00536
Trihydroxybenzoic acids	462	1.78	[M + H]+	315.0713	Norbergenin	$C_{13}H_{14}O_9$	314.064	0.06	CID 73192
Monolignols	309	1.76	[M + NA]+	395.1316	Syringin	$C_{17}H_{24}O_9$	372.142	0.89	C01533
	372	1.78	[M + NA]+	365.1212	Coniferin	$C_{16}H_{22}O_8$	342.1315	1.46	C00761
Amino alcohols	367	2.90	[M + H]+	280.2639	Crucigasterin E	$C_{18}H_{33}NO$	279.256	2.22	CID 46937368
1-monopalmitoylglycero derivative	333	4.11	[M+NA]+	353.2669	3-hexadecanoyl-sn-glycerol	$C_{19}H_{38}O_4$	330.277	-2.03	CHEBI:64757
Lumazine derivative	100	1.51	[M + K]+	369.0804	reduced 6-(hydroxymethyl)-8-(1-D-ribityl)lumazine	$C_{20}H_{16}O_7$	330.1176	-1.03	CHEBI:70986
2-benzofurans derivative	39	3.42	[M + H]+	149.0232	phthalic anhydride	$C_8H_4O_3$	148.016	0.74	CHEBI:36605
	268	3.42	[M + H]+	205.0860	3-Butylidene-7-hydroxyphthalide	$C_{12}H_{12}O_3$	204.0786	0.34	C09921
Others	495	1.78	[M + NA]+	507.0752	4-O,6-O-Digalloyl-D-glucopyranose	$C_{20}H_{20}O_{14}$	484.085	2.02	CID 101630404
	29	0.41	[M + H]+	203.0529	N-(3-Nitramido-4,5-dihydropyridazin-4-yl)nitramide	$C_4H_6N_6O_4$	202.045	3.07	CID 419333
	40	2.15	[M + H]+	494.1245	(2S)-2-amino-5-[[(1S)-2-(carboxymethylamino)-1-[(8-hydroxy-3-methyl-1,4-dioxo-2-naphthyl)sulfanylmethyl]-2-oxo-ethyl]amino]-5-oxo-pentanoic acid	$C_{21}H_{23}N_3O_9S$	493.116	2.47	CID 52942238
	49	12.83	[M + NA]+	356.8936	5-[(3,5-dichloro-4-pyridyl)sulfanyl]-4-nitro-thiophene-2-carbaldehyde	$C_{10}H_4Cl_2N_2O_3S_2$	333.904	1.14	CID 87562543
	50	2.44	[M + H]+	359.1494	2-[2-[(3-cyano-2-pyridyl)oxy]anilino]-N-(p-tolyl)acetamide (a)	$C_{21}H_{18}N_4O_2$	358.143	-2.46	CID 60537784
	81	2.44	[M + NA]+	381.1314	2-[2-[(3-cyano-2-pyridyl)oxy]anilino]-N-(p-tolyl)acetamide (b)	$C_{21}H_{18}N_4O_2$	358.143	-2.29	CID 60537784
	51	3.24	[M + NH4]+	335.2798	N-[1-(3-Aminophenyl)ethylideneamino]undecanamide	$C_{19}H_{31}N_3O$	317.247	-3.34	CID 3616475
	102	0.42	[M + H]+	247.0425	1-(3-hydroxy-2-thienyl)-3-phenyl-propane-1,3-dione	$C_{13}H_{10}O_3S$	246.035	0.89	CID 13004301
	121	0.44	[M + H]+	229.0317	9-hydroxy-2-thioxo-3H-phenalen-1-one	$C_{13}H_8O_2S$	228.025	-2.41	CID 88740582
	258	3.13	[M + NA]+	391.1649	Phthalic acid, 2-chloroethyl decyl ester	$C_{20}H_{29}ClO_4$	368.175	1.85	CID 6424038
	297	4.92	[M + H]+	359.3165	18-(2,3-dihydroxypropoxy)octadecan-2-one	$C_{21}H_{42}O_4$	358.308	3.32	CID 21475594

	298	4.67	[M + H]+	359.3165	18-(2,3-dihydroxypropoxy)octadecan-2-one	$C_{21}H_{42}O_4$	358.308	3.32	CID 21475594
	303	12.03	[M + H]+	247.1669	2,4-Diamino-6,7-diisopropylpteridine	$C_{12}H_{18}N_6$	246.1593	-1.32	CHEBI:73908
	379	1.84	[M+NA]+	563.1380	(3S,4S)-5,6,7-Trihydroxy-4-(4-hydroxy-3,5-dimethoxyphenyl)-3-[[(2R,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxymethyl]-3,4-dihydroisochromen-1-one	$C_{24}H_{28}O_{14}$	540.1479	-1.62	101437170
	455	2.05	[M + H]+	124.0869	2,5-dimethylpyrimidin-4-amine	$C_6H_9N_3$	123.0796	-0.02	chebi:46546
	20	0.42	[M + H]+	261.0582	8-methoxy-4,5-dihydrobenzo[g]benzothiophene-2-carboxylic acid	$C_{14}H_{12}O_3S$	260.051	-0.35	CID 81605969
	461	0.42	[M + H]+	185.0534	1-azido-4-nitro-2H-triazin-5-amine	$C_3H_4N_8O_2$	184.046	0.65	CID 22213878

Supplementary Table 6 ANOVA results for comparison of metabolites regulation between *Nepenthes* species (n = 4) and in response to the environmental conditions.

	Sum of squares	df	Mean square	F	p (same)
Species:	$1.56E^{+12}$	3	$5.20E^{+11}$	71.71	$5.08E^{-46}$
Metabolites:	$1.47E^{+14}$	248	$5.94E^{+11}$	81.83	< 0.001
Interaction:	$5.36E^{+13}$	744	$7.21E^{+10}$	9.928	< 0.001
Within:	$1.01E^{+14}$	13944	$7.26E^{+09}$		
Total:	$3.04E^{+14}$	14939			

Supplementary Table 7 The top 77 key metabolites with VIP scores over 1.

Metabolites	Comp. 1	Comp. 2
2S-2-amino-5-1S-2-carboxymethylamino-1-8-hydroxy-3-methyl-1,4-dioxo-2-naphthylsulfanylmethyl-2-oxo-ethylamino-5-oxo-pentanoic acid	2.4790	2.3770
6,8-Dihydroxykaempferol 3-rutinoside	2.3869	2.2928
1-naphthoic acid	2.3828	2.2852
Unknown 191.0705mz	2.2763	2.1846
Unknown 316.0751mz	2.2643	2.2018
Unknown 267.0506mz	2.2633	2.1994
Unknown 515.1534mz	2.2571	2.2026
Berberastine	2.2081	2.1228
Rutin	2.2073	2.1201
Unknown 495.1506mz	2.1677	2.1614
Unknown 286.0644mz	2.1382	2.0876
Unknown 366.1244mz	2.0804	2.0551
Unknown 297.0611mz	2.0500	1.9926
Quercetin 3-2"-galloyl-alpha-L-arabinopyranoside b	2.0391	1.9743
Unknown 634.1472mz	2.0381	1.9610
Quercetin 3-2"-galloyl-alpha-L-arabinopyranoside a	1.9638	1.8981

Unknown 329.0874mz	1.8615	1.7847
2R,3R-2-2-Methoxybenzoyloxy-3-hydroxysuccinic acid	1.8574	1.8037
Norbergenin	1.8392	1.7973
Unknown 376.1092mz	1.8334	1.7596
5-Galloylshikimic acid b	1.8310	1.7733
4,2'-Dihydroxy-4',6'-dimethoxychalcone 4-glucoside	1.8161	1.7864
Epiafzelechin 3-O-gallate-4beta-6-epigallocatechin 3-O-gallate	1.8126	1.7384
Peruvianoside II	1.7582	1.7704
myrciacitrin I	1.7509	1.6787
Unknown 143.0337mz	1.7267	1.6669
Unknown 435.0930mz	1.6919	1.6227
3,2-hydroxypropylbenzoic acid	1.6790	1.6851
Unknown 360.1661mz	1.6691	1.6703
3,5,7-trihydroxy-2-3,4,5-trihydroxy-2-2,3,4-trihydroxy-6-3,5,7-trihydroxychroman-2-ylphenylphenylchromen-4-one a	1.6672	1.6350
Unknown 884.1764mz	1.6525	1.5869
Unknown 553.2040mz	1.6072	1.5413
Unknown 281.0659mz	1.5821	1.5299
Unknown 496.1824mz	1.5566	1.5120
Unknown 163.0755mz	1.5285	1.4782
5-Galloylshikimic acid a	1.521	1.4624
Unknown 283.0817mz	1.4954	1.4742
18-2,3-dihydroxypropoxyoctadecan-2-one a	1.4693	1.4571
Unknown 153.0182mz a	1.4682	1.4320
Unknown 543.1846mz	1.4638	1.4164
Anatoxin as	1.4606	1.5070
Unknown 147.0652mz	1.4561	1.4633
Unknown 585.5947mz	1.4403	1.3993
2-2-3-cyano-2-pyridyloxyanilino-N-p-tolylacetamide a	1.4221	1.5039
2-2-3-cyano-2-pyridyloxyanilino-N-p-tolylacetamide b	1.4177	1.4271
Unknown 223.0093mz	1.4163	1.3824
Unknown 391.0810mz	1.4024	1.3900
Coniferin	1.3637	1.3711

Unknown 611.1618mz	1.355	1.3003
Unknown 650.1421mz	1.3438	1.2883
Unknown 582.5836mz	1.2629	1.3075
Unknown 502.0681mz	1.2352	1.1892
miquelianin b	1.2242	1.1754
Unknown 197.0897mz	1.2183	1.1744
Unknown 521.1271mz	1.2094	1.1612
Unknown 513.1807mz	1.2046	1.1563
Kaempferol 3-O-beta-D-xyloside b	1.2009	1.1545
Unknown 360.3245mz a	1.1563	1.1198
Unknown 341.1389mz	1.1357	1.1694
Unknown 360.3245mz b	1.1313	1.0974
Unknown 376.1758mz	1.1137	1.1103
Unknown 884.1767mz	1.1086	1.0639
Quercetin 3-O-beta-D-xylopyranoside	1.0940	1.0496
Unknown 303.2902mz b	1.0900	1.0831
Unknown 407.0548mz	1.0587	1.0188
Unknown 303.2902mz a	1.0430	1.0405
Unknown 554.5522mz	1.0357	1.0864
Unknown 341.3058mz b	1.0350	1.1868
Unknown 153.0182mz c	1.0336	0.99525
Unknown 189.0392mz	1.0266	0.99264
Unknown 107.0490mz	1.0254	1.0145
Unknown 341.3058mz a	1.0229	1.1808
2S-2-5-chloro-2-thienyl-1-hydrazino-propan-2-ol	1.0202	0.97918
Unknown 588.1073mz	1.0146	0.99209
Unknown 153.0182mz d	1.0101	1.0085
Unknown 651.1454mz	1.0011	0.96712

Supplementary Table 8 Two-way ANOVA results for comparison of metabolite profiles between *Nepenthes* species (n = 2).

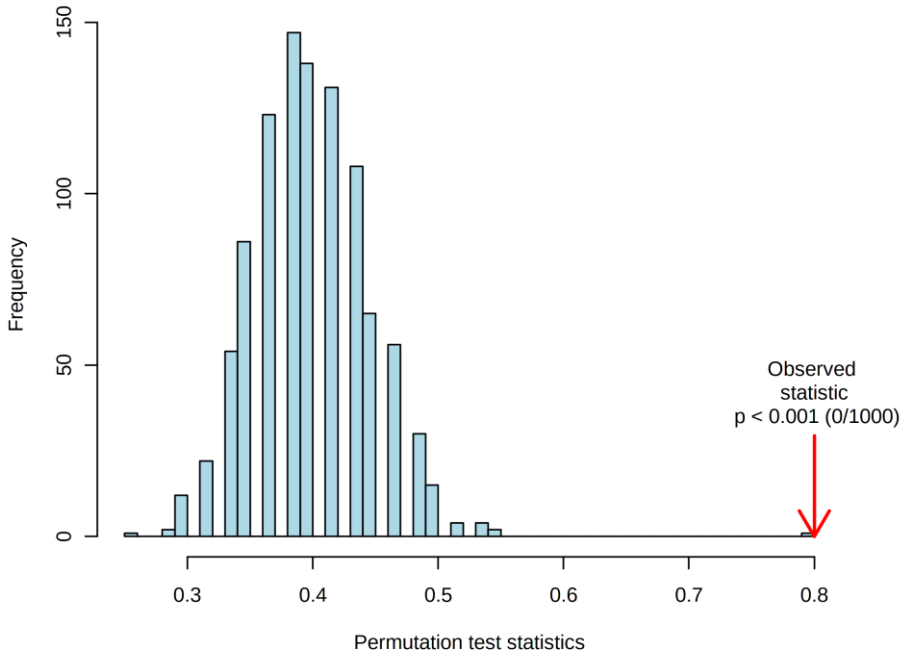
	Sum of squares	**df**	**Mean square**	**F**	**P-value**
***Nepenthes* species:**	38.03	1	38.03	19.38	< 0.001
Metabolite:	69.28	3	23.09	11.77	< 0.001
Interaction:	1.88	3	0.63	0.32	0.81
Within:	62.80	32	1.96		
Total:	171.98	39			

Supplementary Table 9 Tukey's post-hoc test results for comparison of antibacterial properties of the methanolic leaf extracts of *Nepenthes ampullaria* within the bacterium species.

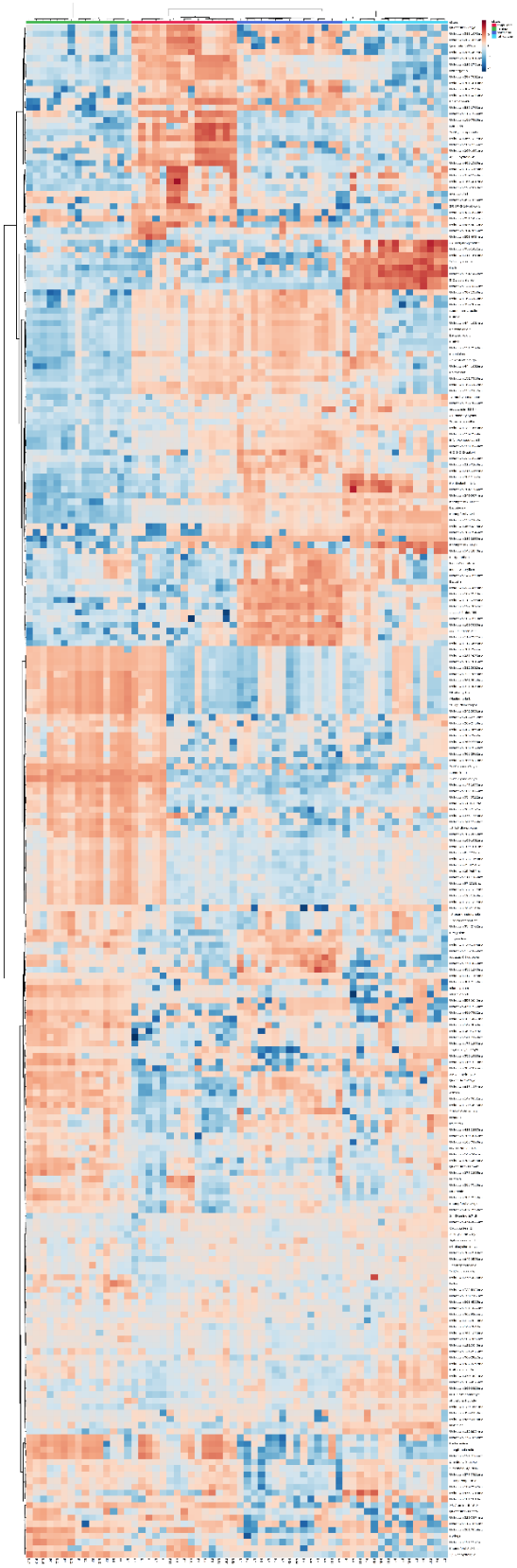
	Bacillius cereus	***Pseudomonas aeruginosa***	***Staphylococcus epidermidis***	***Bacillius subtilis***
Bacillius cereus		0.3241	0.02059	0.8134
Pseudomonas aeruginosa	0.3241		0.435	0.07443
Staphylococcus epidermidis	0.02059	0.435		0.003435
Bacillius subtilis	0.8134	0.07443	0.003435	

Supplementary Table 10 Tukey's post-hoc test results for comparison of antibacterial properties of the methanolic leaf extracts of *Nepenthes rafflesiana* within the bacterium species.

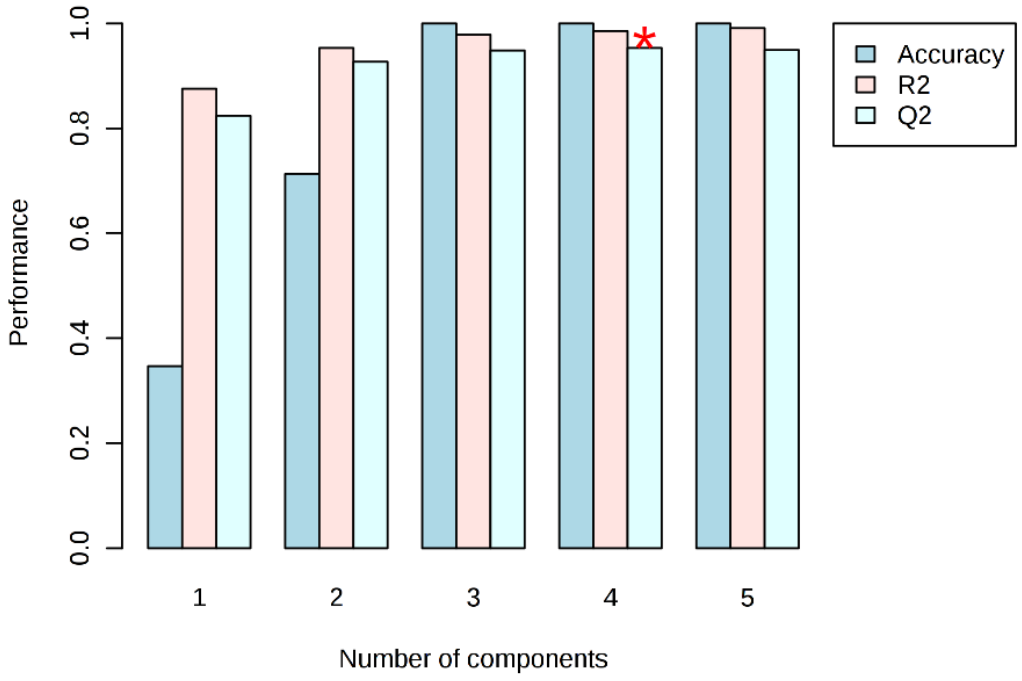
	Bacillius cereus	***Pseudomonas aeruginosa***	***Staphylococcus epidermidis***	***Bacillius subtilis***
Bacillius cereus		0.6625	0.02415	0.9955
Pseudomonas aeruginosa	0.6625		0.202	0.5255
Staphylococcus epidermidis	0.02415	0.202		0.0152
Bacillius subtilis	0.9955	0.5255	0.0152	



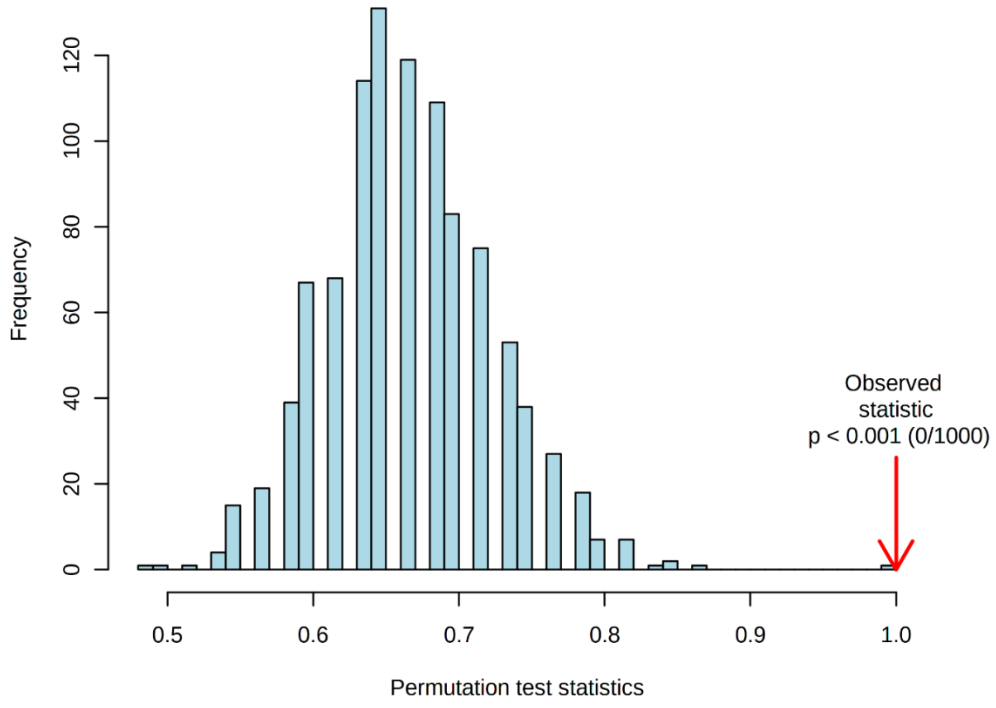
Supplementary Figure 1 Permutation test for model PLS-DA model validation.



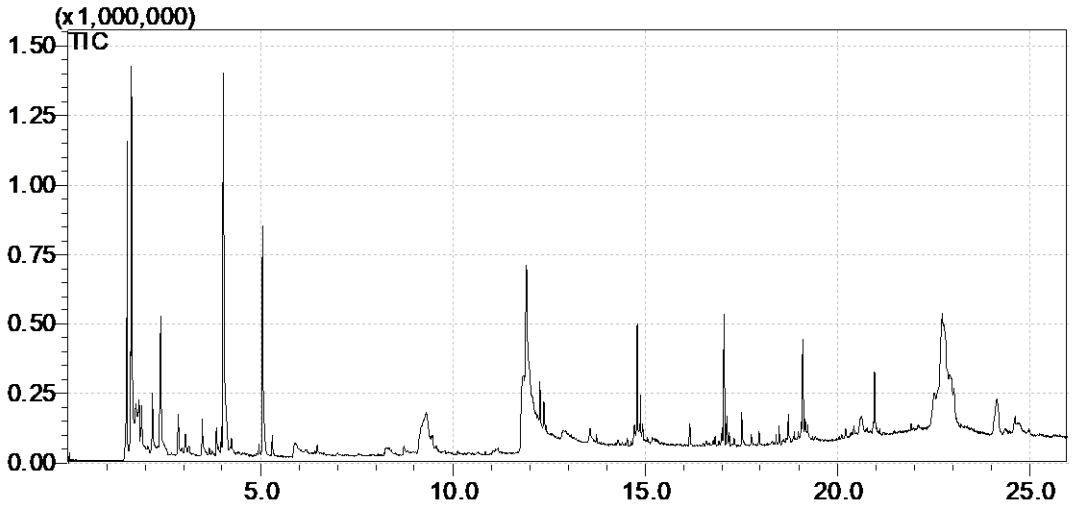
Supplementary Figure 2 Heatmap of 249 metabolites from the 4 *Nepenthes* species based on Euclidean distances and Ward clustering. The metabolites concentrations is represented on a log scale.



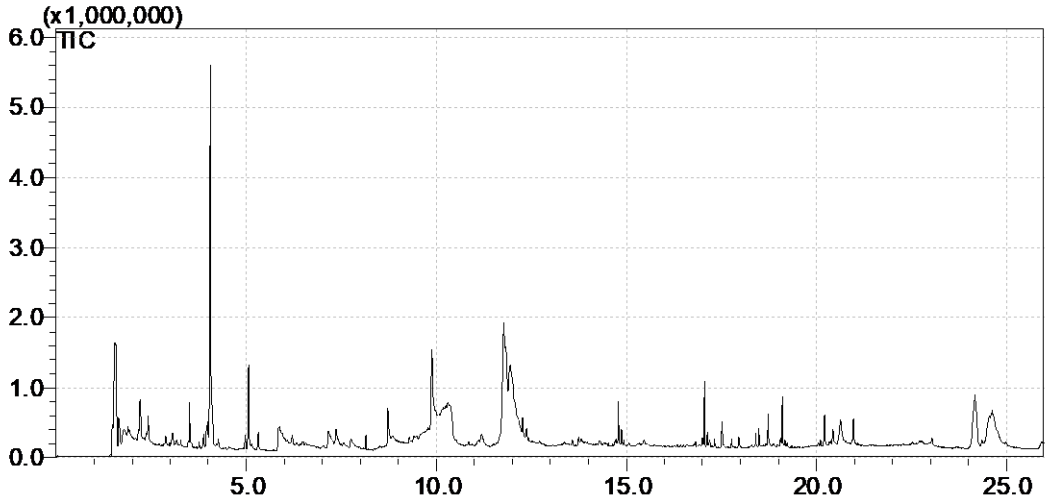
Supplementary Figure 3 Permutation test for PLS-DA model validation.



Supplementary Figure 4 Permutation test for PLS-DA model validation.



Supplementary Figure 5 GC–MS chromatogram of the methanolic leaf extract of *Nepenthes ampullaria*.



Supplementary Figure 6 GC–MS chromatogram of the methanolic leaf extract of *Nepenthes rafflesiana*.