Ethnobotany and the Biological Activities of Iningai and Mbabaram Medicinal Plants

Submitted by

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Disclaimer: This thesis contains information on medicinal plants used by the Mbabaram and Iningai Aboriginal communities. The information has been collected as per the Informed Consent Agreement, the Human Ethics Number (H8523), and Biodiscovery Act approval number (BIBC20200417-2).

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Statement of the Contribution of Others

Nature of assistance	Contribution	Name and affiliation
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Executive summary of the thesis

The Australian continent separated from Antarctica in the mid-Paleogene, c. 45 Ma, in the last act in the breakup of Gondwanaland, resulting in six biomes: desert, grassland, savannah, temperate forest, rainforest and Mediterranean heath. Australia became one of 17 mega-diverse countries in the world due to the wealth of unique and endemic plant species that now exist. The Aboriginal and Torres Strait Islanders have occupied this island continent for 65,000 years and are recognised as two distinct cultural groups with their own cultural practices, languages and beliefs. Aboriginal people mainly occupy the mainland, while the Torres Strait region is located between the tip of Cape York and Papua New Guinea and is made up of over two hundred islands. Prior to colonisation, there were over 250 different Aboriginal and Torres Strait Islander Nations clan groups and family lines with at least 300 distinctive Indigenous languages, cultures, and beliefs who learnt to adapt and live sustainably within nature through climatic and environmental changes. Aboriginal bush medicine knowledge and practices have survived in many regions, particularly in remote areas where cultural practices are still being performed despite the influence of colonial contact. However, due to elderly people passing away, there is a danger of losing bush medicine knowledge.

In our continuous efforts to document, preserve, promote and scientifically study Aboriginal bush medicine knowledge, the Tropical Indigenous Ethnobotany Centre (TIEC) has been established to safeguard biocultural knowledge. TIEC has been closely working with many Aboriginal communities of Queensland, especially Iningai and Mbabaram Aboriginal communities in Central and Far North Queensland. In this study, I conducted a literature review on Queensland medicinal plants, botanically identified medicinal plants used by two Aboriginal communities, and assessed them for their phytochemical and biological properties.

The review found that while many medicinal plants were recorded from the Aboriginal communities in Northern Territory, New South Wales, South Australia, and Western Australia, not much had been reported on Aboriginal medicinal plants of Queensland. This review identified 135 species of Queensland Aboriginal medicinal plants, including those used by the two communities selected for this study. These medicinal plants belong to 53 families (Myrtaceae representing the highest) and 103 genera. Many of these medicinal plants are native to Australia and are also used as bush food by Aboriginal peoples. Most widely used plants belong to the life form of trees, with their leaf part being most commonly collected for treating as many as 62 different types of ailments. Skin sores, wounds, cuts and infections were the most treated ailments. More than 48 medicinal plants were reported to be in use by Iningai and Mbabaram Aboriginal communities. In this study, I botanically identified more than 20 medicinal plants used by these two communities. However, given the time and the feasibility of my MPhil project, I collected eight species from each community and investigated

them for their major classes of phytochemicals, and the antioxidant and anti-inflammatory activities of their crude extracts.

From the Mbabaram medicinal plants study, three plants - *Persoonia falcata*, *Corymbia citriodora*, and *Tephrosia turpinii*, showed very promising preliminary results and were selected for ongoing biodiscovery projects. *B. oblongifolia* leaves also significantly suppressed all four pro-inflammatory cytokines, indicating good potential for a biodiscovery project. *Exocarpus latifolius* did not show significant suppression of any of the pro-inflammatory cytokines. From the Iningai medicinal plants study, seven plant extracts showed good antioxidant activities by significantly inhibiting the four pro-inflammatory cytokines TNF, IFN-γ, MCP-1, and IL-23 as determined by LPS-activated human PBMCs assay. The three most active crude leaf extracts were from TB75, *G. australe, V. microcalyx*, *S. lanceolatum* and TB79. *Dodonaea tenuifolium* showed the weakest anti-inflammatory capacity. These plants have future applications in biodiscovery projects.

In conclusion, these studies validated Indigenous Biocultural Knowledge by identifying major classes of phytochemicals, toxicity profiles and antioxidant and anti-inflammatory properties. It is recommended that further research on these medicinal plants to be undertaken to identify antioxidant and anti-inflammatory drug lead compounds which may lead to novel therapeutics.

List of Abbreviations

ATH	Australian Tropical herbarium
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DPPH	2,2-Diphenyl-1-picrylhydrazyl
FBS	Bovine foetal serum
FNQ	Far North Queensland
GAE	Gallic acid equivalent
GC-MS	Gas chromatography mass spectrometry
GI	Gastrointestinal tract
HBD	Healthy blood donors
IC_{50}	50% inhibitory concentration
IBD	Inflammatory bowel diseases
IFN-γ	Interferon-gamma
IL	Interleukin
LPS	Lipopolysaccharide
MCP-1	Monocyte chemotactic protein 1
NO	Nitric oxide
PBS	Phosphate buffered saline
PBMC	Peripheral blood mononuclear cell
ROS	Reactive oxygen species
SD	Standard deviation
TNF	Tumor necrosis factor
TPC	Total phenolic content
WHO	World health organization

Publications from the Thesis

1. **Turpin, G.,** Ritmejerytė, E., Jamie, J., Crayn, D., and Wangchuk, P. (2022). Aboriginal medicinal plants of Queensland: ethnopharmacological uses, species diversity, and biodiscovery pathways. *Journal of Ethnobiology and Ethnomedicine* 18: article 54. https://doi.org/10.1186/s13002-022-00552-6
2. **Turpin, G.**, Crayn, D., Thompson, S., Yeshi, K., and Wangchuk, P. (2024). Phytochemical content and antioxidant and anti-inflammatory activities of Iningai Aboriginal medicinal plants of central Queenslan, Australia. *Heliyon* (**Submitted**)
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Publications during candidature not included in the thesis

1. Cooke, P., Fahey, M., Ens, E.J., Raven, M., Clarke, P.A., Rossetto, M. and **Turpin, G.** (2022). Applying biocultural research protocols in ecology: Insider and outsider experiences from Australia. *Ecological Management and Restoration*, 23: 64-74. https://doi.org/10.1111/emr.12545.
2. Cooke, P., Ens, E., Clarke, P., Chang, H., Rossetto, M., Crayn, D., **Turpin, G**. and Ferrier, A. (2024). Not all edible nuts are eaten: Evidence for continued Aboriginal cultural use and dispersal of Bunya Pine (*Araucaria bidwillii*) in southern but not in northern Queensland. *Journal of Ethnobiology*, 44(2): 129-140. https://doi.org/10.1177/02780771241246853.
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Awards and Presentations During Candidature

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Presentations

1. **Keynote Speaker**. "Tropical Indigenous Ethnobotany Centre –Indigenous Biocultural Knowledge". Cairns Indigenous Art Festival. 2022.
2. **Chair.** Indigenous Symposium. Ecological Society of Australia. Wollongong, NSW. November 2022.
3. **Plenary Speaker**. "Tropical Indigenous Ethnobotany Centre and it's work on Indigenous Engagement and Research, Protection of Indigenous Biocultural Knowledge and Qld's Biodiscovery Act ". 2nd First Nations Self-Determination, Representation & Wellbeing: Cultural integrity and economic development. Twin Waters, Sunshine Coast, Qld.2023
4. **Panel Speaker.** "Indigenous Knowledge Databases – Sovereignty, Protocols & Future Food Webinar. University of Technology, Sydney, NSW. November 2021.
5. Invited Forum Participant. Food and Agricultural Organisation of the United Nations. Rome, Italy. January 2024.

6. Visiting Researcher. CHS Seminar. “Tropical Indigenous Ethnobotany Centre – Saving Ancient Indigenous Biocultural Knowledge to help provide solutions for current global problems”. University of Adelaide. November 2023.
7. Visiting Researcher. Delivered seminar. “Tropical Indigenous Ethnobotany Centre”. SA State Herbarium and Adelaide Botanic Gardens. November 2023.
8. Visiting Researcher. Invited Speaker. “Tropical Indigenous Ethnobotany Centre”. Whyalla community workshop, SA. November 2023
9. 2023 JCU Library – SAGE Publishing Grant Winner. Seminar.” Ethnobotanical Identification and Biological Activities of Selected Mbabaram Medicinal Plants and its Phytochemicals. James Cook University, Townsville. October 2023.
10. Queensland Herbarium & Biodiversity Science Symposium speaker. “Tropical Indigenous Ethnobotany Centre”. November 2023
11. Healing Our Spirit Worldwide Conference. Workshop Participant. Vancouver, Canada. September 2023.
12. Inaugural Biri Bulmba Budang National Custodians of Country Gathering. Panel Speaker. “Tropical Indigenous Ethnobotany Centre”. Djabugay Country, Tjapukai (Djabugay) Cultural Park Cairns, QLD. August 2023.
13. Indigenous Symposium. Chair. Ecological Conference of Australia. Darwin, NT. July 2023.
14. NAIDOC event. Invited Speaker. James Cook University, Smithfield. July 2023.
15. Invited Speaker. TESS seminar. Tropical Indigenous Ethnobotany Centre – “Saving Ancient Indigenous Biocultural Knowledge” James Cook University, Smithfield. May 2023
16. Invited Speaker. Australian Bushfoods Conference. “Tropical Indigenous Ethnobotany Centre & Aboriginal Bushtucker and Medicinal Plants Project”. Australian Technology & Agricultural College’ North Maclean, Qld. May 2023.
17. Invited Speaker. “Tropical Indigenous Ethnobotany Centre (Intellectual Property & Biodiscovery Act)”. National Museums, Scotland, Powell-Cotton Museum), Royal Botanic Gardens, Kew. Edinburgh, Scotland. November 2022.
18. Forum Participant. Joint Society for Economic Botany and International Society of Ethnobiology Conference. Kingston, Jamaica. May 2022.
19. Invited Speaker. Indigenous Science Experience Open Day, National Science Week. Redfern, Sydney, NSW. August 2022

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Chapter 1. Introduction

1.1 General Introduction

The isolation of the Australian landmass in the Southern Ocean occurred when, in the last act in the breakup of Gondwanaland, it separated from Antarctica in the mid-Paleogene, c. 45 Ma. Since then, climatic and geological changes on the Australian continent have resulted in six biomes: desert, grassland, savannah, temperate forest, rainforest, and Mediterranean forest. These plant communities are a wealth of unique and endemic plant species, making Australia one of 17 mega-diverse countries worldwide (Williams et al., 2001).

This mega-diverse island continent of Australia has been occupied by the Aboriginal and Torres Strait Islander people for at least 65,000 years (Clarkson et al., 2017). The Aboriginal and Torres Strait Islander people are recognised as two distinct cultural groups with their cultural practices, languages and beliefs. However, both groups have similar close connections with their country, waterways, and nature. The Torres Strait region is located between the tip of Cape York and Papua New Guinea and comprises over two hundred islands. Seventeen of these islands are inhabited. There are also two Torres Strait Islander communities, Bamaga and Seisia, on the northern peninsula of mainland Australia. While the mainland is the homeland of the Aboriginal people, the Aboriginal Kaurareg Nation exists in the Torres Strait.

Prior to colonisation, there were over 250 different Aboriginal and Torres Strait Islander Nations, clan groups and family lines with at least 350 distinctive Indigenous languages, cultures, and beliefs who have learnt to live sustainably within nature. They have adapted through climatic and environmental changes, developing an intimate and profound relationship with the environment, and accumulated knowledge largely determined by their environment. Aboriginal cultural and language practices have been disrupted by the arrival of Europeans, who superimposed Western systems over Aboriginal traditions(Simpson et al, 2013) Consequently, Aboriginal people have lost a lot of their language and biocultural knowledge (Packer et al., 2019). Today, only 123 languages remain, with 12 spoken fluently, and all are endangered or critically endangered according to the Nation Indigenous Language Report 2020 (Dinku, et al., 2020). For example, the Mbabaram language of Far North Queensland has only 300 words left and is critically endangered.

Despite the influence of colonial contact, Aboriginal bush medicine knowledge and practices have survived in many remote areas where cultural practices are still being performed using native flora, fauna and abiotic materials (Packer, Gaikwad, et al., 2012). Their bush medicine knowledge differs in areas of Australia since it is shaped by the environments in which communities reside, and most medicinal plants remain unstudied using a Western scientific lens. Thus, ethnopharmacological research is a largely untapped resource (Packer, Brouwer, et al., 2012). Additionally, some long-lost biocultural information from the communities can be found in archival sources, such as early botanical textbooks,

explorer journals, archives, and old newspaper articles. Some of these records reveal extensive use of Aboriginal medicinal plants across Australia (Barr, 1988). They are widely used in the pockets of places like the Northern Territory and the Tropical Far North Queensland (FNQ) (Simpson et al., 2011). Denis Consiten, the first assistant surgeon to Surgeon-General John White in the first fleet, was the first European medical practitioner to 'discover' Indigenous medicinal plants. However, his records do not clarify whether he interacted with Indigenous informants.

Bush medicine practices are orally passed down from father to son or master to apprentice. As a result of oral transmission, there is a risk of diluting the knowledge or getting lost because of a lack of acceptance and recognition from the government. Therefore, there is an urgent need to document the surviving customary knowledge of the Aboriginal and Torres Strait Islander communities, especially the ethnomedical knowledge of remaining Elders before the knowledge is lost forever (Hill et al., 2011). The Tropical Indigenous Ethnobotany Centre (TIEC), housed within the Australian Tropical Herbarium (ATH), is an Indigenous-driven initiative for engaging and supporting the Traditional Owner groups in Queensland to document and add value to some of these unrecorded medicinal plants, especially those plants used by Aboriginal people in Queensland.

1.2 Rationale

In efforts to preserve Aboriginal bush food and bush medicine knowledge, TIEC researchers have been working very closely with Mbabaram and Iningai Aboriginal communities in Queensland for a decade to document and commercialise their bush medicine knowledge. While the Mbabaram (Bar-Barrum) community live in Far North Queensland, the Iningai community resides in Central Queensland; therefore, their environment, vegetation, and biocultural knowledge are different. The Mbabaram peoples' traditional lands extend to the top of the Great Dividing Range near the Walsh River Spring, bounded by the Walsh River near Dimbulah and includes the present towns of Watsonville, Irvinebank, Almaden and south to Mt Garnet. On the other hand, the Iningai country is situated in the desert uplands bioregion. It spans about 50,500 km^2 west of the Dividing Range to Longreach, including Aramac and Muttaburra to the north and south along Alice River. The Iningai community is one of the largest Aboriginal nations in Central West Queensland.

These two nations' biocultural knowledge remains little documented and less studied scientifically. Documenting their knowledge is important for preserving Aboriginal culture and traditional medical knowledge for future generations, but also beneficial for guiding the next generation of drug discovery from tropical medicinal plants. More than 70% of modern drugs were discovered from the global medicinal plants used in traditional medicine (Wangchuk, 2018). Since many Mbabaram and Iningai medicinal plants have not been thoroughly investigated previously for their chemical and biological activities, there is huge potential for the biodiscovery of novel drug lead molecules. In addition, there is huge potential for their ethnobotanical knowledge to guide the development of over-the-counter (OTC) products such as food supplements, energy drinks, nutraceuticals, essences, and

beauty products. The communities aspire to add value to their Bush medicine knowledge and utilise it to benefit not only themselves through income generation but also all humanity in general, using their medical knowledge to guide drug discovery and development, especially for treating chronic inflammation and healthy ageing.

In this study, I hypothesised that Iningai and Mbabaram medicinal plants used to detox and treat inflammation-related diseases may possess antioxidant and anti-inflammatory properties. This study aimed to identify 8-15 potential bush medicinal plants and collect, extract, and validate the traditional uses of Aboriginal medicinal plants (bush medicine) using Western scientific protocols.

1.3 Objectives

To validate my hypothesis, my project aims to achieve the following objectives:

- To conduct an extensive literature review on medicinal plants used by Aboriginal people in Queensland.
- To identify medicinal plants and determine major classes of phytochemicals and biological properties of the crude extracts of five selected Iningai Aboriginal medicinal plants.
- To identify medicinal plants and determine major classes of phytochemicals and biological properties of the crude extracts of eight selected Mbabaram Aboriginal medicinal plants.

1.4 Logical framework approach and expected outcomes

To achieve the above aims, I have approached my project in the following manner. First, I conducted an extensive literature review on Queensland medicinal plants, including those used by Iningai and Mbabaram Aboriginal communities. Then, I chose two Aboriginal communities in Queensland that I had worked closely with before the present project. Since Aboriginal communities use many medicinal plants, I developed the following selection criteria for investigating the plant candidates for the present project.

Criterion 1. Medicinal plants are used for treating inflammation and wound healing.

Criterion 2. Medicinal plants that have not been studied before for their phytochemicals and biological activities.

Once potential study species were selected, the research proceeded in the following stages:

i. Human ethics, informed consent, and collection permit approvals were obtained from James Cook University, the Iningai and Mbabaram Aboriginal communities, and the Queensland government.
ii. Fieldwork was undertaken on Iningai and Mbabaram Country, and target plants were botanically identified and collected.
iii. Voucher specimens were deposited at the Australian Tropical Herbarium.

iv. Collected plants were dried, and extractions were performed using both water (as used by aboriginal people) and ethanol (a universal solvent for extracting a wide range of phytochemicals).
v. Crude extracts were investigated for their major classes of phytochemicals and were tested for antioxidant and anti-inflammatory properties as per the traditional uses of medicinal plants.

My project has the potential to usher the following short-term and long-term outcomes:

i. Generate data to provide scientific validation of the Aboriginal ethnomedicinal uses of selected medicinal plants.
ii. Identify medicinal plant candidates for isolating novel drug lead molecules.
iii. Generate publications, including scientific papers and a bush medicine handbook on selected medicinal plants.
iv. Inspire researchers and pharmaceutical companies to work with the Aboriginal communities.
v. Garner respect for and appreciation of Aboriginal bush medicine knowledge among non-Aboriginal communities and Western medical professions.
vi. Develop commercial antioxidant and anti-inflammatory bush medicine products.
vii. Scientific data is archived in the Miromaa database to preserve knowledge and serve as scholarly resources for communities and researchers.
viii. Furnish information that could help set up herbal and agricultural ventures.

1.5 The layout of the thesis

This thesis comprises an introductory chapter, three data chapters, and a concluding chapter.

Chapter 1 introduces the thesis and provides the framework/layout of the thesis.

Chapter 2 reviews the literature on Australian Queensland Aboriginal medicinal plant information sourced from published books and archival material, including botanical textbooks and explorer journals. It provides the background of the Australian Queensland Aboriginal peoples' who have lived on this continent for millennia and a description of the intimate biocultural knowledge of the landscape and environment that was accumulated by living within the ecology and adapting through climatic and environmental changes. The review identified 135 medicinal plant species, with the majority being native to Australia, and identified 62 different diseases that were treated with the medicinal plants, mostly skin sores and infections.

Chapter 3 investigates the phytochemical content and the antioxidant and anti-inflammatory activities of Aboriginal medicinal plants of Central Queensland, Australia. It describes the collaboration between The Tropical Indigenous Ethnobotany Centre, the Australian Tropical Herbarium, the Australian

Institute of Tropical Health and Medicine (AITHM), James Cook University, and key members of the Iningai community near Barcaldine, Queensland, Australia. It describes the Iningai community, their environment, fieldwork, botanical identification and collection of selected medicinal plants. It highlights the phytochemical content, cell viability effect and anti-inflammatory activities of eight Iningai medicinal plants. The manuscript from this chapter was submitted as a research article to the *Heliyon* journal and is currently under review.

Chapter 4 investigates selected Mbabaram medicinal plants for their phytochemicals and antioxidant and anti-inflammatory activities. The manuscript for this chapter is prepared and submitted to the *Journal of Ethnopharmacology* (JEP).

Chapter 5 summarises the findings from this project, discusses the broader context of Indigenous-led biodiscovery research on medicinal plants, and offers some suggestions for future research directions.

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Chapter 2. Aboriginal medicinal plants of Queensland: ethnopharmacological uses, species diversity, and biodiscovery pathways

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REVIEW Open Access

Aboriginal medicinal plants of Queensland: ethnopharmacological uses, species diversity, and biodiscovery pathways

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Abstract

Background: Aboriginal peoples have occupied the island continent of Australia for millennia. Over 500 different clan groups or nations with distinctive cultures, beliefs, and languages have learnt to live sustainably and harmoniously with nature. They have developed an intimate and profound relationship with the environment, and their use of native plants in food and medicine is largely determined by the environment they lived in. Over 1511 plant species have been recorded as having been used medicinally in Australia. Most of these medicinal plants were recorded from the Aboriginal communities in Northern Territory, New South Wales, South Australia, and Western Australia. Not much has yet been reported on Aboriginal medicinal plants of Queensland. Therefore, the main aim of this review is to collect the literature on the medicinal plants used by Aboriginal peoples of Queensland and critically assess their ethnopharmacological uses.

Methods: The information used in this review was collected from archival material and uploaded into the Tropical Indigenous Ethnobotany Centre (TIEC) database. Archival material included botanist's journals/books and old hard copy books. Scientific names of the medicinal plant species were matched against the 'World Flora Online Plant List', and 'Australian Plant Census' for currently accepted species names to avoid repetition. An oral traditional medical knowledge obtained through interviewing traditional knowledge holders (entered in the TIEC database) has not been captured in this review to protect their knowledge.

Results: This review identified 135 species of Queensland Aboriginal medicinal plants, which belong to 103 genera from 53 families, with Myrtaceae being the highest represented plant family. While trees represented the biggest habit, leaves were the most commonly used plant parts. Of 62 different diseases treated by the medicinal plants, highest number of plants are used for treating skin sores and infections. Few plants identified through this review can be found in other tropical countries but many of these medicinal plants are native to Australia. Many of these medicinal plants are also used as bush food by Aboriginal peoples.

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2.1. Abstract

Background: Aboriginal peoples have occupied the island continent of Australia for millennia. Over 500 different clan groups or nations with distinctive cultures, beliefs and languages have learnt to live sustainably and harmoniously with nature. They have developed an intimate and profound relationship with the environment and their use of native plants in food and medicine is largely determined by the environment they lived in. Over 1,511 plant species have been recorded as having been used medicinally in Australia. Most of these medicinal plants were recorded from the Aboriginal communities in Northern Territory, New South Wales, South Australia and Western Australia. Not much has yet been reported on Aboriginal medicinal plants of Queensland. Therefore, the main aim of this review was to collect the literature on the medicinal plants used by Aboriginal peoples of Queensland and critically assess their ethnopharmacological uses.

Methods: The information used in this review was collected from archival material and uploaded into the Tropical Indigenous Ethnobotany Centre database. Archival material included explorer's journals, bulletins, proceedings, reports, books and old newspapers. We also collected information from journal papers. Scientific names of the medicinal plant species were compared against the 'The Plant List', and 'Australian Plant Census' for currently accepted species names to avoid repetition. The data coverage was from the earliest recorded data until February 2022. A limited amount of oral traditional medical knowledge from TIECs ethnobotany projects has been captured in this review where permission has been given to use the knowledge.

Results: This review identified 135 species of Queensland Aboriginal medicinal plants, which belong to 103 genera from 53 families, with Myrtaceae being the highest represented plant family. While trees represented the biggest life form, leaves were most used plant parts. Of 62 different diseases treated by the medicinal plants, highest number of plants are used for treating skin sores and infections. Few plants identified through this review can be found in other tropical countries but many of these medicinal plants are native to Australia. Many of these medicinal plants are also used as bush food by Aboriginal peoples.

Conclusion: Through extensive literature review, we found that 135 medicinal plants native to Queensland are used for treating 62 different diseases especially skin sores and infection. Since these medicinal plants are also used as bush food and are rarely studied using the scientific protocols, there is a huge potential for bioprospecting and bush food industry.

2.2. Introduction

Globally, Indigenous Biocultural Knowledge (IBK) is gaining increasing recognition for its potential value in contemporary biodiversity conservation, land management and biodiscovery (Ens et al., 2015). Despite recent scientific medical advancements, Traditional Medicines (TM) based on IBK have gained significant attention due to growing healthcare demands and are considered as a primary

healthcare modality. The World Health Organisation (WHO) acknowledges the importance of TM and describes it as the "sum total of the knowledge, skill, and practices based on the theories, beliefs, and experiences indigenous to other cultures, whether explicable or not, used in the maintenance of health, as well as in the prevention, diagnosis, improvement or treatment of physical and mental illnesses" (WHO, 2019). Other terms used for traditional medicines are complimentary, alternative, integrative, and customary medicines.

The WHO uses the term Traditional and Complimentary Medicines (TCM) (WHO, 2019) and it considers TCM an important and often underestimated health resource, particularly in the prevention and management of lifestyle related chronic diseases (WHO, 2019). The WHO Traditional Medicine Strategy 2014–2023, focusses on developing norms, standards, and technical documents based on reliable information and data. These will be used to provide support to its Member States in providing TCM services and its integration into their health systems. Currently, more than 85–90% of the world's population uses Indigenous medicines for primary health care and approximately 50,000–70,000 plant species are used in Indigenous medicines (Wangchuk, 2018). In India and China, 20% and 19% of the local flora is used for treating various disorders, respectively (Wangchuk & Tobgay, 2015) and more than 25% of prescription drugs worldwide are derived from plants with many more synthetic drugs obtained from phytochemical precursors (Rates, 2001; Wangchuk, 2018).

Aboriginal peoples have occupied the island continent of Australia for millennia. Over 500 different clan groups or nations with distinctive cultures, beliefs and languages have learnt to live harmoniously within the varied eco-floristic zones: Wet Tropics, Savannahs, Evergreen forests, Shrublands, Grasslands, and Wetlands (Australian Government, 2021; Keith, 2017). While there is a large number of different tribal groups in these vastly different regions, the commonality of all these groups is their intimate and profound relationship with the environment and the use of their resources (Maclean et al., 2021). Their knowledge and use of native plants plays significant and multiple roles in their lives, providing people with resources to make food, medicine, narcotics, stimulants, adornments, ceremonial objects, weapons, clothing, shelter, tools, and artwork (Clarke, 2007; Stump, 2018). This Aboriginal knowledge lore has evolved over thousands of years, using native flora, fauna and abiotic materials (Packer et al., 2019). Without a written language, knowledge is maintained and shared as Aboriginal lore, the customs and stories have been transferred intergenerationally through songs, stories, dance, and art.

Despite the disruption of oral traditions and lore practices by colonial contact, there are still thousands of Aboriginal people who speak their traditional language and retain the knowledge, songs and customs of their ancestors with English being the second or third language (Marmion, Obata, & Troy, 2014; Pearn, 2005). Aboriginal ethnomedicinal knowledge is still widely used by various Aboriginal clan groups but the extent to which it is practiced varies widely amongst communities across

Australia and between urban and rural regions (Oliver, 2013). Aboriginal plant knowledge can be considered the oldest living pharmacopoeia, with many plants still being consumed as bush food and bush medicine within Aboriginal communities, especially in remote areas (Packer et al., 2012).

Overall, over 1,511 plant species have been recorded as having been used medicinally in Australia (Packer et al., 2012; Wangchuk & Tobgay, 2015). Most of the medicinal plant knowledge recorded in the literature belongs to the Aboriginal communities in Northern Territory, New South Wales, South Australia and Western Australia (B. Simpson, Claudie, M Smith, McKinnon, & Semple, 2013). Not much has yet been reported on Aboriginal medicinal plants of Queensland, with only limited publications on Aboriginal medicinal plants of Cape York Peninsula (Ndi et al., 2016; B. S. Simpson et al., 2011). There is still a large number of Aboriginal medicinal plants that are either unrecorded in the literature or not available in the public domain (Simpson et al., 2013). Much of this knowledge has been underexplored in a 'Western scientific' sense, and like much Indigenous knowledge worldwide, Australian Aboriginal knowledge is being eroded and is in critical danger of being lost forever (Jamie, 2020). This review describes and discusses 135 medicinal plants used customarily by Aboriginal people of Queensland (Figure 2.1).

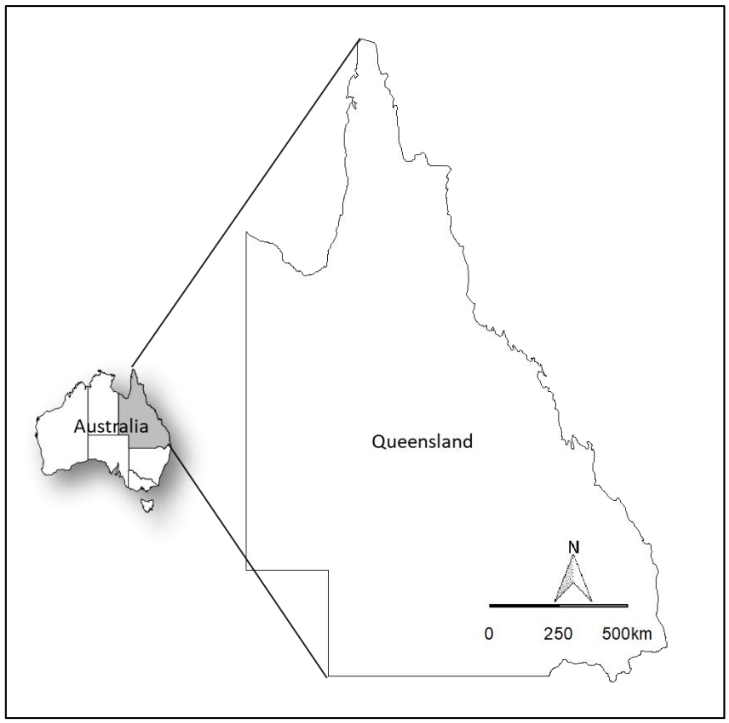

Figure 2.1 Map of Australia and the State of Queensland.

2.3. Methods

The information used in this review was collected from archival material (uploaded into the Tropical Indigenous Ethnobotany Centre database -TIEC) (Turpin, 1995), which included old explorer's journals, journal papers, bulletins, proceedings, reports, books and old newspapers. We also thoroughly searched the online databases and platforms such as Web of Science, Scopus, PubMed and journal

websites using the key words such as Aboriginal medicinal plants, Queensland medicinal plants, bush medicine and bush food. A list of medicinal plants containing important information such as family, life form, parts used, and ethnopharmacological uses was tabulated. Scientific names of the medicinal plant species were compared against the 'The Plant List' (The Plant List, 2013) and 'Australian Plant Census' (CHAH, 2021) for currently accepted species names to avoid repetition. The data coverage was from the earliest recorded data until February 2022. A limited amount of oral traditional medical knowledge from TIEC ethnobotany projects has been captured in this review where permission has been given to use the knowledge. It is worth mentioning here that a number of medicinal plants that are not available in the public domain were excluded from this review as the Aboriginal communities want to protect their intellectual property rights. Since this review is on ethnobotanical aspects purely based on the information that is available online, we did not include voucher specimen numbers and other scientific information such as phytochemical and pharmacological activities of medicinal plants.

2.4. Results and discussion

2.4.1. Historical perspective and contribution of Aboriginal medicines

To put this review into overall Australian ethnomedical context, we analysed the literature to see what the contribution of Aboriginal health system in Australia was. Historical studies and records of Aboriginal medicinal plants (e.g. Barr et al., 1988; Clarke, 2013; Collins, Culvenor, Lamberton, Loder, & Price, 1990; Cribb & Cribb, 1981; Lassak & McCarthy, 1983; Reid & Betts, 1979; Webb, 1969) reveal their extensive use across Australia. While the medical practices of Aboriginal communities in Australia involve the use of plants (Covacevich, Irvine, & Davis, 1988; Lassak & McCarthy, 1992), there is little documentation of European use of this knowledge in early botanical texts. Stack (1989) writes that two hundred years ago, Aboriginal traditional medicine played no part in the lives of the European immigrants since they brought their own diseases and used their own traditional remedies. However, there are records, which show that during the early years of European settlement, some medical practitioners and botanists interacted with Aboriginal healers and experimented with native flora for medicinal purposes. For example, Denis Considen (1788-1794) (first assistant surgeon to Surgeon-General John White in the first fleet) claimed to be the first European medical practitioner to discover Indigenous medicinal plants; however, his methods of discovery are unknown and it is unclear if he involved Indigenous informants (MacPherson, 1927). Considen documented some Indigenous medicinal plant efficacy including myrtle (possibly *Eugenia australis*) and yellow gum (possibly *Xanthorrhoea hastilis*) for dysentery, and native sarsaparilla (*Smilax glycyphylla*) as an antiscorbutic (MacPherson, 1927). MacPherson (1927) further suggested that native sarsaparilla was not only therapeutic but was considered more pleasant than Jamaican or Central American sarsaparilla. He also stated that prior to 1927 it had been a common article of trade among Sydney herbalists. One of the widely traded plants by both the Aboriginal people and the European settlers was macadamia nuts (*Macadamia* sp.).

Despite disruption of Aboriginal medical practices by Europeans, there have been some major contributions by Aboriginal Peoples to world medicinal knowledge. For example, Pearn (2005) suggests that Aboriginal child care ethnomedicinal knowledge represents the "The world's longest surviving paediatric practices" (Pearn, 2005). Even today, Aboriginal ethnomedical practices form a living treasure trove of many Aboriginal communities. The use of medicinal plants by different communities across Australia is determined by the vegetation and environment they live in (Oliver, 2013). For example, the fruits of the native shrubs *Solanum laciniatum* is used in southern Australia while *Solanum aviculare* (Kangaroo Apple) was used in the eastern parts of Australia (Stack, 1989). Both species were used as poultices for joint swellings (Stack, 1989). Both of these *Solanum* species contain an alkaloid solasodine, which is the precursor of cortisone and other steroids used in production of oral contraceptives (i.e. 'the pill') (Manosroi, Manosroi, & Sripalakit, 2003). These plants have been imported into Russia and eastern Europe where they are now cultivated at large scale, due to their capacity to biosynthesise this valuable phytochemical (Stack, 1989). Similarly, the native Aboriginal narcotic shrub, pituri (*Duboisia hopwoodii)* of the arid interior region of Australia led to the research of congeneric, *Duboisia myoporoides*, which proved to be highly beneficial. Joseph Banks, the first European botanist to visit the east coast of Australia (in 1770), observed 'pituri' being chewed by Aboriginals similarly to tobacco or East Indian betele (Foley, 2006). A century later (in 1872), Ferdinand von Mueller, the Victorian Government botanist, identified the plant as *Duboisia hopwoodii* (Ratsch, Steadman, & Bogossian, 2010). Von Mueller suggested that the related *Duboisia myoporoides* should be researched, and it was found to produce hyoscine, currently known as scopolamine, an alkaloid that is a highly effective treatment for motion sickness (Hines, 1947).

2.4.2. Queensland Aboriginal medicinal plants

Of all Australian States, Queensland, located in the north-east, encompasses the widest variety of landscapes, vegetation types and climatic zones - temperate, wet and dry tropics and semiarid to arid – across its 1.73 million square kilometres. The vegetation includes temperate, subtropical and tropical rainforests, eucalypt woodlands, coastal communities and heath, a variety of grasslands and wetlands, and mangroves and marshes (Figure 2.2) (Neldner et al., 2019). Queensland's Aboriginal communities have lived in harmony within these landscapes for thousands of years. Their customary knowledge is shaped by the rich and diverse vegetation communities that are home to a diverse range of medicinal plant species, including that are endemic to the state.

Some of the earliest records of medicinal plant knowledge in Queensland were made by botanists, chief protectors, anthropologists, pastoralists and chemists (Dowe, 2010). For example, a pastoralist, amateur anthropologist and politician Edward Palmer writes that Aboriginal people possess a considerable amount of knowledge of native plants and their uses (Palmer, 1884). Joseph Henry Maiden, who was the Curator of the Australian Museum at that time, wrote that Queensland is by far the richest of the colonies concerning recorded plants with medicinal properties (Maiden, 1889).

However, he did not consult local Aboriginal people for medicinal plant knowledge stating that “In fairness to ourselves we must confess ourselves very little indebted to the Australian aboriginal for information as to the medical (or in fact any other) properties of our plants” (Maiden, 1889). He also suggested that the great majority of these will be common to India and the Archipelago, and to have been employed by the natives of those countries (Maiden, 1889).

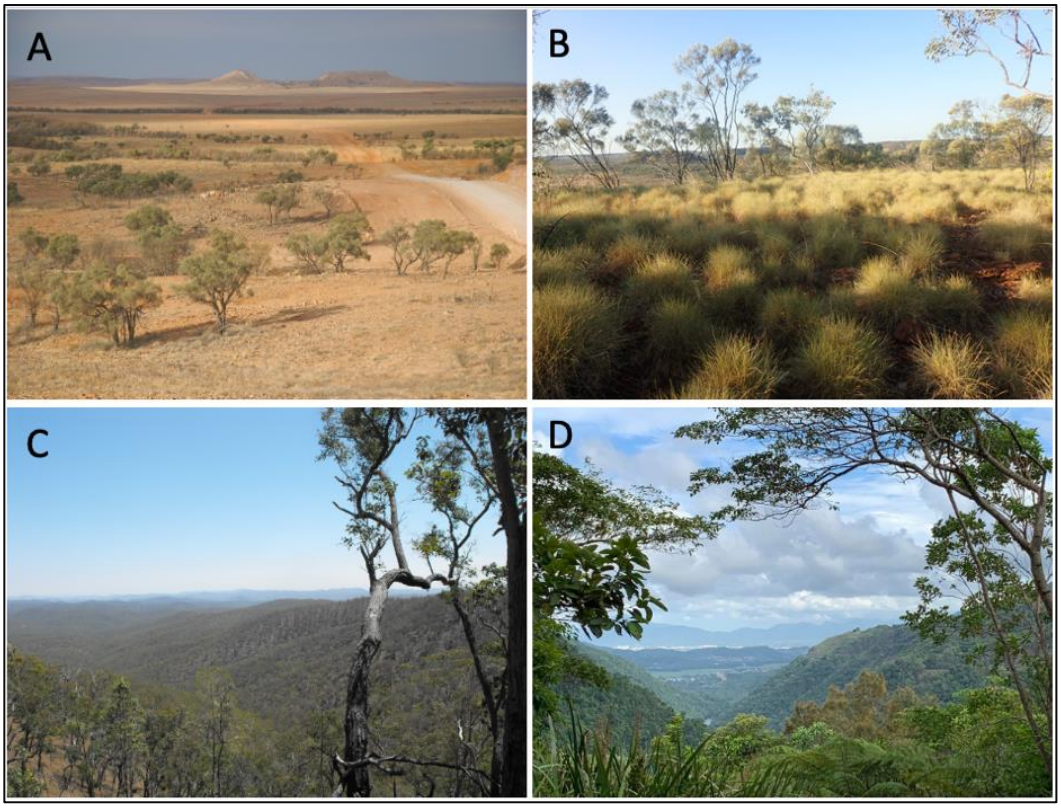

Figure 2.2 Representative vegetations of Queensland. A) Desert. B) Grassland. C) Savanna. D) Wet Tropics (Photo courtesy: Phurpa Wangchuk and Gerry Turpin).

Based on the current available literature and oral medical traditions still practiced by the rural Aboriginal elders and traditional knowledge holders of Queensland, it is fair to say that Maiden’s information on Aboriginal medical knowledge was limited and his perceptions grossly misleading. Walter E. Roth, as Northern Protector of Aboriginals on Cape York Peninsula, recorded extensive information on Aboriginal customs, languages and knowledge (Roth, 1903). Similarly, Sandyl Kyriazis and Nai Begutab Agama Aboriginal Corporation have listed many medicinal plants in their book ‘Bush Medicine of the Northern Peninsula area of Cape York’ (Kyriazis, 1995). Leonard James Webb accompanied a team of anthropologists and Aboriginal people to document Indigenous plant uses at Lockhart River in northern Queensland between 1952 and 1977. He recognised the value of collaboration between scientists, anthropologists and Indigenous peoples, combining the collection of

scientific data with cultural perspectives in the study of plant uses (Webb, 1952, 1969). The inventory list (Table 2.1) also supports the view that Queensland is rich in Aboriginal medicinal knowledge and medicinal plant diversity.

2.4.3. Inventory of Queensland Aboriginal medicinal plants

Based on the archival information from the TIEC database (maintained by an Indigenous Ethnobotanist - Mr. Gerry Turpin, Figure 2.3) and the online information, we have identified a total of 135 species of medicinal plants used by Queensland Aboriginal people. The archival information is presented in Table 2.1, includes botanical name, plant family, life form, plant part used, and diseases/conditions treated.

Figure 2.3 Indigenous Ethnobotanist collecting herbarium specimens of Aboriginal medicinal plants (Photo courtesy - Gerry Turpin).

Table 2.1 List of Queensland medicinal plants and their uses (Barr et al., 1988; Clarke, 2011; Cribb & Cribb, 1981; Kalotas, 1996; Reid & Betts, 1979; Stack, 1989; Webb, 1952).

Botanical name	Family	Life form	Part used	Diseases treated
Abrus precatorius L.	Fabaceae	Climber	Seeds	Abortion
Acacia bivenosa DC.	Fabaceae	Shrub	Bark, ash	Headache, colds and fever
Acacia holosericea A.Cunn. ex G.Don	Fabaceae	Shrub	Root	Headache, colds and fever
Acacia melanoxylon R.Br.	Fabaceae	Tree	Bark	Headache, colds and fever
Acalypha wilkesiana Müll.Arg.	Euphorbiaceae	Shrub	Shoot	Sores/skin lesions/wounds/cuts
Acmella grandiflora (Turcz.) R.K.Jansen	Asteraceae	Herb	Root	Toothache
Aegiceras corniculatum (L.) Blanco	Primulaceae	Tree	Leaves, juice of leaves	Earache
Ageratum conyzoides L. *subsp. conyzoides*	Asteraceae	Herb	Whole plant	Sores/skin lesions/wounds/cuts
Alphitonia excelsa (A.Cunn. ex Fenzl) Benth.	Rhamnaceae	Tree	Bark, wood, young leaves, leaves, roots	Headache, colds, fever, stomach upset, snake bite, body ache, muscle pain, eye sores, skin lesions, wounds, cuts, toothache, diarrhoea, tonic

Botanical name	Family	Life form	Part used	Diseases treated
Alphitonia petriei Braid & C.T.White	Rhamnaceae	Tree	Bark	Body pain
Alstonia constricta F.Muell.	Apocynaceae	Tree	Latex, bark	Skin infection, fever, tonic
Alstonia scholaris (L.) R.Br.	Apocynaceae	Tree	Juice, sap, bark	Neuralgia, toothache, fever, sores, skin lesions, wounds and cuts
Alyxia spicata R.Br.	Apocynaceae	Shrub	Root	Headache, breathlessness, colds and fever
Amyema quandang (Lindl.) Tiegh.	Loranthaceae	Hemi-parasitic shrub	Leaves	Fever, headache and colds
Antidesma bunius (L.) Spreng.	Phyllanthaceae	Tree	Fruit	Colds, fever and headache
Arivela viscosa (L.) Raf.	Cleomaceae	Herb	Whole plant, seeds, leaves	Colds and fever
Asteromyrtus symphyocarpa (F.Muell.) Craven	Myrtaceae	Shrub	Leaves	Colds, fever and headache
Barringtonia calyptrata (Miers) R.Br. ex F.M.Bailey	Lecythidaceae	Tree	Leaves	Fever and chest pain
Barringtonia racemosa (L.) Spreng.	Lecythidaceae	Tree	Bark	Tonic
Basilicum polystachyon (L.) Moench	Lamiaceae	Herb	Whole plant	Fever

Botanical name	Family	Life form	Part used	Diseases treated
Blainvillea acmella (L.) Philipson	Asteraceae	Herb	Inner bark, fruit, root	Muscle sprain, bone aches, dislocation, broken bones
Boerhavia diffusa L.	Nyctaginaceae	Herb	Whole plant	Asthma
Breynia cernua (Poir.) Müll.Arg.	Phyllanthaceae	Shrub	Leaves	Eye soreness
Brucea javanica (L.) Merr.	Simaroubaceae	Shrub	Leaves, roots	Pain
Buchanania obovata Engl.	Anacardiaceae	Tree	Inner bark, sapwood, leaves	Eye sores and toothache
Calamus caryotoides A.Cunn. ex Mart.	Arecaceae	Palm	Shoot	Headache
Callicarpa longifolia Lam.	Lamiaceae	Tree	Bark	Body pain
Calophyllum inophyllum L.	Clusiaceae	Tree	Kernel, fruit	Body pain and purgative
Canarium australasicum (F.M.Bailey) Leenh.	Burseraceae	Tree	Bark	Stomach ache and diarrhoea
Capparis lanceolaris DC.	Capparaceae	Climber	Bark	Sores, skin lesions, wounds and cuts
Capparis mitchellii Lindl.	Capparaceae	Tree	Bark	Sores, skin lesions, wounds and cuts
Carica papaya L.	Caricaceae	Tree	Fruit	Prickly heat

Botanical name	Family	Life form	Part used	Diseases treated
Cassytha filiformis L.	Lauraceae	Climber	Macerated plant	Tonic
Cassytha glabella R.Br.	Lauraceae	Climber	Whole plant, bark, leaves	Colds, fever, body pain, headache, sores, skin lesions, wounds and cuts
Casuarina equisetifolia L.	Casuarinaceae	Tree	Inner bark	Toothache
Centipeda thespidioides F.Muell.	Asteraceae	Herb	Whole plant	Sprains
Cissus hypoglauca A.Gray	Vitaceae	Climber	Fruit	Headache, colds and fever
Clematis glycinoides DC.	Ranunculaceae	Climber	Leaves	Headache, colds and fever
Clematis microphylla DC.	Ranunculaceae	Climber	Whole plant, seeds, leaves	Headache, colds and fever
Clerodendrum floribundum R.Br.	Lamiaceae	Tree	Wood	Pain
Clerodendrum inerme (L.) Gaertn.	Lamiaceae	Shrub	Leaves, roots	Sores, skin lesions, wounds, cuts and sprains
Cocos nucifera L.	Arecaceae	Tree	Oil, bark, coconut jelly	Earache, swelling, sores, skin lesions, wounds and cuts
Coelospermum decipiens Baill.	Rubiaceae	Shrub	Leaves, roots	Pregnancy prevention
Convolvulus erubescens Sims	Convolvulaceae	Herb	Whole plant	Diarrhoea

Botanical name	Family	Life form	Part used	Diseases treated
Corymbia gummifera (Gaertn.) K.D.Hill & L.A.S.Johnson	Myrtaceae	Tree	Leaves, gum	Bleeding control, diarrhoea, ringworm and sexually transmitted diseases
Corymbia polycarpa (F.Muell.) K.D.Hill & L.A.S.Johnson	Myrtaceae	Tree	Kino	Toothache and dysentery
Corymbia terminalis (F.Muell.) K.D.Hill & L.A.S.Johnson	Myrtaceae	Tree	Bark	Dysentery
Corymbia tessellaris (F.Muell.) K.D.Hill & L.A.S.Johnson	Myrtaceae	Tree	Gum	Constipation
Crinum pedunculatum R.Br.	Amaryllidaceae	Herb	Whole plant	Stings (marine)
Crotalaria cunninghamii R.Br.	Fabaceae	Shrub	Leaves, sap, bark	Pain
Croton arnhemicus Müll.Arg.	Euphorbiaceae	Tree	Root	Sores, skin lesions, wounds, cuts and stomach ache
Cyathea australis (R.Br.) Domin	Cyatheaceae	Tree fern	Young leaves	Stomach ache and tonic
Cymbidium canaliculatum R.Br.	Orchidaceae	Orchid	Bulb	Dysentery
Cymbidium madidum Lindl.	Orchidaceae	Orchid	Bulb, seeds	Dysentery, Pregnancy prevention

Botanical name	Family	Life form	Part used	Diseases treated
Cymbonotus lawsonianus Gaudich.	Asteraceae	Herb	Leaves	Sores, skin lesions, wounds and cuts
Cymbopogon ambiguus (Hack.) A.Camus	Poaceae	Grass	Leaves	Colds
Cymbopogon bombycinus (R.Br.) Domin	Poaceae	Grass	Whole plant	Eye soreness
Cymbopogon obtectus S.T.Blake	Poaceae	Grass	Whole plant	Eye soreness
Cymbopogon sp.	Poaceae	Grass	Root	Earache
Cynanchum viminale subsp. australe (R.Br.) Meve & Liede	Apocynaceae	shrub Succulent	Latex, tuber, sap	Sores, skin lesions, wounds, cuts, warts, and gonorrhoea
Deplanchea tetraphylla (R.Br.) F.Muell.	Sapotaceae	Tree	Bark	Colds and influenza
Derris sp.	Fabaceae	Climber	Bark	Sores, skin lesions, wounds and cuts
Dioscorea transversa R.Br.	Dioscoreaceae	Climber	Tuber	Skin cancer
Dodonaea polyandra Merr. & L.M.Perry	Sapindaceae	Tree	Root	Sores, skin lesions, wounds, cuts and toothache
Erythrophleum chlorostachys (F.Muell.) Baill.	Fabaceae	Tree	Bark	Sores, skin lesions, wounds, cuts, pain and sprain

Botanical name	Family	Life form	Part used	Diseases treated
Eucalyptus haemastoma Sm.	Myrtaceae	Tree	Kino	Diarrhoea, wounds, ulcers
Eucalyptus pruinosa Schauer	Myrtaceae	Tree	Bark	Rheumatism and body pain
Eucalyptus resinifera Sm.	Myrtaceae	Tree	Leaves, inner bark, gum	Ringworm
Eucalyptus tetrodonta F.Muell.	Myrtaceae	Tree	Leaves	Fever and headache
Euphorbia tirucalli L.	Euphorbiaceae	Succulent shrub	Latex	Skin cancer
Euphorbia mitchelliana Boiss.	Euphorbiaceae	Shrub	Flowers	Diarrhoea
Excoecaria agallocha L.	Euphorbiaceae	Mangrove tree	Latex	Stings (marine)
Excoecaria parvifolia Müll.Arg.	Euphorbiaceae	Tree	Bark	Body pain
Exocarpos aphyllus R.Br.	Santalaceae	Succulent shrub	Bark, roots	Boils
Ficus fraseri G.Forst.	Moraceae	Tree	Milky juice of young roots	Sores, skin lesions, wounds and cuts
Ficus microcarpa L.f.	Moraceae	Tree	White sap	Stings (fish)

Botanical name	Family	Life form	Part used	Diseases treated
Ficus opposita Miq.	Moraceae	Tree	Latex, leaves, gum	Sores, skin lesions, wounds, cuts and fungal infections including ringworm
Flagellaria indica L.	Flagellariaceae	Climber	Fresh new growth tips	Sores, skin lesions, wounds, cuts and pox
Flueggea virosa (Willd.) Voigt	Phyllanthaceae	Shrub	Root	Toothache
Grevillea mcgillivrayi I.M.Turner	Proteaceae	Tree	Leaves	Sore throat
Grevillea striata R.Br.	Proteaceae	Tree	Charcoal, bark	Sores, skin lesions, wounds, cuts, hives and stings
Grewia retusifolia Kurz	Malvaceae	Shrub	Inner bark of roots, roots, leaves, fruits	Diarrhoea, dysentery, boils, swelling, toothache, stomach ache, sores, skin lesions, wounds, cuts and cough
Haemodorum corymbosum Vahl	Haemodoraceae	Herb	Root	Constipation
Heliotropium ovalifolium Forssk.	Boraginaceae	Herb	Extract	Body wash and fever
Hibiscus vitifolius L.	Malvaceae	Herb	Tuber	Boils
Ipomoea pes-caprae (L.) R.Br.	Convolvulaceae	Climber	Whole plant, leaves, stems	Sexually transmitted diseases, boils, and swelling

Botanical name	Family	Life form	Part used	Diseases treated
Litsea glutinosa (Lour.) C.B.Rob.	Lauraceae	Tree	Leaves, bark	Sores, skin infection, lesions, wounds, cuts, eye sores, body pain, scabies, gastritis, fever, headache, influenza and pedriatic uses
Macaranga tanarius (L.) Müll.Arg.	Rubiaceae	Tree	Red sap	Sores, skin lesions, wounds and cuts
Manihot esculenta Crantz	Euphorbiaceae	Shrub	Root	Diarrhoea and stomach ache
Melaleuca leucadendra (L.) L.	Myrtaceae	Tree	Young leaves, bark	Cough and cold, headache, tonic, sinusitis, sores, skin lesions, wounds and cuts
Melaleuca quinquenervia (Cav.) S.T.Blake	Myrtaceae	Tree	Young leaves	Cold, headache and tonic
Melaleuca viridiflora Sol. ex Gaertn.	Myrtaceae	Tree	Leaves	Cough
Melicope vitiflora (F.Muell.) T.G.Hartley	Rutaceae	Tree	Juice, resin, gum, bark	Toothache, bodyache and toothache
Mentha australis R.Br.	Lamiaceae	Herb	Whole plant	Cough and cold
Morinda citrifolia L.	Rubiaceae	Tree	Fruit	Cough and cold, sore throat
Mucuna gigantea (Willd.) DC.	Fabaceae	Climber	Seed	Colds, fever, headache

Botanical name	Family	Life form	Part used	Diseases treated
Musa banksia F.Muell.	Musaceae	Tree	Sap, juice	Stings (stinging tree), paralysis and headache
Myristica globosa subsp. muelleri (Warb.) W.J.de Wilde	Myristicaceae	Tree	Gum from bark	Ringworm
Nauclea orientalis (L.) L.	Rubiaceae	Tree	Bark	Rheumatism, colds, stomach ache, and snake bite
Ocimum tenuiflorum L.	Lamiaceae	Herb	Leaves, stems	Influenza, labour pain, and stomach ache
Pandanus sp.	Pandanaceae	Palm	Base of leaf	Body pain, sore throat, sores, skin lesions, wounds and cuts
Pandanus spiralis R.Br.	Pandanaceae	Palm	Sap, base of leaf	Sores, skin lesions, wounds and cuts
Persicaria subsessilis (R.Br.) K.L.Wilson	Polygonaceae	Herb	Whole	Sores, skin lesions, wounds and cuts
Persoonia falcata R.Br.	Proteaceae	Shrub	Leaves, bark, wood	Cough and cold, sore throat and eye sores
Petalostigma pubescens Domin	Picrodendraceae	Shrub	Fruit, root	Toothache
Phyllanthus urinaria L.	Phyllanthaceae	Herb	Leaves	Colds
Piper hederaceum (Miq.) C.DC.	Piperaceae	Climber	Whole plant	Sore gums

Botanical name	Family	Life form	Part used	Diseases treated
Planchonella pohlmaniana (F.Muell.) Pierre ex Dubard	Sapotaceae	Tree	Twigs and leaves	Boils
Planchonia careya (F.Muell.) R.Knuth	Lecythidaceae	Tree	Bark, leaves	Tonic and body pain
Coleus congestus R.Br.	Lamiaceae	Herb	Leaves and branches	Body pain and syphilis
Coleus parviflorus Willd.	Lamiaceae	Herb	Leaves	Syphilis
Plumeria rubra L.	Apocynaceae	Tree	Leaves	Swelling
Pseudognaphalium luteoalbum (L.) Hilliard & B.L.Burtt	Asteraceae	Herb	Whole plant	Fever and tonic
Psidium guajava L.	Myrtaceae	Tree	Fruit	Constipation and stomach ache
Pterocaulon serrulatum (Montrouz.) Guillaumin	Asteraceae	Herb	Leaves	Chest congestion, fever, colds, headache, sores, skin lesions, wounds and cuts
Ripogonum album R.Br.	Ripogonaceae	Climber	Bark, roots	Stings (stingray)
Santalum lanceolatum R.Br.	Santalaceae	Tree	Scraped outer wood, inner moist bark, leaves	Chest ailments, purgative, sexually transmitted diseases, arthritis, insect bites, sores, skin lesions, wounds and cuts

Botanical name	Family	Life form	Part used	Diseases treated
Santalum obtusifolium R.Br.	Santalaceae	Shrub	Wood	Constipation and pain
Scoparia dulcis L.	Plantaginaceae	Herb	Whole plant	Influenza, sores, stomach ache, skin lesions, wounds and cuts
Senna alata (L.) Roxb.	Fabaceae	Shrub	Leaves	Fungal infections specially ringworm, heat rash, scabies and skin itches
Stemodia viscosa Roxb.	Plantaginaceae	Herb	Whole plant	Body pain and tonic
Sterculia quadrifida R.Br.	Sterculiaceae	Tree	Leaves, branches, barks, roots	Eye sores, skin lesions, wounds, cuts and nausea
Striga curviflora (R.Br.) Benth.	Linderniaceae	Herb	Whole plant	Sores, skin lesions, wounds and cuts
Syzygium suborbiculare (Benth.) T.G.Hartley & L.M.Perry	Myrtaceae	Tree	Bark, roots	Body pain
Tabernaemontana orientalis R.Br.	Apocynaceae	Shrub	Rootbark, sap	Fever, sores, skin lesions, wounds and cuts
Tephrosia turpinii Pedley	Fabaceae	Herb	Tuber	Sores, skin lesions, wounds and cuts
Tephrosia varians (F.M.Bailey) C.T.White	Fabaceae	Herb	Root	Sores, skin lesions, wounds and cuts

Botanical name	Family	Life form	Part used	Diseases treated
Terminalia catappa L.	Combretaceae	Tree	Bark, young green fruit	Sore throat and thrush
Terminalia muelleri Benth.	Combretaceae	Tree	Soft leaves	Scabies, sores, skin lesions, wounds and cuts
Tetrameles nudiflora R.Br.	Datiscaceae	Tree	Leaves	Swelling, sores, skin lesions, wounds and cuts
Timonius timon (Spreng.) Merr.	Rubiaceae	Tree	Inner bark	Colds, fever, influenza
Tribulus cistoides L.	Menispermaceae	Climber	Whole plant	Toothache
Wrightia saligna (R.Br.) F.Muell. ex Benth.	Apocynaceae	Tree	Inner bark, milky sap, root	Boils, sores, skin lesions, wounds and cuts
Xenostegia tridentata (L.) D.F.Austin & Staples	Convolvulaceae	Climber	Whole plant	Sores, skin lesions, wounds and cuts
Xylomelum scottianum (F.Muell.) F.Muell.	Proteaceae	Tree	Leaves, roots	Body pain

2.4.4. Diversity of medicinal plants

Table 2.1 lists the botanical names of 135 species of plants used customarily as medicines by Aboriginal people of Queensland. These plants belong to three plant groups - Angiosperms, Gymnosperms and Pteridophytes – and represent a total of 53 families and 103 genera. Plant families with most species with recorded medicinal properties were Myrtaceae (14), Fabaceae (11), Lamiaceae (8), and Apocynaceae, Asteraceae and Euphorbiaceae (7 each). It is no surprise that Myrtaceae is recorded as having the most medicinal uses of any plant family as it is one of the most common but largely tropical families worldwide, estimated to include about 5,950 species in about 132 genera (Christenhusz & Byng, 2016).

Queensland has 60 genera and 746 species of Myrtaceae (ALA, 2020). Many plants within the Myrtaceae family contain oils in the leaves, and oil is one of the best solvents for medicinal compounds. Within the Myrtaceae family, there are five genera used medicinally in Queensland: *Eucalyptus* (seven species used medicinally), *Melaleuca* (four species), *Corymbia* (two species), *Syzygium* (one species) and the introduced *Psidium guajava*. *Corymbia* and *Eucalyptus* are two of the three genera (the other being *Angophora*) that comprise the 'eucalypts', a group of iconic Australian forest trees including more than 800 species that together dominate 77% of Australia's native forests (Cock, 2011). While fungi, seaweed and bryophytes (liverworts, hornworts and mosses) were commonly used globally as foods and medicines, the record of use, particularly in Queensland, is very poor (Kalotas, 1996; Thurstan et al., 2018). Lichens were also not recorded.

2.4.5. Life forms of Queensland medicinal plants

Of the 135 species of Queensland medicinal plants (Table 2.1), 62 species are trees, followed by herbs (26 species), shrubs (22 species), climbers (16 species), epiphytes and grasses (4 species each), and palms (3 species) (Figure 2.4). The epiphyte category includes orchids and hemiparasitic mistletoes; shrubs include small erect, woody plants and succulent shrubberies; and trees include mangroves and tree ferns.

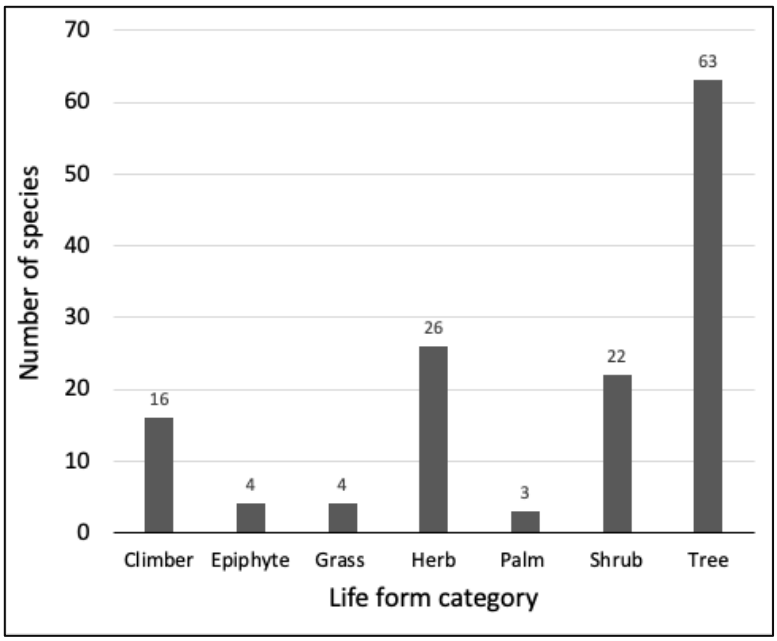

Figure 2.4 Category of life forms of medicinal plants. A) Bar graph showing number of species in each life form category.

2.4.6. Plant parts used for treating diseases

Over millennia, Aboriginal people have determined which plant parts are useful medicinally. Table 2.1 shows different parts of 135 medicinal plants grouped into 10 major categories: 'Branch & Twig', 'Bark', 'Charcoal & Ash', 'Flower', 'Fruit & Seed', 'Leaf', 'Plant exudate', 'Root', 'Whole plant' and 'Wood' (Figure 2.5). The category 'Bark' includes outer bark, inner cambium, and root bark; 'Fruit & Seed' comprises fruit, seed and kernel; 'Leaf' includes young leaf, leaf stalk, leaf tip, and young shoot; 'Plant exudate' includes oil, sap, gum, resin, kino and latex; and 'Root' includes tap root, tuber, and bulb. The category 'Whole plant' is mostly smaller herbaceous plants and vines where it was more productive and easier to collect the roots, stems and leaves together rather than separating plant parts.

Of 10 major plant part categories, 'Leaf' ranked first in terms of percentage use with 24%, followed by bark (20.6%), root (14.9%), whole plant (13.1%), plant exudate (11.4%), fruit & seed (9.1%), branch & twig (2.9%), wood (2.3%), charcoal & ash (1.1%) and flower (0.6%). For example, the leaves of *Aegiceras corniculatum* are used, the barks of *Acacia melanoxylon*, the roots of *Flueggea virosa*, *Alphitonia excelsa* as whole plant, *Corymbia polycarpa* for kino (plant exudate), the fruits of *Morinda citrifolia*, branches and twigs of *Cassytha filiformis*, the wood of *Clerodendrum floribundum*, and the flowers of *Euphorbia mitchelliana*. It is to be expected that leaves and barks show the highest amount of usage in Aboriginal medicine, given that they are in abundance, easily accessed and processed. They are also readily harvested typically without much damage to the plant and readily replenished, especially the leaves and fruits, making their use sustainable. Leaves are an easy target not only for humans but also for herbivores.

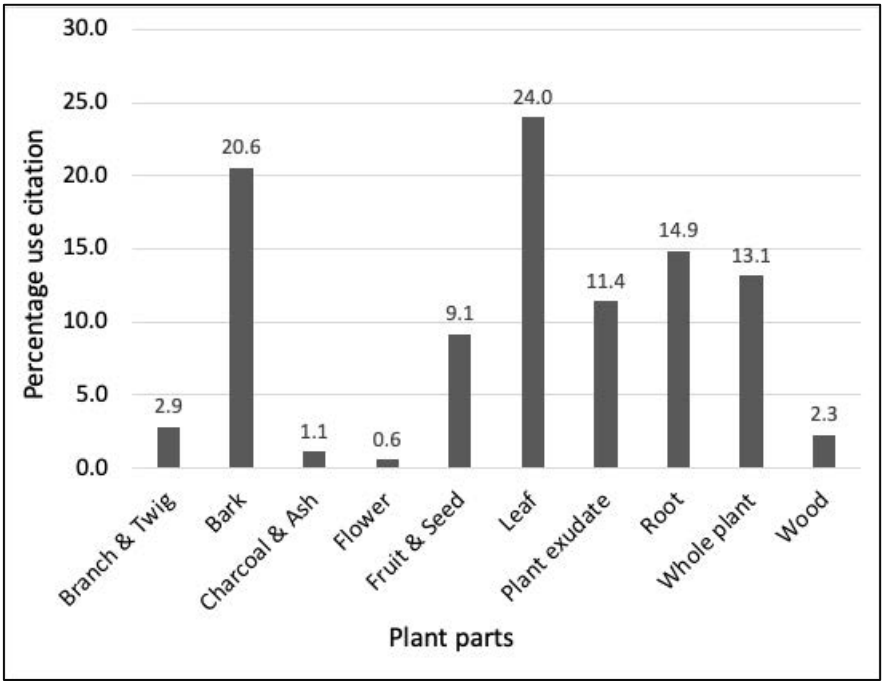

Figure 2.5 Category of medicinal plants parts and their percentage of uses citation.

2.4.7. Types of diseases treated by medicinal plants

A total of 62 types of diseases are treated by 135 medicinal plants listed in Table 2.1. Most medicinal plants are used for treating disease of the nervous system, integumentary system, respiratory system, and digestive system. Over 17% of these medicinal plants have been reported to be used for treating 'Skin sores and infections' (integumentary system), followed by 'Cuts and wounds' (12.6 %), 'Stomach disorders' (digestive system) and 'Pain' (nervous system) (8.5% each), 'Fever' (7.9 %), 'Cough and cold'(respiratory system) (7.6%), 'Headache' (6.3%), 'Toothache' (3.8%), 'Eyesore' (3.8%), and 'Sexually transmitted disease' (1.9%). Interestingly, 3.2% of plants were recorded for their use as tonics. For example, *Cassytha filiformis* and *Barringtonia racemosa* are described as good health tonics for the body. The biggest disease category 'Skin sores and infections' includes sores, boils, ringworm, hives, skin irritation and other fungal and bacterial infections. Similarly, the category 'Stomach disorders' comprises stomach ache, gastritis, constipation, diarrhoea and dysentery. The disease category 'Pain' includes body ache/pain, earache, muscle pain, labour pain, and chest pain; the 'Sexually transmitted diseases' consist of gonorrhoea, syphilis and other infections.

Of 135 medicinal plant species, 53 of them have been cited for the treatment of a single disease (one plant-one disease treatment category). For example, *Acmella grandiflora* and *Euphorbia tirucalli* are used for treating only toothache and skin cancer, respectively. Similarly, while *Coelospermum decipiens* is used as a contraceptive, *Abrus precatorius* is used for abortion. However, the majority of medicinal plants have been used for treating more than one disease. For example, *Alphitonia petriei* is used for treating headache, cough and cold, fever, stomach upset, body ache, muscle pain, snake bite,

eye sore, skin lesion, cuts/wounds, toothache, diarrhoea and as a tonic. Another plant, *Grewia retusifolia*, is used for treating diarrhoea, dysentery, boils, swelling, toothache, stomachache, sores, skin lesions, wounds, cuts and cough. Four plant species are used for treating stings from marine organisms such as rays, fishes, and sea jellies.

For treating the diseases, decoctions are commonly used in herbal medicine, and these are preparations from plants using water. While decoctions, especially the tonics, are frequently ingested for diseases such as gastritis, cough and cold, and fever; poultices are commonly used for external skin diseases. Some medicinal plants are chewed and swallowed. There are a few plant species that are burnt and used as fumigants. Some plant products are used as infusions.

2.4.8. Biodiscovery initiatives involving Queensland medicinal plants

Biodiscovery involves identification of novel drug lead compounds from various natural resources such as plants, animals, fungi, bacteria and extremophiles. Novel and effective drugs can be developed from natural products, especially from medicinal plants using a biorational ethnobotany-guided strategy. Indeed, out of 122 current plant-derived prescription drugs, 80% were discovered from medicinal plants (Wangchuk, 2018). Well known examples are quinine (antimalarial compound isolated from *Cinchona officinalis*), artemisinin (antimalarial compound isolated from *Artemisia annua*), paclitaxel (anticancer compound isolated from *Taxus baccata*), vincristine (anticancer compound isolated from *Catharanthus rosea*), aspirin (anti-inflammatory compound derived from salicylic acid isolated from *Salix babylonica*) and morphine (analgesic compound isolated from *Papaver somniferum*). Rainforest plants are the source of a quarter of pharmaceutical products, and more than 70% of these plant species are found exclusively in the tropical Amazon rainforest (Huizen, 2019).

Queensland harbours the great majority of Australia's tropical rainforest, which represents a warehouse of the continent's medicinal treasure trove (Setzer, Setzer, Jackes, Gentry, & Moriarity, 2001). However, the biodiversity of these rainforests, along with other species rich tropical biomes such as the Great Barrier Reef, is under threat of climate change (Costion et al., 2015; Steffen et al., 2009; Williams, Bolitho, & Fox, 2003). Consequently, the changes in the environment might trigger plant physiological responses as well as adaptations in secondary metabolism to produce either higher concentrations or novel phytochemicals to cope with abiotic stress (Mounter, 2019). It is well-known that such anti-stress biomolecules exhibit potent antioxidant and anti-inflammatory properties (Ruiz-Ruiz, Matus-Basto, Acereto-Escoffié, & Segura-Campos, 2017; Tungmunnithum, Pinthong, & Hano, 2018; Tungmunnithum, Thongboonyou, Pholboon, & Yangsabai, 2018; Yeshi, Crayn, Ritmejerytė, & Wangchuk, 2022), which have potential applications in novel drug development.

The rich and diverse vegetation of Queensland has shaped the development of unique ethnobotanical knowledge of Aboriginal and Torres Strait Islander people, which has huge potential to guide biodiscovery programs. Early investigations of Aboriginal medicinal plant knowledge in

Queensland were carried out by chemists and pharmacologists in the nineteenth century. For example, Joseph Bancroft analysed properties of the pituri narcotic used by inland Aboriginal groups, which was later discovered to contain nicotine alkaloids (Bancroft, 1872; Hicks & LeMessurier, 1935). Commencing in 1944, an Australian Phytochemical Survey was established by Leonard James Webb at the Commonwealth Scientific and Industrial Research Organisation (CSIRO) in Brisbane but few Queensland medicinal plants were screened for their phytochemicals (Webb, 1949, 1952). In 1984, CSIRO staff based in Melbourne began publishing the results of the Phytochemical Survey (Collins et al., 1990). However, it doesn't contain comprehensive information on bioactive chemical constituents of Queensland medicinal plants.

A recent review by Janice Mani and colleagues (Mani et al., 2021) reported the antioxidative and therapeutic potential of selected number of Australian plants, which included only a limited number of Queensland medicinal plants. While some medicinal plants that are distributed globally may have been studied for their phytochemical and pharmacological activities, there are hundreds of Queensland native plants that remain unexplored for therapeutic applications. Indeed, Australian medicinal plants in general remain underexplored in terms of biological, medicinal and economic resources. Given that the globally popular natural medicines and their related products from other parts of the world (worth an estimated US$83 billion (Robinson & Zhang, 2011; Wassie, Aragie, Taye, & Mekonnen, 2015)) has created lucrative marketplaces and that the Australian agriproducts are already enjoying an international reputation for their high quality and clean image, there are exclusive demands for the Queensland medicinal plants. More elaborate studies are therefore required to develop quality parameters to monitor the quality of medicinal plants (both cultivated and wild type) and to identify biomarker and bioactive compounds. This will not only improve our knowledge on the phytochemistry of Queensland medicinal flora, but also generate data and ideas for developing herbal industries related to health-promoting, pharmaceutical, nutraceuticals, cosmetics, and functional food products. This has a huge scope for the development of a sustainable regional development of Queensland, Indigenous workforce development, and promotion of plant-based biotechnological innovations both locally and worldwide.

2.4.9. Biodiscovery frameworks, community engagement and research approaches

In the past, especially in the 1970s-1990s, biopiracy has commonly occurred and the innovations and intellectual property rights belonging to many Indigenous peoples around the world have been exploited and compromised by the researchers, institutions, companies and pharmaceutical industries (Drahos, 2000). This was partly due the lack of proper protocols, ethical rules and regulations, and biodiscovery acts. It has left Indigenous peoples dry and void of their rights, which has partly led Indigenous communities to step back from collaborating with the researchers and companies. In order to investigate the biocultural knowledge of medicinal plants used by Indigenous communities, consents and approvals from Traditional Owners/corporations, must be adequately addressed (Packer et al., 2019). At times, negotiations and signing collaborative/benefit sharing agreement with the communities

can take longer, which will impede the access to plant materials and progress of research. This partly explains the relative scarcity of documented ethnomedicinal knowledge about plants used by the Australian Aboriginal and Torres Strait Islander peoples, especially in the biodiscovery space. However, if the biodiscovery research project is built upon the trust and the long-term relationship of the parties, this gap can be bridged easily.

We found that it is important to conduct any bioprospecting/biodiscovery projects in line with global ethical guidelines and intellectual property rights practices advocated by the following national and international bylaws and regulations.

- World Intellectual Property Organization (relevant section on Indigenous knowledge)(WIPO, 2022).
- Nagoya Protocol on Access and Benefit-sharing and Traditional Knowledge(CBD, 2014).
- NHMRC ethical guidelines for research with Aboriginal and Torres Strait Islander Peoples (NHMRC, 2018).
- The AIATSIS Code of Ethics for Aboriginal and Torres Strait Islander Research (AIATSIS, 2020).
- Queensland Biodiscovery Act 2004 on using traditional knowledge for biodiscovery, which provides step-wise traditional knowledge guidelines and biodiscovery resources tool kits (Government, 2020).
- Relevant university's research code of conduct such as James Cook University Aboriginal and Torres Strait Islander Research Ethics (JCU, 2022).

The World Health Organisation realises the importance of traditional medicines both as the source of health care and novel drug leads for modern medicine and therefore their long-term survival and sustainability is imperative. Australia's Biodiversity Conservation Strategy (2010-2030) has acknowledged that preservation and sustainable maintenance of Indigenous knowledge is a priority area and therefore it is essential to actively engage Indigenous people through employment, partnership and transfer of scientific knowledge that actively supports its sustainable use.

2.4.10. Building community relationships and engagement

It is important to develop collegial relationship with the Indigenous communities and propose the project to the funding bodies together as a team. The community members should be involved as a partner in shaping the research activities and the project must facilitate two-way exchange of knowledge, skills and benefits and should provide capability building opportunities. Informed consent and ethics from the Indigenous community must be obtained prior to any information collection or documentation or product development based on traditional knowledge. It is also imperative to sign proper memoranda of understandings or collaborative agreements or benefit sharing agreement with

the relevant Indigenous community. To build better trust and relationships, researchers must identify the cultural broker (Indigenous background) and provide the community with: a) results from each aim of the project on a yearly basis, b) study tours to research stations, c) community-researchers' seminars where both sides present their ideas, challenges and needs, d) staying connected with the community, and d) co-authorship on relevant publications. During engagement with the communities, the National Indigenous Science Education Program developed by Jamie and her group at Macquarie University can be adapted to improve the enrolment of Indigenous people in the higher education sector and guide them to become scientists, health workers and policy changers.

2.4.11. Benefits to the Indigenous communities

The project design should find the needs/benefits/requirements of the Indigenous communities prior to biodiscovery grant applications. The benefits to the Indigenous community would include: a) community development support fees to help the community in conducting administrative and legal duties relevant to the projects, b) employment opportunities, c) training the Indigenous communities on preservation of customary medical knowledge, collection and cultivation of quality medicinal plants, and intellectual property rights protection, d) transfer of skills and knowledge to enable the community to carry on similar biodiscovery initiatives on their own even after this project has ended, e) developing commercial products for their own use and marketing, and f) fair distribution or share of the income from the sale of their new drug lead molecules to the pharma companies. Many Indigenous communities in Queensland live in remote areas with limited access to conventional modern medicine, which is available in larger towns and cities. These remote communities still use their culturally accustomed plants as bush food and bush medicines. Therefore, the biodiscovery projects must generate ethically sound scientific data to support their uses by the communities, while at the same time discover novel drug leads with commercial prospects. In addition, the toxicity data on medicinal plants should help the Indigenous communities to make more informed decisions on the safe use of those medicinal plants. Achieving these objectives will add value, confidence and pride to their cultural identity and will improve the preservation and sustainability of their healthcare knowledge, and also that of the medicinal biota of the rainforests. To ensure that the precious first-hand customary knowledge about medicinal biota is preserved and promoted for future generations, the community-authored bush medicine handbooks and a passworded online database on Customary Medicinal Knowledgebase, must be supported through the projects.

2.5. Conclusion and future directions

This review identified 135 species of Queensland Aboriginal medicinal plants, which belong to 103 genera from 53 families, with Myrtaceae being the highest represented plant family. While trees represented the biggest life form, leaves were most used plant parts. Of 62 different diseases treated by the medicinal plants, highest number of plants are used for treating skin sores and infections. Few plants identified through this review can be found in other tropical countries but many of these medicinal plants

are native to Australia. Many of these native medicinal plants are rarely studied for their phytochemical and pharmacological properties and have a huge potential for discovering novel drug lead compounds.

Therefore, there is an urgent need to study the biota with these unexplored medicinal plants for developing novel plant-based drugs. However, it is vital that the biodiscovery projects should benefit the Indigenous communities in Queensland fairly and equitably in accordance with the international and national biodiscovery bylaws and regulations. Prior to any biodiscovery project proposals, it is recommended that the Indigenous communities are consulted first and engaged in the beginning as collaborators/partners and equal decision makers for how grants are applied for if the biodiscovery project funding is limited to only laboratory experiments and consumables (which is the case in most of the project funds), the research organisations should explore means to help the collaborating communities with the community development support services. It is necessary that we build this awareness so that research groups can apply for this funding and ensure that collaboration provides value to all the participants in a project.

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Chapter 3. Phytochemical content and antioxidant and anti-inflammatory activities of Iningai Aboriginal medicinal plants of central Queensland, Australia

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Phytochemical content and antioxidant and anti-inflammatory activities of Aboriginal medicinal plants of Central Queensland, Australia

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Abstract:	Background and aim: Indigenous Australians have vast ethnopharmacological knowledge of their native plants and have been using them for millennia. The Tropical Indigenous Ethnobotany Centre, Australian Tropical Herbarium, and the James Cook University have been working with key members of traditional custodian families of the Iningai people near Barcaldine, Queensland, Australia. This collaboration presents an effective means to preserve their rich ethnopharmacological knowledge and add scientific validations. Thus, this study aims to evaluate crude extracts from their eight medicinal plant species for major phytochemical contents, antioxidant properties, and anti-inflammatory properties. Methods and results: Crude extracts of leaf from eight Iningai medicinal plant species were assessed for their phytochemicals, including total (poly)phenolic content by the Folin-Ciocalteu and phytochemical testing methods and antioxidant activity using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. Furthermore, crude extracts were evaluated for the cell viability effect and anti-inflammatory activity by the lipopolysaccharide (LPS)-activated human peripheral blood mononuclear cells (PBMCs) assay. The phytochemical content (alkaloids, flavonoids, and terpenoids) of crude extracts of the eight medicinal plant species was determined. The species showed moderate to promising antioxidant activity through scavenging DPPH radical, with IC50 values ranging from 37.37 ± 1.01 mg/mLto 206.50 ± 2.44 mg/mL. The crude leaf extract from Pittosporum angustifolium caused the highest cell death (73.97% dead cells) in the cell viability test, indicating potential toxicity to human cells. Seven of eight medicinal plants significantly suppressed the release of four pro-inflammatory cytokines, tumour necrosis factor (TNF), interferon-gamma (IFN-g), monocyte chemoattractant protein-1 (MCP-1), and interleukin (IL)-23. Conclusion: The current study identified major phytochemicals, toxicity profiles, and antioxidant and anti-inflammatory properties, validating the traditional uses of eight medicinal plants from Iningai. Further research on the most promising species is recommended to identify bioactive lead compounds for developing novel therapeutics based on the promising antioxidant and anti-inflammatory results.

3.1. Abstract

Background and aim: Indigenous Australians have vast ethnopharmacological knowledge of their native plants and have been using them for millennia. The Tropical Indigenous Ethnobotany Centre, Australian Tropical Herbarium, and the James Cook University have been working with key members of traditional custodian families of the Iningai people near Barcaldine, Queensland, Australia. This collaboration presents an effective means to preserve their rich ethnopharmacological knowledge and add scientific validations. Thus, this study aims to evaluate crude extracts from their eight medicinal plant species for major phytochemical contents, antioxidant properties, and anti-inflammatory properties.

Methods and results: Crude extracts of leaf from eight Iningai medicinal plant species were assessed for their phytochemicals, including total (poly)phenolic content by the Folin-Ciocalteu and phytochemical testing methods and antioxidant activity using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. Furthermore, crude extracts were evaluated for the effects on cell viability and anti-inflammatory activity by the lipopolysaccharide (LPS)-activated human peripheral blood mononuclear cells (PBMCs) assay. The phytochemical content (alkaloids, flavonoids, and terpenoids) of crude extracts of the eight medicinal plant species was determined. The species showed moderate to promising antioxidant activity through scavenging DPPH radical, with IC_{50} values ranging from 37.37 ± 1.01 μg/mL to 206.50 ± 2.44 μg/mL. The crude leaf extract from *Pittosporum angustifolium* caused the highest cell death (73.97% dead cells) in the cell viability test, indicating potential toxicity to human cells. Seven of eight medicinal plants significantly suppressed the release of four pro-inflammatory cytokines, tumour necrosis factor (TNF), interferon-gamma (IFN-γ), monocyte chemoattractant protein-1 (MCP-1), and interleukin (IL)-23.

Conclusion: The current study identified major phytochemicals, toxicity profiles, and antioxidant and anti-inflammatory properties, validating the traditional uses of eight medicinal plants from Iningai. Further research on the most promising species is recommended to identify bioactive lead compounds for developing novel therapeutics based on the promising antioxidant and anti-inflammatory results.

3.2. Introduction

Medicinal plants and Traditional Medicine (TM) play an important role in managing and preventing many chronic diseases (WHO, 2019a), with about 50,000–70,000 plant species recorded as having medicinal value (Wangchuk, 2018). More than 170 member states of the World Health Organization (WHO) use various forms of TMs for treating many lifestyle-related chronic diseases (Turpin et al., 2022; Wangchuk, 2018; Yeshi et al., 2022a). Those who practice various forms of TM constitute about 80% of the developing world's population (Wangchuk, 2018). Recently, the WHO emphasised the importance of TMs and held the first-ever WHO traditional medicine global summit in

conjunction with the G20 health ministerial meeting in 2023 in Gujarat, India (WHO, 2023). The WHO continues to support member states in promoting or integrating TM with modern healthcare systems. However, a lack of consolidated response from some member countries has hindered the WHO's efforts in TM promotion in many countries, including Australia (Panzironi, 2013; WHO, 2019b).

Australian Indigenous people have inhabited the Australian continent for at least 65,000 years (Clarkson et al., 2017) and currently constitute 3.3% of the Australian population (ABS, 2019). They connect deeply with the local flora and fauna, using them for medicine, food, clothing, shelter, tools, and weapons (Clarke, 2007; Turpin et al., 2022). Their ethnomedicinal knowledge is still vibrant, although frequencies of use may differ amongst Aboriginal clan groups across Australia (Oliver, 2013). So far, about 1511 medicinal plants have been recorded in Australia (Packer et al., 2012), and a recent review by Turpin et al. (Turpin et al., 2022) identified 135 medicinal plant species used by various Aboriginal communities in the State of Queensland, including the Iningai Aboriginal community in central Queensland, for treating 62 diseases and conditions, including pain and inflammations from cuts and wounds (Turpin et al., 2022). Moreover, in our review (Yeshi et al., 2022a) of 78 medicinal plants used by Aboriginal communities in the tropics, extracts or compounds from 45 species were found to have anti-inflammatory properties. However, the biological activities and phytochemical contents of many other Queensland medicinal plants, especially those used by the Iningai community in central Queensland, have not yet been studied.

The Iningai community is one of the largest Aboriginal nations in central western Queensland, situated in the desert uplands bioregion (Figure 3.1A). Bioregions are relatively large defined areas based on common climate, geology, landform, native vegetation, and species information (Williamson et al., 2011). The desert upland bioregion has abundant Eucalypt woodlands, *Spinifex* understorey, and *Acacia* woodlands growing on sandstone ranges and sand plains (Queensland Herbarium, 2023) (Figure 3.1B). Iningai country spans 50,504 km^2 of abundant eucalypt woodlands with *Spinifex* understorey and *Acacia* woodlands growing on sandstone ranges and sand plains from west of the Dividing Range to Longreach and north to Aramac and Muttaburra. This community's rich Indigenous Biocultural Knowledge (IBK) remains little studied scientifically and could be a promising source of knowledge for the biodiscovery of potential new medicines.

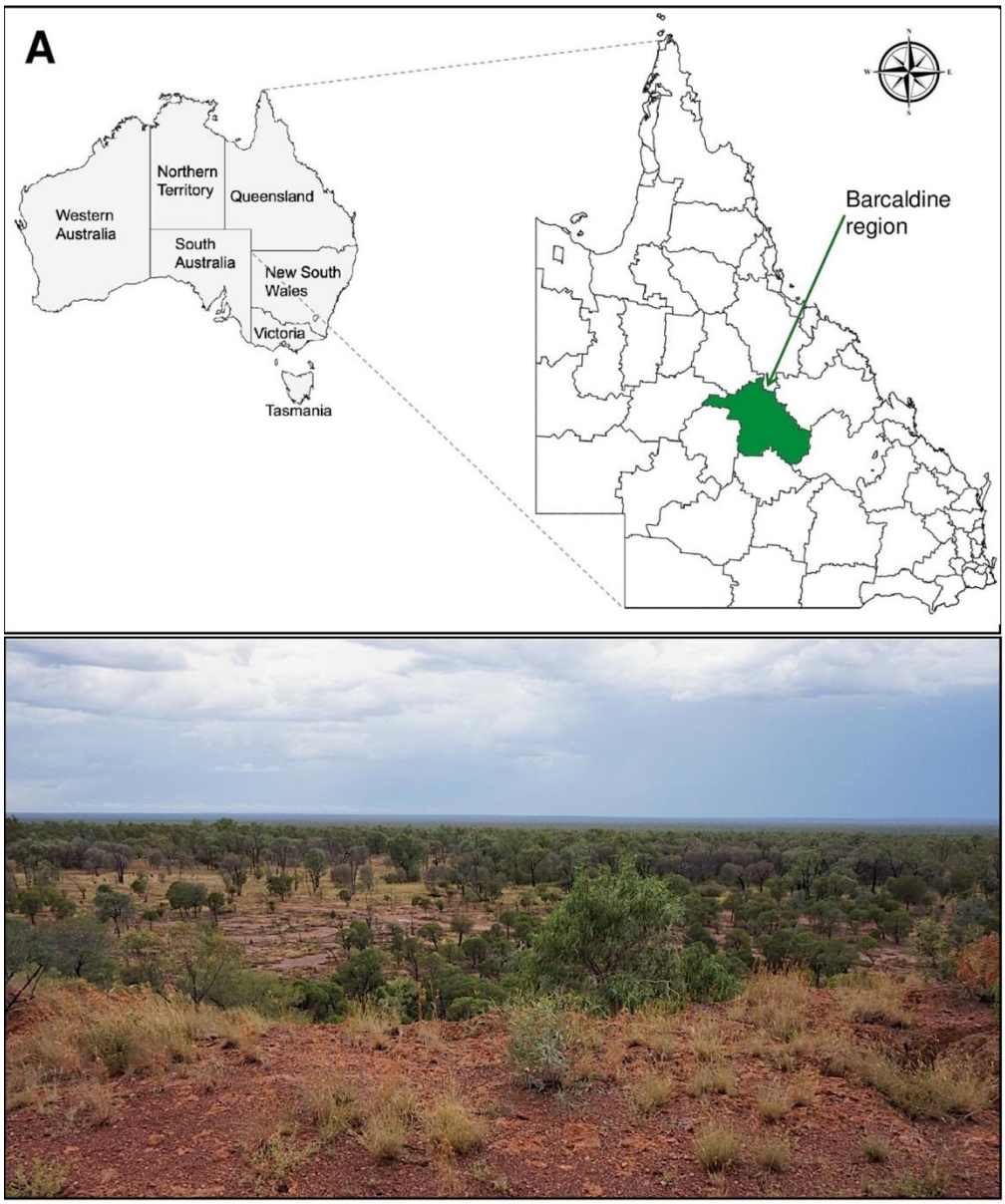

Figure 3.1 (A) A map of Australia showing the State of Queensland with the shaded green area representing the Barcaldine region of the Iningai Aboriginal community – labels and locations are added from a map of local government areas in QLD (DSDILGP, 2021); (B) A typical open woodland community on sandy soil found in the Barcaldine region. (Photo courtesy: G. Turpin).

Recent research has shown that when compared with tropical flora, plants growing in arid zone environments exposed to different environmental stressors, such as low rainfall and high ultraviolet exposure, remain an untapped reservoir of potentially novel bioactive compounds (Simpson et al., 2016). The collaborative approach (cross-cultural or two-way) of IBK working with modern science has proven to be one of the promising biodiscovery strategies (Ens et al., 2016; Ens et al., 2015; Yeshi et al., 2022a),

with more than 25% of currently available prescription drugs sourced from natural products associated with IBK (Rates, 2001; Wangchuk & Tobgay, 2015). Thus, we hypothesised that Iningai medicinal plants used to treat inflammation, and related diseases may offer a new avenue for developing natural antioxidant supplements or anti-inflammatory drugs. In this study, we assessed crude leaf extracts of eight medicinal plant species found in Iningai country for major phytochemical contents, antioxidant properties, and anti-inflammatory properties.

3.3. Materials and methods

3.3.1. Ethics

Ethical and cultural clearance for research on medicinal plants was obtained from the Iningai Aboriginal community. *In vitro* assays with human peripheral blood mononuclear cells (PBMCs) were conducted under ethics approval number H8523, Human Research Ethics Committee, James Cook University.

3.3.2. Chemicals and reagents

The chemicals and reagents used in this study were all AR grades. Aluminium chloride, ammonia, butylated hydroxytoluene (BHT), Dragendorff reagent, ferric chloride, ferric sulphate, Folin-Ciocalteu (F-C) reagent, gallic acid, glacial acetic acid, hydrochloric acid (HCl), sodium carbonate, sulphuric acid, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma Aldrich (Melbourne, VIC, Australia). chloroform, ethanol, and methanol were purchased from ChemSupply (Gillman, SA, Australia).

3.3.3. Instruments

To determine total phenolic content (TPC) and antioxidant activity of samples, absorbance was obtained using a microplate reader (SPECTROstar® Omega, BMG Labtech, VIC, Australia). To determine the effect on cell viability and anti-inflammatory activity, samples were run through the LSRFortessa™ X20 (BD Biosciences), and the data (mean fluorescence intensity, MFI) obtained were exported in the FCS (flow cytometry standard) format for further analysis.

3.3.4. Plant material collection

All plant species evaluated in this study were collected from Iningai country in central Queensland, Australia, by the first author and Iningai Traditional Custodians in June 2022. Taxonomic identification was confirmed by botanists at the Queensland Herbarium (Index Herbarium code: BRI). Herbarium specimen numbers were assigned to each plant species (Table 3.1), and voucher specimens were deposited at the Australian Tropical Herbarium (CNS). Fresh leaves were washed under running tap water and dried in an oven (40 °C) for one week.

3.3.5. Crude extraction and analysis for major classes of phytochemicals

For each plant species, 10 grams (g) of dried leaves were ground into a coarse powder using a NutriBullet grinder (NutriBullet, Australia), transferred to a clean flask, and 50 mL methanol was added. The mixture was constantly stirred for 20 minutes (min) to obtain the optimum extract. Fresh solvent was added every 20 min (thrice with a total extraction time of 1 h). The three-cycle extract was pooled and filtered using a Sterricup quick vacuum-driven disposable filtration system (0.22 µM PES, 500 mL, Merck, Kenilworth, USA), and the filtrate solvent was concentrated using a rotary evaporator (Heidolph, Germany) to 16 mL. The 16 mL of concentrated extract was divided into eight tubes, with each volume of 2 mL to test for the absence or presence of seven major phytochemical classes (Table 3.2) using a method adapted from Wangchuk et al. (Wangchuk et al., 2011), Iqbal et al. (Iqbal et al., 2017), Yeshi et al. (Yeshi et al., 2022b) and Shah and Hossain (Shah & Hossain, 2014). Briefly, qualitative phytochemical analysis was conducted as below:

i. Alkaloid test: 1 mL of Dragendorff reagent (1 mL) was added to 2 mL of crude extract, and the presence of alkaloid was confirmed by the development of orange/white precipitate.

ii. Steroid test: 2 mL of chloroform was added to 2 mL of crude extract, followed by a few drops of acetic anhydride. The test tube containing the reaction mixture was heated in a water bath (40 °C) for 10 min, followed by cooling on ice. Then, 1 mL of sulphuric acid (conc.) was added, and the presence of steroid was confirmed by forming a brown ring at the junction and the upper green layer.

iii. Terpenoid test: 2 mL of chloroform was added to 2 mL of crude extract, followed by a few drops of sulphuric acid (conc.). The presence of terpenoids was confirmed by the formation of a reddish-brown colour at the interface of the reaction mixture.

iv. Tannin test: a few drops of 10% ferric chloride (aqueous) were added to 2 mL of crude extract, and the presence of tannin was confirmed by the formation of black precipitates/blue-green colour.

v. Saponin test: the presence of saponin was confirmed by the formation of persistent foam/froth after shaking with distilled water. The test was considered negative when foam/froth disappeared after warming in a water bath (37 °C) for 5 min.

vi. Anthraquinone glycoside test: dilute sulphuric acid (5%), and chloroform were added to 2 mL of crude extract. Then, a dilute ammonia solution was added to the lower layer of the reaction mixture with slight shaking. The formation of a rose-pink to-red colour confirmed the presence of anthraquinone glycoside.

vii. Cardiac glycoside test: 2 mL of glacial acetic acid was added to 2 mL of crude extract, followed by a few drops of ferric chloride (10%) and dilute sulphuric acid (5%). The presence of cardiac glycosides was confirmed by the formation of a brown ring at the interface.

3.3.6. Determination of total phenolic content (TPC)

3.3.6.1. Determination of maximum absorption wavelength (λmax) and optimum retention time

The maximum absorption wavelength (λ_{max}) and optimum reaction time were determined following the methods (with slight modifications) described by Molole et al. (Molole et al., 2022) and Adusei et al. (Adusei et al., 2019) before evaluating the samples for the total phenolic content. Briefly, 0.5 mL of crude leaf extract samples (200 and 400 µg/mL) and the standard gallic acid in methanol (50 and 100 µg/mL) were transferred to clean test tubes. Then, 2 mL Folin-Ciocalteu (F-C) reagent (diluted with distilled water in a 1:1 ratio) was added to the sample/standard and vortexed. After 3 min but not more than 8 min, 4 mL of Na_2CO_3 (7.5% w/v in distilled water) was added and mixed well. The reaction mixture (200 µL) was then transferred to a 96-well flat bottom plate (Falcon®, Corning, NJ, USA), and the absorbance in the wavelength range of 400 to 900 nm was measured at 5 min intervals until 60 min. Methanol was used as a blank. The absorbance readings were used to determine the λ_{max} and the optimum reaction time.

3.3.6.2. Total phenolic content (TPC)

After obtaining λ_{max} and the optimum reaction time, TPC in the crude extract of leaves from eight medicinal plants was determined following the Folin-Ciocalteu (F-C) method described by Molole et al. (Molole et al., 2022), Yeshi et al. (Yeshi et al., 2022b), and Adusei et al. (Adusei et al., 2019) with slight modifications. The TPC was determined in triplicates at two concentrations (500 and 1000 µg/mL). The standard calibration curve was prepared using a gallic acid solution with concentrations from 0 to 150 µg/mL in triplicates. The reaction mixtures were prepared as described above, and the absorbance was recorded at previously determined λ_{max} (760 nm) and incubation time (30 min). The average absorbance values were used to draw the calibration curve, and the R^2 (coefficient of determination) value was used to evaluate the linearity of the curve. The regression equation obtained from the curve was used to calculate the gallic acid concentration in each crude extract. Finally, TPC was expressed in terms of milligrams of gallic acid equivalent per gram of dry extract (mg GAE/g dry extract) using the equation below (Siddiqui et al., 2017):

$$C = C1 \times \frac{V}{m}$$

Where ‘C’ is TPC in mg GAE/g extract, ‘C1’ is the concentration of gallic acid (in mg/mL) calculated from the calibration curve, ‘V’ is the extract sample volume (in mL), and ‘m’ is the mass of the dry plant extract (g).

3.3.6.3. 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The 1 mg/mL stock solutions of the crude leaf extract from eight medicinal plants and standard compounds were prepared. Then, the working solution was prepared with 2-fold serial dilutions (13-500 µg/mL) in methanol. Subsequently, each sample/standard compound was mixed with 0.1 mM of DPPH solution (1:2 ratio vol/vol) in a 96-well flat-bottom plate and mixed well. The control sample was prepared by mixing the methanol with DPPH in the same ratio. The reaction mixture was then incubated in the dark at room temperature for one hour. After one hour of incubation, the absorbance was measured at the previously determined λ_{max} (517 nm). Finally, the percentage of DPPH radical scavenged in the reaction mixture was calculated using the equation below:

$$\text{Percentage (\%) of DPPH scavenged} = \frac{\text{Ao} - \text{As}}{\text{Ao}} \times 100$$

Where A_0 is the absorbance of the DPPH solution without antioxidants and A_s is the absorbance of the sample (Fadda et al., 2014). The obtained % of DPPH radical scavenged was plotted against the concentration of the samples in GraphPad prism (v.10.2.2), and IC_{50} values were determined by interpolating from a sigmoidal standard curve, four-parameter logistic (4PL) regression model, where 'X' is concentration.

3.3.7. Effect of crude extracts on cell viability and their anti-inflammatory activity

3.3.7.1. Human peripheral blood mononuclear cells (PBMCs) culture and sample treatment

Human blood from healthy donors was sourced from the Australian Red Cross Lifeblood. Subsequently, PBMCs were isolated from the whole blood using the Ficoll-Paque PLUS (GE Health) density gradient method described by the STEMCELL technologies. PBMCs were then cryopreserved in heat-inactivated FBS (foetal bovine serum) (Corning #35-076-CV) with 10% DMSO (dimethyl sulfoxide, Sigma-Aldrich). On the day of the experiment, PBMCs were thawed in a water bath (37 °C) and washed with pre-warmed R-10 media (RPMI-1640, Gibco), containing 10% heat-inactivated FBS, 100 U/mL penicillin, and 100 µg/mL streptomycin (Gibco). Approximately 1×10^6 cells in 100 µL of R-10 media were seeded per well in 96-well U-bottom culture plates (Falcon®, Corning, NY, USA). Then, PBMCs were activated with 10 ng/mL of bacterial-derived lipopolysaccharide (LPS) (Sigma-Aldrich) and incubated at 37 °C in a 5% CO_2 incubator for 2 h. After 2 h, LPS-activated PBMCs were treated in triplicate with methanolic crude leaf extracts of eight medicinal plants at 100 µg/mL concentration in R-10 media with 0.5% DMSO. Dexamethasone (Dexa) (100 µg/mL in R-10 media) was used as positive control, and R-10 media with 0.5% DMSO was used as negative control. The final culture volume in each well was adjusted to 200 µL using the R-10 media. Finally, the culture plate was incubated overnight at 37 °C in a 5% CO_2 incubator. The next day, the plate was centrifuged (277× g force, 4 °C for 5 min), and the supernatants were collected for cytokine analysis. Cells were used for cell viability assay, as described in the subsequent section.

3.3.7.2. Effect of crude extracts on cell viability

Before quantifying pro-inflammatory cytokines in the PBMCs culture soup, PBMCs were analysed for cell health/viability (cytotoxicity) (Yeshi et al., 2022b). After harvesting the supernatant, PBMCs were washed (1×) using a PBS with 2% FBS and then stained with live/dead viability dye (LIVE/DEAD™ fixable near-IR dead cell stain kit, for 633 or 635 nm excitation, cat. no. L34975) following the manufacturer's manual. Stained cells were kept on ice in the dark for 30 min. After 30 min, stained cells were washed (2×) with PBS containing 2% FBS. Finally, cells were resuspended in 100 µL PBS buffer and run through a LSRFortessa™ X20 flow cytometer (BD Biosciences) to differentiate live cells from dead cells. The reactive dye binds to surface proteins on live cells, giving a dim fluorescence. In contrast, in dead cells, due to loss of membrane integrity, dye enters inside the dead cell, binds with interior proteins, and fluoresces more brightly than in live cells. To determine the percentage (%) of viable cell population, cell viability data (in FCS format) from the flow cytometer was determined using a FlowJo 10.8.1 version software (Ashland, OR, USA). Before the analysis, a forward scattering (FSC) gating (FSC-H versus FSC-A) was applied to exclude doublet cells. After knowing the effect of crude extracts on cell viability, the supernatant of non-toxic crude extracts was further analysed for pro-inflammatory cytokines/chemokines profile.

3.3.7.3. Quantification of pro-inflammatory cytokines

Pro-inflammatory cytokines/chemokines in the PBMCs culture supernatant were quantified (in pg/mL) using a customised LEGENDplex™ multi-analyte flow assay kit (cat. no. 740808 and lot no. B291817, BioLegend®, USA) following the manufacturer's instructions. This assay is bead-based and enables simultaneous analysis of 13 pro-inflammatory cytokines/chemokines, including interleukin (IL)-1β, interferon (IFN)-α, IFN-γ, tumour necrosis factor (TNF), monocyte chemoattractant protein-1 (MCP-1), IL-6, IL-8 (CXCL8), IL-10, IL-12p70, IL-17A, IL-18, IL-23, and IL-33 using the LSRFortessa™ X20 flow cytometer (BD Biosciences). The data obtained from the flow cytometry (in FCS format) was further analysed using cloud-based software from BioLegend® (San Diego, CA, USA), accessible from this link: https://legendplex.qognit.com/user/login?next=home. Finally, the quantity of each cytokine in the culture soup treated with eight crude extracts was expressed as mean ± standard deviation (SD) in pg/mL.

3.3.8. Data analysis

All data were expressed as the mean ± SD. Statistical analysis was performed using GraphPad Prism version 10.2.2 (San Diego, CA, USA), whereby mean values of samples were compared to the control using unpaired t-test ($p < 0.05$).

3.4. Results

3.4.1. Ethnopharmacological uses of eight Iningai medicinal plants

Recently, we have documented Iningai bush medicine knowledge and identified 45 medicinal plants from the Barcaldine area of the Iningai country. While some plants are common to other regions

of Queensland and are known for their strong antioxidant and anti-inflammatory activities (e.g., *Pittosporum* and *Corymbia* species), we found that more than 14 unique medicinal plants were used for treating bowel disorders and inflammation. These plants have never been studied from a Western scientific perspective, which adds novelty to this project. Building on the field results and in consultation with the Iningai community and the Tropical Indigenous Ethnobotany Centre (TIEC), we collected eight Iningai medicinal plants (Table 3.1) from the Barcaldine region by engaging the Iningai community and evaluated their phytopharmaceutical properties using various approaches (Yeshi et al., 2022b; Molole et al., 2022; Adusei et al., 2019; Iqbal et al., 2017; Fadda et al., 2014; Wangchuk et al., 2011). These species are TB75 ((Intellectual Property rights protected (IP) protected)), TB79 (IP rights protected), *Pittosporum angustifolium, Gossypium australe*, *Velleia macrocalyx*, *Petalostigma pubescens* and *Santalum lanceolatum* (Figure 3.2).

Table 3.1 Eight medicinal plant species with their specimen voucher number and ethnopharmacological uses (Mani et al., 2021); Turpin et al., 2022).

Botanical name and family	Voucher specimen number	Part used for extraction	Part(s) used for treatments	Ethnopharmacological properties
Dodonaea tenuifolia Lindl. (Sapindaceae)	BRI AQ1030932	Leaf	Leaf	Used for treating toothache and skin infections.
*TB75 (Myrtaceae)	BRI AQ1034525	Leaf	Leaf, bark	Used for treating sores and cuts.
Gossypium australe F.Muell. (Malvaceae)	BRI AQ1034548	Leaf	Leaf	Used for treating sores, cuts, and cough and colds.
Velleia macrocalyx de Vriese. (Goodeniaceae)	BRI AQ103453	Leaf		Used against digestive disorders.
*TB79 (Myrtaceae)	BRI AQ1034545	Leaf		Used for treating cuts, nail fungus, and relieving insect bites.
Pittosporum angustifolium G.Lodd. (Pittosporaceae)	BRI AQ1034549	Leaf	Leaf, fruit	Used as poultice or decoction for healing bruises, muscle aches, eczema, and other forms of pruritis, cough and cold, cramps, eczema, and skin diseases, and inducing lactation in mothers of newborns.
Petalostigma pubescens Domin. (Euphorbiaceae)	BRI AQ1030888	Leaf	Fruit, bark	Used as an antiseptic, and for treating fever, malaria, toothache, and sore eyes.
Santalum lanceolatum R.Br (Santalaceae)	BRI AQ1034547	Leaf	Leaf; Outer wood; inner moist bark	Used as a purgative, and for healing chest pain, arthritis, sores, wounds, cuts, skin lesions, and sexually transmitted diseases.

*Further research is ongoing for two species, which have been coded as TB75 and TB79 due to intellectual property (IP) sensitivity.

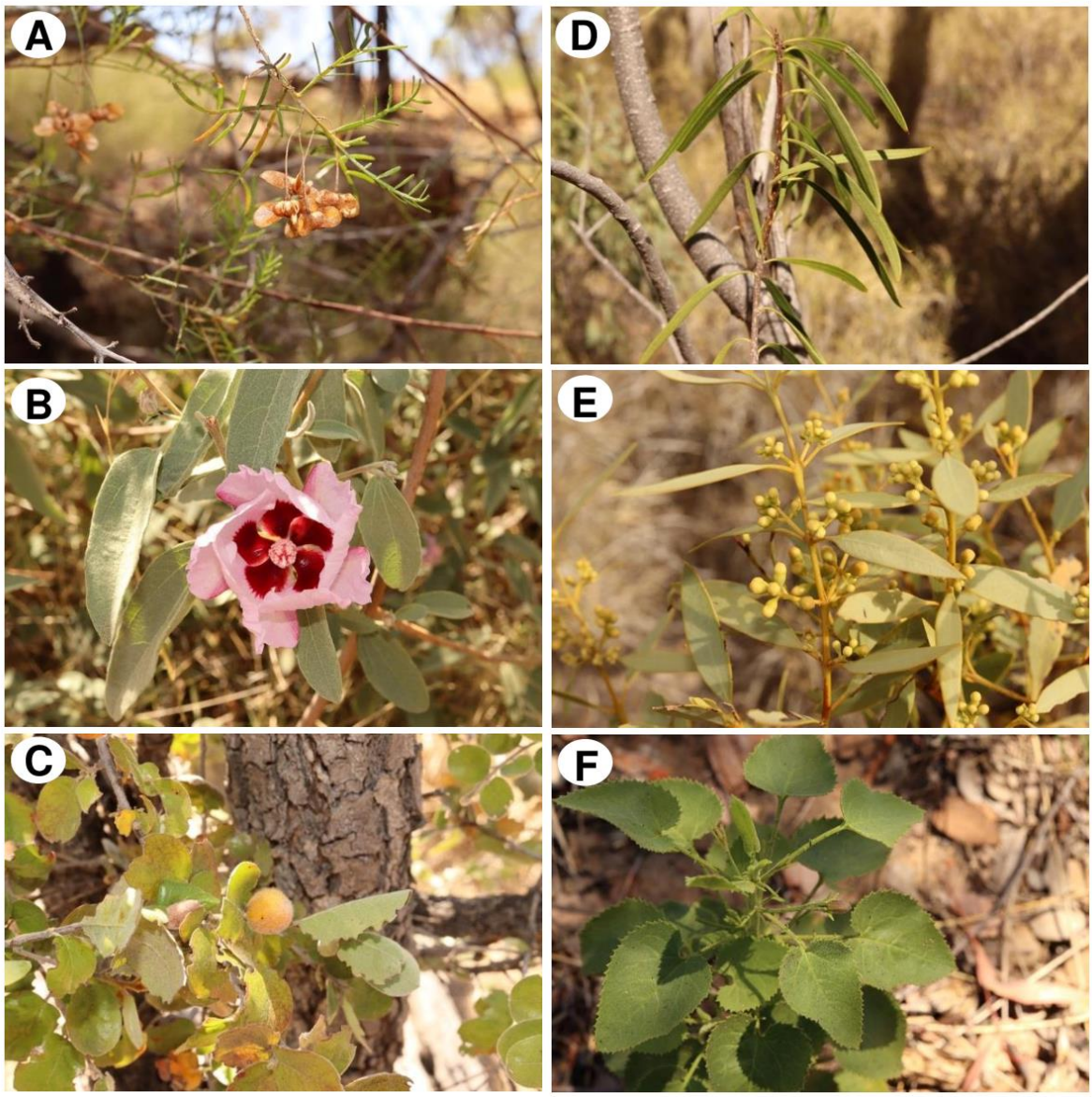

Figure 3.2 Medicinal plant species included in the study with their aerial parts: A) *Dodonaea tenuifolia*; B) *Gossypium australe*; C) *Petalostigma pubescens*; D) *Pittosporum angustifolium;* E) *Santalum lanceolatum*; F) *Velleia macrocalyx.* Photographs of two species, which have been coded as TB75 and TB79, were excluded due to intellectual property (IP) sensitivity. (Photo courtesy: G. Turpin).

3.4.2. Qualitative screening for major classes of phytochemicals in crude extracts

Plants generally produce diverse classes of phytochemicals, and most possess interesting biological activities (Geyid et al., 2005; Wangchuk et al., 2011). The diversity and intensity of a plant's biological activities may be related to the diversity and quantity of the chemicals it contains. Thus, we assessed crude leaf extracts of eight medicinal plants for the presence of the six major classes of phytochemicals.

Table 3.2 Qualitative screening for the major phytochemical classes.

Plant species	Sample tested	Alkaloids	Flavonoids	Steroids		Tannins	Saponins	Glycosides	
				Salkawski test	Liebermann Buchard's test			Anthraquinone glycosides	Cardiac glycosides
D. tenuifolia	Leaf	+	+	+	–	+	+	+	–
TB75	Leaf	+	+	–	–	–	+	–	+
G. australe	Leaf	–	–	+	+	–	–	+	–
V. macrocalyx	Leaf	–	+	+	–	–	+	–	–
TB79	Leaf	+	+	–	–	+	+	–	–
P. angustifolium	Leaf	+	+	–	–	–	–	–	–
P. pubescens	Leaf	+	+	–	–	+	+	–	–
S. lanceolatum	Leaf	+	+	+	+	+	–	–	–

Symbols: – indicates negative test for the phytochemical class tested; + indicates positive test for the phytochemical class tested.

All tests were performed following methods (with slight modifications) described by Molole et al. (Molole et al., 2022) and Adusei et al. (Adusei et al., 2019).

Of the eight medicinal plants tested, *D. tenuifolia* tested positive for all six major classes of phytochemicals, namely, alkaloid, flavonoid, tannin, steroid, saponin, and glycoside (Table 3.2). Four of the six classes were detected in the following species: TB75 (alkaloid, flavonoid, saponin, and glycoside), TB79 (alkaloid, flavonoid, tannin, saponin), *P. pubescens* (alkaloid, flavonoid, tannin, saponin), and *S. lanceolatum* (alkaloid, flavonoid, steroid, and tannin). *V. macrocalyx* showed the least positive results, testing positive for only flavonoids, steroids, and saponins. Only TB75, *G. australe,* and *D. tenuifolia* tested positive for glycosides. Except for *G. australe* and *V. macrocalyx,* crude extracts from all medicinal plants tested positive for the alkaloid test.

3.4.3. Total phenolic content (TPC) and antioxidant activity of crude extracts

3.4.3.1. Total phenolic content (TPC)

The Folin-Ciocalteu (F-C) method is a widely used assay for quantifying total (poly)phenols in food (Pérez et al., 2023). The F-C reagent is a complex mixture of phosphomolybdic and phosphotungstic acids, which the phenolic compounds reduce, forming blue complexes with maximum absorbance (λ_{max}) at 765 nm (Ainsworth & Gillespie, 2007; Rosbash, 1949; Singleton & Rossi Jr, 1965). However, λ_{max} may vary depending upon how the F-C reagent responds to the compounds/substrates, as polyphenols (antioxidants) are of diverse chemical structures (Blainski et al., 2013; Galvão et al., 2018). Such variations in the wavelength of maximum absorption may affect the quantitative estimation of total (poly)phenolic compounds. Therefore, crude leaf extracts from eight medicinal plants were compared with the standard compound, gallic acid, to determine λ_{max}. All eight crude leaf extracts showed a similar absorption spectrum (Figure 3.3A). The maximum absorption (λ_{max}) lies between 700 and 800 nm, as shown in Figure 3.3A. Moreover, there were insignificant variations (coefficient of variation, CV, ≤ 3.67%) in the absorbance values between 700 and 800 nm, as shown in Table 3.3. Thus, to determine the TPC, a wavelength (λ_{max}) of 760 nm was chosen in accordance with previous study (Yeshi et al., 2022) and other studies (Blainski et al., 2013; Molole et al., 2022; Singleton et al., 1974).

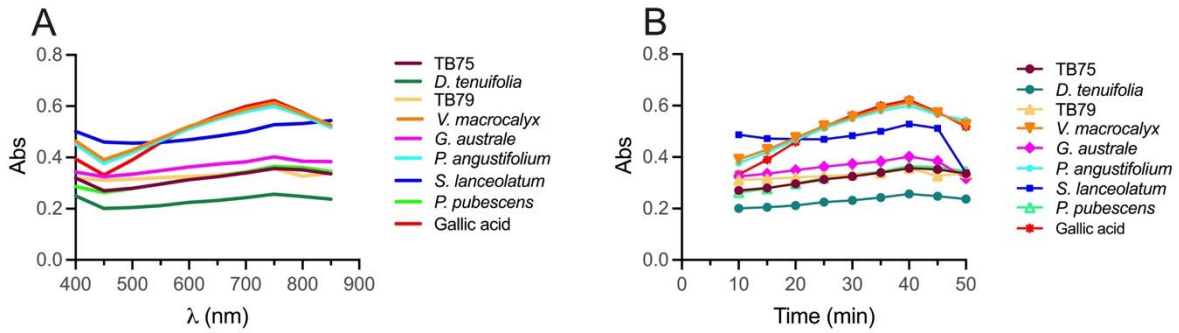

Figure 3.3 Maximum absorbance (λmax) and optimum reaction time for total phenolic content: A) absorption spectra (400-850 nm) of the product of the reaction of F-C reagent with crude leaf extracts of eight medicinal plant species (400 µg/mL) and gallic acid (100 µg/mL); B) change in absorbance with a reaction time of F-C reagent with crude leaf extracts of eight medicinal plant species and gallic acid.

After selecting the maximum absorption wavelength (760 nm), the optimum time required by the crude leaf extracts to develop/change the colour (from yellow to blue) with the F-C reagent was determined at the selected wavelength. The reaction kinetics showed an increase in the absorption by all samples from 10 to 40 min, then became steady or dropped progressively, as shown in Figure 3.3B. For all samples, including the gallic acid, the optimum response was observed between 30 to 40 min, indicating that at least 30 min of incubation is required before measuring the absorbance. Our result was consistent with other similar optimisation studies on the F-C method (Blainski et al., 2013; Galvão et al., 2018; Molole et al., 2022). Thus, λ_{max} of 760 nm and an optimum reaction time of 30 min was used to determine the TPC in the crude leaf extracts of eight medicinal plants.

Table 3.3 The absorbance of solutions of leaf crude extracts of eight medicinal plant species and gallic acid after 30 min of reaction with F-C reagent in a wavelength range of 740-770 nm.

Sample	Conc. (ug/mL)	Absorbance at the wavelength (nm) of							CV (%)
		740	745	750	755	760	765	770	
D. tenuifolia	200	0.151	0.145	0.145	0.145	0.145	0.146	0.144	2.73
	400	0.195	0.195	0.196	0.197	0.198	0.196	0.194	0.66
TB75 (IP protected)	200	0.185	0.171	0.173	0.173	0.173	0.174	0.172	1.60
	400	0.217	0.214	0.216	0.215	0.214	0.216	0.213	0.69
G. australe	200	0.136	0.131	0.131	0.13	0.13	0.13	0.129	3.67
	400	0.167	0.161	0.163	0.164	0.163	0.165	0.162	1.46
V. macrocalyx	200	0.132	0.126	0.126	0.127	0.131	0.125	0.125	1.81
	400	0.179	0.18	0.182	0.183	0.181	0.181	0.18	0.56
TB79 (IP protected)	200	0.14	0.127	0.128	0.128	0.126	0.128	0.129	2.26
	400	0.154	0.148	0.151	0.15	0.148	0.148	0.15	0.74
P. angustifolium	200	0.165	0.157	0.159	0.158	0.157	0.158	0.157	0.36
	400	0.244	0.232	0.236	0.235	0.235	0.234	0.231	0.68
P. pubescens	200	0.267	0.252	0.255	0.257	0.257	0.257	0.256	1.76
	400	0.386	0.381	0.381	0.383	0.38	0.38	0.381	1.22
S. lanceolatum	200	0.266	0.265	0.266	0.266	0.268	0.266	0.267	1.81
	400	0.391	0.391	0.397	0.396	0.393	0.392	0.39	1.80
Gallic acid	50	0.297	0.293	0.294	0.298	0.294	0.295	0.293	0.66
	100	0.488	0.486	0.489	0.491	0.489	0.489	0.485	0.42

*Sample: crude leaf extract; Coefficient of variance (CV, %) was calculated dividing the standard deviation (σ) by the mean (μ) of absorbance values of each sample between 740 to 770 nm wavelength multiplied by 100% ($CV = \sigma/\mu \times 100\ \%$) (Molole et al., 2022).

To quantify TPC in crude leaf extracts, a calibration curve was plotted using the reference compound, gallic acid, with concentration ranging from 0 to 150 µg/mL. The obtained R^2 (coefficient of determination) value of 0.9988 (y = 0.004x + 0.0084), approximated perfect linearity. Total phenolic content (TPC) was measured at two different concentrations (500 µg/mL and 1000 µg/mL). The results were expressed as mg gallic acid equivalent (GAE)/g dry crude extract, as shown in Figure 3.4. At both low (500 µg/mL) and high concentrations (1000 µg/mL), *S. lanceolatum* had the highest phenolic content (51.95 ± 2.38 and 89.87 ± 3.02 mg GAE/g extract, respectively), followed by *P. pubescens* (46.21 ± 2.01 and 87.45 ± 2.36 mg GAE/g extract). TB75, *G. australe*, and *P. angustifolium* had almost equivalent total phenolic content at 1000 µg/mL concentration (37.46 ± 4.95, 37.84 ± 1.02, 37.69 ± 1.00 mg GAE/g extract, respectively). However, at a lower concentration (500 µg/mL), *P. angustifolium* had a higher content (22.39 ± 1.94 mg GAE/g extract), followed by TB75 (20.89 ± 1.22 mg GAE/g extract). TB79 had the lowest phenolic content at both concentrations (Figure 3.4).

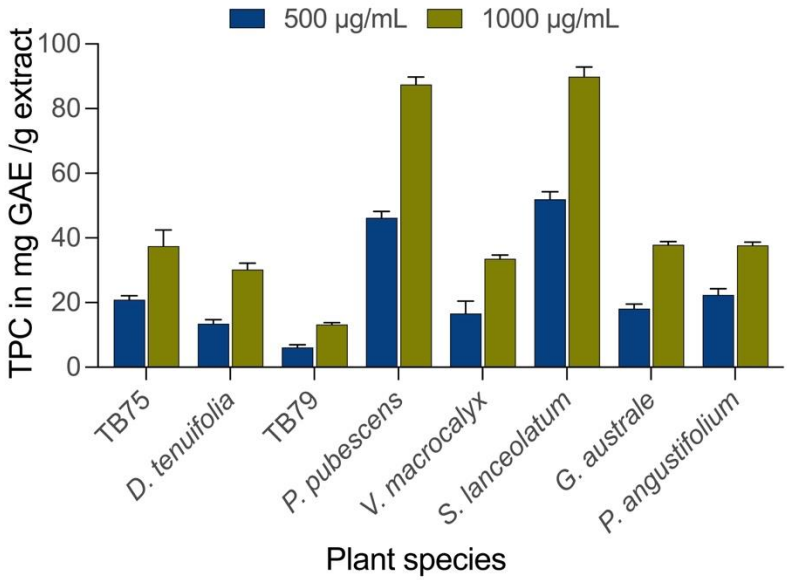

Figure 3.4 Total phenolic content of crude leaf extracts from eight medicinal plant species at two concentrations (500 and 1000 µg/mL). Data were expressed as mean ± SD [n (instrumental × sample replicate) = 9].

3.4.3.2. DPPH radical scavenging activity

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) is a widely used free radical to assess the antioxidant properties of crude extracts from different parts of plants. The ability of plant extracts to scavenge DPPH radical is mostly determined spectrophotometrically by measuring the absorbance at a specific wavelength (Amorati & Valgimigli, 2015; Molole et al., 2022). Thus, it is crucial first to

determine the maximum absorption wavelength and instrumental response to the various DPPH concentrations for the assay. As shown in Figure 3.5A, the maximum absorption was at 517 nm wavelength (λ_{max}). There was also a linear relationship between the DPPH radical concentrations and their respective absorbance at 517 nm (Figure 3.5B).

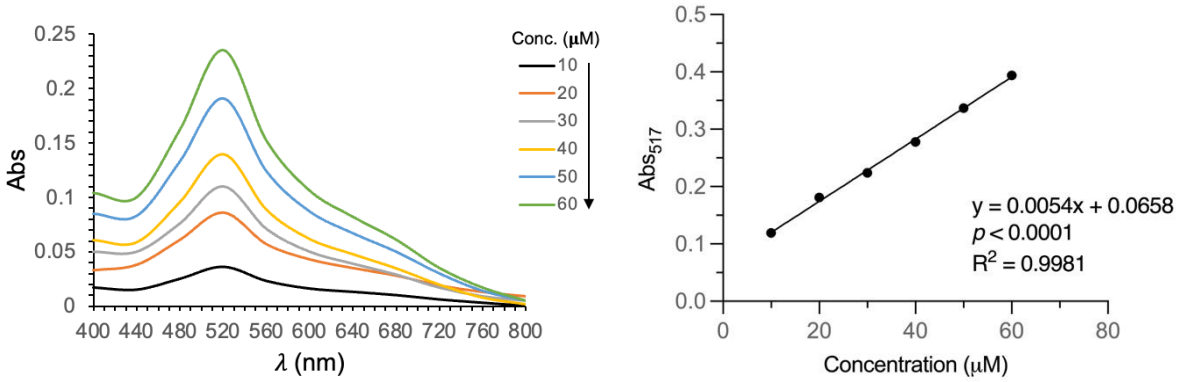

Figure 3.5 (A) Absorption spectra (400 – 800 nm) for solutions containing 10, 20, 30, 40, 50, and 60 μM DPPH and (B) the linear relationship between different concentrations of DPPH and their absorbance measured at 517 nm.

In this study, the antioxidant activity of crude leaf extracts was evaluated by measuring their absorbance at 517 nm in line with other studies (Brand-Williams et al., 1995; Yeshi et al., 2017) at various concentrations between 50 and 400 μg/mL. Gallic acid was used as a reference compound. The DPPH radical scavenging effect of all crude extracts increased as their concentration was increased (Figure 3.6). The DPPH radical scavenging activity of crude leaf extracts of eight medicinal plants at a concentration of 400 μg/mL was ordered as follows: *V. microcalyx* (77.09 ± 0.26%) > TB75 (75.28 ± 1.42%) > *P. angustifolium* (74.35 ± 0.95%) > *S. lanceolatum* (72.23 ± 2.57%) > *P. pubescens* (71.83 ± 3.59%) > TB79 (70.96 ± 0.45%) > *D. tenuifolia* (67.91 ± 4.03%) > *G. australe* (66.97 ± 5.01%). The reference compound, gallic acid, showed only 76.60 ± 1.18 % at 400 μg/mL of the initial DPPH concentration. Unlike all crude samples except for *V. macrocalyx*, gallic acid showed high and consistent scavenging activity at all concentrations (Figure 3.6).

The current study also determined the inhibitory concentration that scavenged 50% of the initial DPPH concentration (IC_{50} values). The antioxidant capacity of the substance is indirectly proportional to its IC_{50} value (Brand-Williams et al., 1995). That means the substance with a lower IC_{50} value has better antioxidant activity. Among the crude extracts from eight medicinal plant species, *V. macrocalyx*

had the lowest IC_{50} value of 37.37 ± 1.01 µg/mL, followed by *P. angustifolium* (48.33 ± 1.07 µg/mL), TB75 (119.90 ± 1.11 µg/mL) and *S. lanceolatum* (134.30 ± 2.49 µg/mL). *D. tenuifolia* had the highest IC_{50} value of 206.50 ± 2.44 µg/mL.

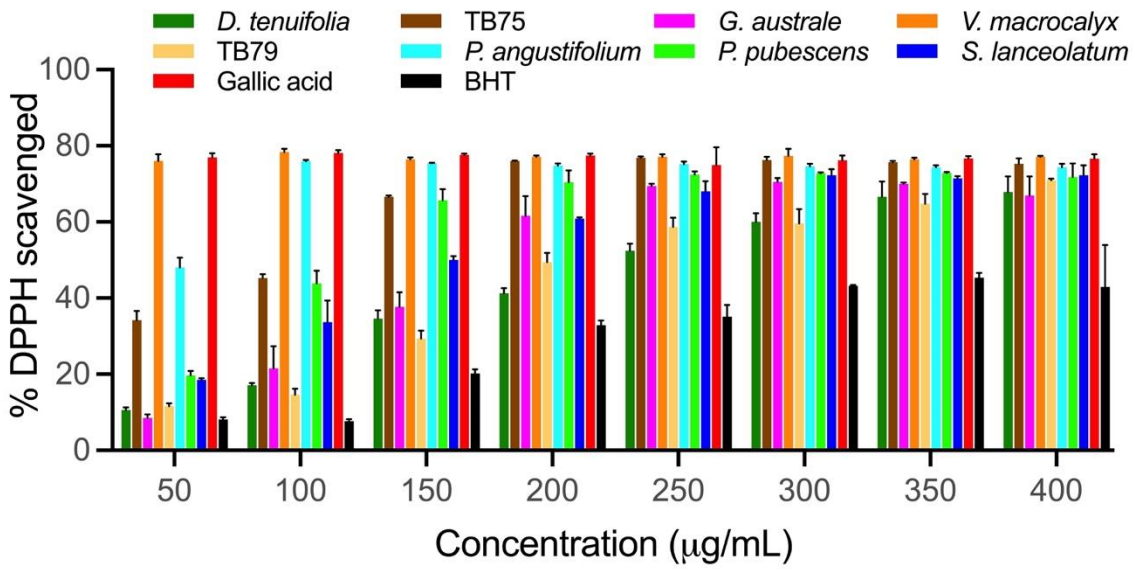

Figure 3.6 DPPH radical scavenging effects of methanolic leaf crude extracts from eight medicinal plant species, gallic acid, and butylated hydroxytoluene (BHT). Results were expressed as mean ± SD of three means (n = 3).

3.4.3.3. Correlation between TPC and antioxidant activity

Antioxidant activity is attributed to the phenolic and flavonoid contents. We conducted a Pearson correlation coefficient (PCC, Pearson r) using GraphPad Prism (v.10.2.2) between TPC and DPPH radical scavenging activity (IC_{50} values) (Figure 3.7). There was a significant correlation (*p* value = ** < 0.0027) between TPC and antioxidant activity of crude leaf extracts from eight medicinal plant species.

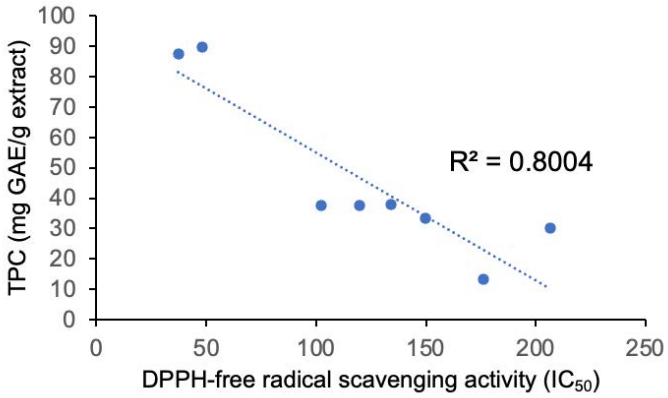

Figure 3.7 A Pearson correlation scatter plot showing the positive relationship but the negative correlation between the TPC and DPPH radical scavenging activity (IC_{50}) (i.e., higher TPC correlated with lower IC_{50} values).

3.4.4. Cell viability and anti-inflammatory activity of crude extracts

3.4.4.1. Cell viability

Of the eight medicinal plants tested, the crude leaf extract of *P. angustifolium* affected the viability of PBMCs (73.97% dead cells), whereas the remaining seven samples did not (mean dead cells <37%) (Figure 3.8) when compared with the stimulated control. Therefore, the culture supernatants from these seven species were further analysed to quantify pro-inflammatory cytokines.

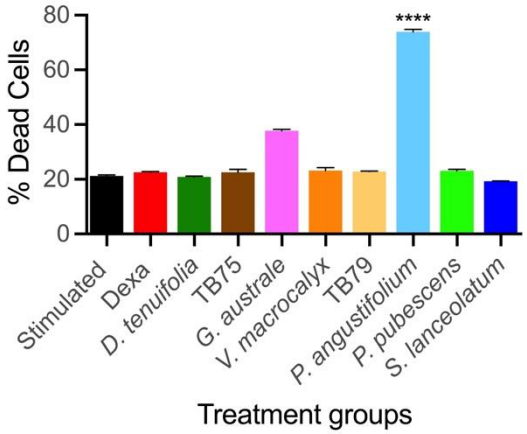

Figure 3.8 The percentage of dead cells in LPS-stimulated human PBMCs treated with the methanolic crude leaf extracts (overnight) obtained from eight medicinal plant species at a 100 µg/mL concentration. Data were expressed as mean ± SD (n = 3). Statistical significance was determined by unpaired t-test using GraphPad Prism (v.10.2.2), where ****$P < 0.0001$. Dexamethasone (Dexa) was used as a positive drug control. The % of dead cells < 50% was considered non-toxic to the cells (Yeshi et al., 2022b).

3.4.4.2. Anti-inflammatory activity

The inhibitory effect of crude leaf extracts (100 µg/mL) from seven species on 13 pro-inflammatory cytokines and chemokines in the supernatant of LPS-stimulated PBMCs was investigated using a LEGENDplex™ flow assay described in the methods section. Out of the 13 pro-inflammatory cytokines and chemokines tested (IL-1β, IFN-α, IFN-γ, TNF, MCP-1, IL-6, IL-8 (CXCL8), IL-10, IL-12p70, IL-17A, IL-18, IL-23, and IL-33), the crude leaf extracts of seven plant species (Figure 3.9) significantly reduced the production of IFN-γ (Figure 3.9A), TNF (Figure 3.9B), MCP-1 (Figure 3.9C), and IL-23 (Figure 3.9D). Except for the crude extract of *D. tenuifolia*, the remaining plant species significantly suppressed at least four pro-inflammatory cytokines. Dexamethasone (Dexa) was used as the positive control drug. Based on significant suppression (*p* values) of four cytokines (Figure 3.9), the overall anti-inflammatory effect of seven medicinal plant species tested ordered as follows: TB75 > *G. australe* > *V. microcalyx* > *S. lanceolatum* > TB79 > *P. pubescens* > *D. tenuifolium*. The crude leaf extracts of TB75, *G. australe,* and *V. microcalyx* were the three most active, followed by *S. lanceolatum* and TB79 (Figure 3.9). *Dodonaea tenuifolium* was the least active of all seven species tested.

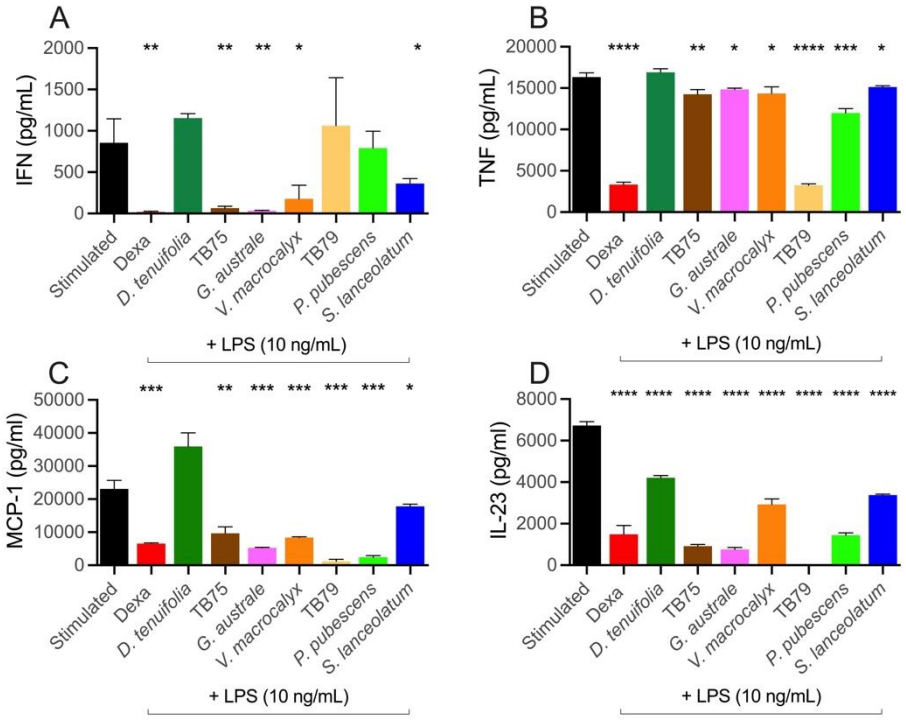

Figure 3.9 Inhibitory effects of methanolic crude leaf extracts (100 µg/mL) obtained from seven medicinal plant species on the secretion of pro-inflammatory cytokines (A) tumor necrosis factor (TNF), (B) interferon-gamma (IFN-γ), (C) monocyte chemoattractant protein-1 (MCP-1), and (D) interleukin 23 (IL-23) by human peripheral blood mononuclear cells (PBMCs) stimulated with bacterial-derived lipopolysaccharide (LPS) after overnight incubation. Data were expressed as mean ± SD (n = 3). Statistical significance was determined by unpaired t-test using GraphPad Prism (v.10.2.2), where *P = 0.0332, **P = 0.0021, ***P = 0.0002, ****P < 0.0001. TB 75, *Dodonaea tenuifolia*

(TB76), TB79, *Petalostigma pubescens* (TB81), *Velleia macrocalyx* (TB84), *Santalum lanceolatum* (TB87), *Gossypium australe* (TB88), and *Pittosporum angustifolium* (TB89). Dexamethasone (Dexa) was used as a positive control drug.

3.5. Discussion

Many medicinal plants and other native plants used by the Aboriginal people of Australia have exhibited antioxidant and anti-inflammatory potentials (Akter et al., 2016; Ali et al., 2023; Mani et al., 2021; Yeshi et al., 2022a; Yeshi et al., 2022b). The antioxidant potential of medicinal plants is mainly attributed to polyphenols, including phenolic acids, flavonoids, stilbenes, and lignans (Manach et al., 2004; Oreopoulou et al., 2019; Pandey & Rizvi, 2009). These antioxidant compounds are capable of preventing/ or minimising the generation of free radicals in living cells (Cai et al., 2004; Diaz et al., 2012; Dragland et al., 2003), as excessive free radicals are toxic and cause damage to important biomolecules, such as DNA, lipids, and proteins (Diaz et al., 2012; Zhang et al., 2011). If the body's antioxidant defence system fails to control the production of free radicals, it may lead to oxidative stress, which can gradually result in inflammation (Biswas, 2016; Hussain et al., 2016; Ramos-González et al., 2024) and multiple diseases, including neurodegenerative diseases (Chen et al., 2012; Wojtunik-Kulesza et al., 2016), cancer (Valko et al., 2006), diabetes (Bhatti et al., 2022; Giacco & Brownlee, 2010), arthritis (Hadjigogos, 2003; Zahan et al., 2020), atherosclerosis (Rajendran et al., 2014), and fast ageing (Getoff, 2007; Maldonado et al., 2023). Under such conditions, supplementary antioxidants are required to support the body's defence mechanism. There has been more interest in natural antioxidants over synthetic ones in recent years due to possible side effects associated with the latter (Lourenço et al., 2019; Pokorný, 2007).

Therefore, in this study, we evaluated the antioxidant and anti-inflammatory properties of medicinal plant species used by the Iningai Aboriginal people of central Queensland, Australia, for treating pain and inflammation and diseases associated with oxidative stress, such as arthritis. Methanolic leaf crude extracts of eight medicinal plant species were initially investigated for the major phytochemical classes and total (poly)phenolic content. The extracts tested positive for flavonoid and alkaloid except for *G. australe* (which tested negative for flavonoid and alkaloid) and *V. microcalyx* (which tested negative for alkaloid). Such test results may vary based on the polarity of extract solvents used, as different solvents have varying affinity with secondary metabolites (Abubakar & Haque, 2020; Mahato & Sen, 1997; Odebiyi & Sofowora, 1978). For instance, ether is recommended to extract alkaloids and chloroform for flavonoids and terpenoids (Pandey & Tripathi, 2014; Tiwari et al., 2011), and alcohols are mostly preferred for extracting polar secondary metabolites (Das et al., 2010). Irrespective of the extract solvents used in this study, studies have indicated that alkaloids are least present/investigated in the Australian native species under the Goodeniaceae family (Kerr, 2012). The saponin test was positive for five of the eight species; *Pittosporum angustifolium* and *S. lanceolatum* tested negative. Although a few studies have reported saponins from seeds of *Pittosporum* species

(Backer et al., 2014; D'Acquarica et al., 2002; Seo et al., 2002), in our study, *P. angustifolium* tested negative for saponins and steroids. The amount and distribution of saponins among plants and within tissues and organs of individual plants greatly vary based on their functions/maturation process and seasonal fluctuations (Moses et al., 2014), mostly synthesised in the underground parts, such as roots and tubers (Park et al., 2005; Seki et al., 2008; Teng et al., 2009). Thus, the qualitative screening of results of such phytochemicals may be affected by the plant part used for the test and other factors, such as collection season and plant maturity.

Based on the qualitative phytochemical screening results, we next searched the literature on these eight medicinal plants to ascertain whether they had been explored for their biodiscovery potential. Out of eight species, only *Pittosporum angustifolium* has been studied. A total of 30 compounds (28 triterpene glycosides and 2 triterpene saponins) have been isolated from the leaves and seeds of *P. angustifolium* (Bäcker et al., 2015; Backer et al., 2013; Bäcker et al., 2014). Additionally, 20 secondary metabolites have been identified from leaves and stems using gas chromatography-mass spectrometry (GC-MS/MS) (Mani et al., 2024). Some of these compounds were tested for anti-microbial and DNA-topoisomerase I inhibitory activity (Backer et al., 2016); however, they were least studied for their anti-inflammatory activity.

All eight medicinal plants tested showed the presence of (poly)phenols in the quantitative TPC assay. Based on this result, we next evaluated their capacity to scavenge DPPH radicals, as the antioxidant potential of medicinal plants is mainly attributed to the presence of polyphenols (Manach et al., 2004; Oreopoulou et al., 2019; Pandey & Rizvi, 2009). They showed a percentage DPPH radical scavenging capacity ranging from 66.97 to 77.09% and IC_{50} values between 37.37±1.01 to 206.5±2.44 μg/mL. Based on the IC_{50} values, *Velleia macrocalyx* (Goodeniaceae) and *Pittosporum angustifolium* (Pittosporaceae) showed the best DPPH radical scavenging activity with IC_{50} values of 37.37±1.01 and 48.33±1.07 μg/mL, respectively. The IC_{50} values of DPPH radical scavenged also showed a significant ($p^{**} < 0.0027$) negative correlation to their TPC (i.e., higher TPC correlated with lower IC_{50} values). Moreover, five polyphenolic compounds, including rutin and isoquercitrin, were also isolated from the chloroform extract of leaves of *P. angustifolium* (Bäcker et al., 2014), which could be responsible for the scavenging effect, as many studies (Li et al., 2016; Patil et al., 2013; Yang et al., 2008) have reported excellent DPPH radical scavenging effect by these two compounds.

However, TPC was highest in the leaf crude extracts of *P. pubescens* and *S. lanceolatum,* which showed higher IC_{50} values for DPPH assay with 102.30±2.35 and 134.30±2.49 μg/mL, respectively, than the two medicinal plants showing the highest antioxidant activity. Such variations in the antioxidant activity among the plant species may be due to their different phenolic profiles and their nature of interaction with DPPH radicals. Antioxidant phenolics mostly quench DPPH radicals by transferring electrons or donating hydrogen atoms (Matthäus, 2002; Yamauchi et al., 2024), and

phenolic compounds with more hydroxyl groups possess better scavenging capacity (Mathew et al., 2015). Variations in the antioxidant activity of crude plant extracts may also be attributable to the synergistic effects of mixtures of compounds present in the crude extracts. For instance, when Joshi et al. (Joshi et al., 2022) compared the DPPH radical scavenging effect of five phenolic compounds, rutin hydrate and resveratrol showed better scavenging activity than other combinations. The moderate to promising antioxidant activity shown by all medicinal plants may be related to the traditional usage of these medical plants by Aboriginal people in central Queensland. Although Aboriginal people apply rigorous detoxification processes before using medicinal plants (Yeshi et al., 2022b), they deserve scientific validation, as anything toxic could be harmful for consumption/application. Thus, the cytotoxicity of the crude leaf extracts of eight medicinal plants was determined through their effect on the viability of human PBMCs after overnight incubation.

Except for the crude extract of *P. angustifolium* (73.97% dead cells), none of the species caused more than 50% cell death. Several studies have isolated and reported cytotoxic saponins from *Pittosporum* species (Backer et al., 2014; D'Acquarica et al., 2002; Seo et al., 2002). Recently, Bäcker et al. (Backer et al., 2014) isolated seven saponins/triterpene glycosides from seeds, and all compounds showed cytotoxicity against three cancer cell lines, namely urinary bladder carcinoma (5637), breast cancer (MCF7), and glioblastoma IV (LN18) with IC_{50} values between 1.7±0.1 to 34.1±2.3 μM. The high percentage of dead PBMCs caused by the methanolic leaf extract of *P. angustifolium* indicates that the extract may contain other cytotoxic compounds, as it tested negative for saponins and glycosides. Thus, it is worth considering the use of this plant for consumption/treatment with caution.

Antioxidants can suppress the expression of transcription factors that induce the secretion of pro-inflammatory cytokines and chemokines (Borquaye et al., 2020; Ferreira et al., 2018; Yahfoufi et al., 2018). When crude extracts (except for *P. angustifolium*) were tested for anti-inflammatory activity using the LPS-activated human PBMCs assay, the crude extracts from *V. microcalyx,* TB79*,* and TB75 most significantly suppressed the release of pro-inflammatory cytokines, namely TNF, IFN-γ, MCP-1, and IL-23. Tumour necrosis factor (TNF) is a key cytokine that orchestrates inflammatory responses by directly triggering inflammatory gene expression or indirectly inducing cellular apoptosis (Jang et al., 2021; van Loo & Bertrand, 2023). Excessive expression of TNF is associated with several inflammatory-related diseases, including inflammatory bowel diseases (IBD), rheumatoid arthritis, and ankylosing spondylitis (Bradley, 2008; Popa et al., 2007). Currently, biologics such as adalimumab, certolizumab, and golimumab that neutralise TNF or block its production are considered the most effective drugs for treating inflammatory and autoimmune diseases (Jang et al., 2021; van Loo & Bertrand, 2023). Many studies have reported the inhibition of TNF by crude extracts from medicinal plants (Arora et al., 2022; Campana et al., 2015; Ye et al., 2016). Interferon-gamma (IFN-γ) is a pleiotropic cytokine with a function similar to TNF and additionally induces the production of nitric oxide (NO) free radicals (Lee et al., 2017). All crude extracts also significantly suppressed IL-23, and

one of the main roles of IL-23 in inflammation is in the expression/differentiation of Th17 (T helper type 17) cells (Benveniste, 2014; Verstockt et al., 2023). The promising anti-inflammatory exhibited by all crude extracts is intriguing; however, it will be difficult to pinpoint the exact cause of suppression of these cytokines, as crude extracts contain a mixture of several compounds. Thus, future research should focus on purifying and characterising pure compounds from anti-inflammatory crude extracts of promising medicinal plant species identified in this study, including TB75, *G. australe,* and *V. macrocalyx*. These three medicinal plants are traditionally used for inflammation, cuts, sores, and digestive disorders; thus, they have a high potential for yielding novel anti-inflammatory molecules for treating inflammatory-related diseases, including inflammatory bowel diseases (IBD), which currently have no cure.

3.6. Conclusions and future directions

As an initial collaborative work towards scientifically validating the indigenous biocultural knowledge (IBK) of the Iningai Aboriginal community in central Queensland, this study evaluated the crude extracts from eight medicinal plants for phytochemical content and antioxidant and anti-inflammatory properties. Four *(D. tenuifolia,* TB75, TB79*,* and *P. pubescens*) out of eight medicinal plants tested positive for alkaloids, flavonoids, and saponins. Except for *G. australe* (which tested negative for flavonoids), all plant species contained flavonoids and phenolics. All tested species showed moderate to promising antioxidant activity through scavenging DPPH radicals, with IC_{50} values ranging from 37.37 ± 1.01 μg/mL to 206.50 ± 2.44 μg/mL. *Velleia macrocalyx* exhibited the highest antioxidant activity (IC_{50} = 37.37 ± 1.01 μg/mL), followed by *P. angustifolium* (48.33 ± 1.07 μg/mL) and TB75 (119.90 ± 1.11 μg/mL). In the human PBMCs viability test, crude extract from *P. angustifolium* caused the highest cell death (73.97% dead cells), indicating potential toxicity to human cells. Other plants tested did not show high cell death (dead cells <50%) and may therefore be safe to use naturally.

Seven medicinal plants also exhibited promising anti-inflammatory activity (*P. angustifolium* was not tested due to the cell viability results) by suppressing the release of four pro-inflammatory cytokines (viz. TNF, IFN-γ, MCP-1, and IL-23) by the LPS-activated human PBMCs. Based on the significance level of cytokine suppression, TB75, *G. australe*, and *V. microcalyx* were the three most active species. Two species – TB75 and TB79 – are currently under investigation to identify novel drug lead molecules for treating inflammatory bowel disease. Further research also holds promise for targeted isolation and characterisation of antioxidant and anti-inflammatory lead compounds for developing novel therapeutics.

The study benefited researchers and Traditional Owners by establishing collaboration, partnerships, engagement, and relationship ties through a research co-design process. The immediate benefits to the Iningai people include reinvigorating knowledge, supporting the passing of Indigenous

Biocultural Knowledge (IBK) from elders to the younger generation, engaging with researchers to discover scientific value in their IBK, and, thus, protecting Iningai intellectual property.

3.7. References

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Chapter 4. Antioxidant and anti-inflammatory activities of five medicinal plants used by Mbabaram indigenous community in northeastern Australia

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Antioxidant and anti-inflammatory activities of five medicinal plants used by Mbabaram Indigenous community in northeastern Australia

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Abstract:	Ethnopharmacological relevance The Mbabaram community in Atherton Tableland has been closely working with The Tropical Indigenous Ethnobotany Centre (Queensland Herbarium, Australian Tropical Herbarium) and the College of Public Health Medical & Veterinary Sciences (CPHMVS), James Cook University in the areas of documenting traditional biocultural knowledge and biodiscovery projects. The current study investigated five medicinal plants from the Mbabaram community for their phytochemical contents and biological activities. Materials and methods In this study, crude extracts of five medicinal plants from the Mbabaram community (Breynia oblongifolia, Cajanus reticulatus, Dodonaea lanceolata, Exocarpos latifolius, and Coleus amoenus) were assessed for their phytochemicals contents and antioxidant activity using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. Furthermore, crude extracts were evaluated for their effect on cell viability and anti-inflammatory activity using the human peripheral blood mononuclear cells (PBMC) assay. Results While some plants tested positive for flavonoids and saponins, Breynia oblongifolia and Coleus amoenus did not test positive for saponins. Only Cajanus reticulatus and Exocarpos latifolius tested positive for alkaloids. None of the medicinal plants tested positive for anthraquinones. The ethanol extract of B. oblongifolia leaf (128.36±14.09 GAE/g extract) showed the highest TPC content, followed by Coleus amoenus root and stem with 119.67±3.03 and 94.23±10.16 GAE/g extract, respectively. However, the water extract of C. amoenus exhibited the highest TPC (99.88±4.47 GAE/g extract), followed by Dodonaea lanceolata var. subsessilifolia stem extract and Exocarpos latifolius root extract. The crude extract of Cajanus reticulatus leaf showed the best DPPH-free radical scavenging activity, followed by Breynia oblongifolia, Coleus amoenus, and Exocarpos latifolius. Conclusions

3.8. Abstract

Ethnopharmacological relevance: The Mbabaram community in Atherton Tableland has been closely working with The Tropical Indigenous Ethnobotany Centre (Queensland Herbarium, Australian Tropical Herbarium) and the College of Public Health Medical & Veterinary Sciences (CPHMVS), James Cook University in the areas of documenting traditional biocultural knowledge and biodiscovery projects. The current study investigated five medicinal plants from the Mbabaram community for their phytochemical contents and biological activities.

Materials and methods: In this study, crude extracts of five medicinal plants from the Mbabaram community (*Breynia oblongifolia, Cajanus reticulatus, Dodonaea lanceolata, Exocarpos latifolius,* and *Coleus amoenus*) were assessed for their phytochemical contents and antioxidant activity using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. Furthermore, crude extracts were evaluated for their effect on cell viability and anti-inflammatory activities using the human peripheral blood mononuclear cells (PBMC) assay.

Results: While some plants tested positive for flavonoids and saponins, *Breynia oblongifolia* and *Coleus amoenus* did not test positive for saponins. Only *Cajanus reticulatus* and *Exocarpos latifolius* tested positive for alkaloids. None of the medicinal plants tested positive for anthraquinones. The ethanol extract of *B. oblongifolia* leaf (128.36±14.09 GAE/g extract) showed the highest TPC content, followed by *Coleus amoenus* root and stem extracts with 119.67±3.03 and 94.23±10.16 GAE/g extract, respectively. However, the water extract of *C. amoenus* exhibited the highest TPC (99.88±4.47 GAE/g extract), followed by *Dodonaea lanceolata var. subsessilifolia* stem extract and *Exocarpos latifolius* root extract. The crude extract of *Cajanus reticulatus* leaf showed the best DPPH-free radical scavenging activity, followed by *Breynia oblongifolia*, *Coleus amoenus*, and *Exocarpos latifolius*.

Conclusions: Since the Mbabaram Aboriginal community uses water extract for treating wounds and sores, we have tested the water extracts of five medicinal plants. The water extract of *B. oblongifolia* leaf showed the best anti-inflammatory activity by significantly reducing the levels of four pro-inflammatory cytokines, namely IL-1β, IL-6, IL-8, and TNF. *Coleus amoenus* stem extract also significantly reduced IL-6, IL-8, and TNF. *Cajanus reticulatus* leaf extract also significantly reduced IL-1β. The promising antioxidant and anti-inflammatory results warrant further investigation on *Cajanus reticulatus, Breynia oblongifolia*, and *Coleus amoenus* as potential sources of novel anti-inflammatory drug lead compounds.

3.9. Introduction

Australian Indigenous people have been accumulating ethnobotanical knowledge and using it for thousands of years. Nature and human culture, while often considered as two separate domains in environmental stewardship, are significantly interlinked with similar evolutionary paths, mutual adaptation and co-evolution (Larsen. et al., 2017). The Australian Aboriginal and Torres Strait Islander

people have lived within the ecology of Australia for thousands of years, adapting to changing climates and environments while developing a highly complex and intimate knowledge of the lands, seas and skies. This knowledge is known as Indigenous Biocultural Knowledge (IBK). One of the pillars of IBK is ethnomedical plant knowledge which is often called customary medicine (CM) in Australia, a term that acknowledges the integration of traditional and contemporary use of medicinal plants. CM, especially as practised by the Mbabaram community in the Atherton Tablelands of Far North Queensland (FNQ), is a relatively untapped source of wisdom in biodiscovery projects (Turpin et al., 2022). The Mbabaram (Bar-Barrum) peoples' traditional lands extend to the top of the Great Dividing Range near the Walsh River Spring, bounded by the Walsh River near Dimbulah and includes the present towns of Watsonville, Irvinebank, Almaden and south to Mt Garnet. There have been various references to the Mbabaram people and culture, and different spellings of the name, in Western literature. Dixon (1991, p. 352) discussed and documented these: "…Parry-Okeden (1897) locates the 'Oombabaram' on his map; Mathews (1898) mentions the 'Boomuram'; Richards (194-6:265) states that the 'Woombabaram' tribe was at Dimbulah; McConnell (1930-1940) includes a map which locates 'Wumbabaram' along the Walsh River; Tindale (1941) discusses 'Barbaram'.". Dixon himself (Dixon, 1991) uses the name 'Mbabaram', as he was told by the last speaker of the Mbabaram language, who passed away in the 1940s.

At the time of colonisation, over 250 Australian languages were spoken by Aboriginal and Torres Strait Islander people (Anonymous, 2019); Mbabaram is one of 110 that are now severely or critically endangered. Only around 300 words and 300 phrases were recorded from three Mbabaram informants, and most of these words were used to describe biocultural items, including lands, waters, plants, and animals. Dixon described the language as "*the strange monosyllabic language that looked quite unlike any other Australian tongue*." Mbabaram is a difficult language to speak, so the Mbabaram people often learned other languages to communicate with other tribes. The last Mbabaram speaker passed away in the 1940s, and the few remaining Elders of the Mbabaram tribe didn't speak the language or had very little cultural knowledge. One of the recent knowledge holders who provided some knowledge has recently passed away. We have recorded Mbabaram knowledge from other non-Mbabaram and non-Indigenous people.

In our continuous efforts to document, preserve and add commercial value to Mbabaram IBK, we identified Mbabaram words in Dixon (Dixon, 1991) relating to plants and documented the associated species names, common names, and cultural uses with a focus on Mbabaram bush medicine plants. Additional knowledge on Mbabaram plant names and uses was sourced from literature such as explorer journals and botanical textbooks, and from museum artefacts. This knowledge was entered into a Miromaa database (Miromaa Aboriginal Language and Technology Centre, 2019). Created by Aboriginal people for Aboriginal people, Miromaa is a simple, password-protected software platform

enabling the control by knowledge holders of language and cultural knowledge. Its use for this project was motivated by a desire to protect intellectual property (IP) rights over medicinal plant utilities.

Research protocols were established between the community and TIEC to govern the maintenance, control, protection, and development of Mbabaram Traditional Knowledge. Development of these protocols was undertaken in the context of the Queensland Biodiscovery Act 2004. Reform of this Act resulted in changes enacted in 2020 that include the establishment of legal protections for First Nations peoples' traditional knowledge and requirements for biodiscovery entities to follow traditional knowledge guidelines and code of practices (Queensland, 2020). This has improved the alignment of legislation with international standards such as the Nagoya Protocol on Access and Benefit Sharing (Buck & Hamilton, 2011), which requires: i) biodiscovery to be undertaken only with the prior informed consent of Indigenous communities who hold traditional knowledge about the resources, and ii) the benefits of biodiscovery be fairly and equitably shared.

These protective and supportive initiatives towards Indigenous knowledge and our documentation projects ignited the Mbabaram community's interest and aspirations to think about preparing bush food and bush medicines for commercial benefits. In response to these interests and aspirations of the Watsonville Mbabaram clan group, the Tropical Indigenous Ethnobotany Centre (TIEC) at James Cook University (JCU) initially led collaborative small medicinal plant projects with Southern Cross University (NSW) and the National Institute of Complimentary Medicine Health Research Institute (NICM) at Western Sydney University, NSW. While sporadic collaboration between these universities and the Mbabaram community did not bear major outcomes, their projects have established formal close collaboration between TIEC, the Mbabaram community, and James Cook University since 2019.

The joint Mbabaram community and JCU biodiscovery project was funded by an NHMRC Ideas grant awarded in 2020. This project has steered many developments within JCU, including i) building the relationship with the Mbabaram Aboriginal community, ii) framing biodiscovery pathways at JCU and Queensland government in accordance with Queensland Biodiscovery Act 2004, iii) developing community engagement protocols in accordance with International Standards for Biodiscovery, The Human Rights Act 2019 (QLD) and NHMRC guidelines for Aboriginal researchers; and iv) developing benefit sharing agreements as per the United Nations Declaration on the Rights of Indigenous Peoples (Article 31), and the Nagoya Protocol.

Through this project, collaborators were invited to meet the Mbabaram community for discussions where researchers provided information about their backgrounds, discussed capabilities, aims, and visions of the collaborative project, and the possible benefits to the Mbabaram community. Researchers were also invited to visit Mbabaram country. While JCU has benefited from the Indigenous biocultural knowledge (IBK) shared by the community and TIEC and the scientific publications, the Mbabaram community has benefitted through i) employment opportunities for young Mbabaram land managers, ii) exposure visits to the high-tech JCU laboratories, and iii) training Indigenous researchers

in western science techniques and methods to further understand how their medicinal plants may provide solutions for current diseases.

This study is one of the outcomes of the NHMRC-funded project. Based on our extensive documentation of the Mbabaram Aboriginal community's IBK, we have botanically identified and collected eight plants used for healing and treating inflammation and inflammatory-related conditions from the Watsonville region of far north Queensland. We dried the various parts of selected medicinal plants. We tested their crude extracts for their phytochemical contents and biological activities, and the results for five medicinal plants are presented and discussed here.

4.3. Materials and methods

4.3.1. Reagents and chemicals

Aluminium chloride, gallic acid, glacial acetic acid, ferric chloride, Folin-Ciocalteu (F-C) reagent, hydrochloric acid (HCl), sodium carbonate, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and were purchased from Sigma Aldrich (Victoria, Australia). Ethanol and methanol were purchased from ChemSupply (Gillman, Australia).

4.3.2. Instruments

Total phenolic content (TPC) and antioxidant activity were determined with the help of a microplate reader (SPECTROstar® Omega, BMG Labtech, Mornington, Australia). The LSRFortessa X20 flow cytometer (BD Biosciences) was used to acquire data for determining the anti-inflammatory activity.

4.3.3. Ethical consideration

The human ethics approval (H8523) for conducting *in-vitro* assays using human peripheral blood mononuclear cells (PBMCs) was obtained from the Human Research Ethics Committee, JCU. The ethical clearance/consent for collecting and studying five medicinal plants were obtained from the Mbabaram Aboriginal Corporation (MAC) in Atherton Tableland.

4.3.4. Medicinal plant collection and crude extraction

The biodiscovery approval and the plant collection permits (BIBC20200417-2) were obtained from the Department of Environment and Science, Queensland government. The leaves, stems, wood, and roots of the five medicinal plant species (Figure 4.1) traditionally used by Mbabaram Indigenous communities for healing/treating inflammation and inflammatory-related conditions were collected from Watsonville region, Queensland, Australia (Figure 4.2). Plants were identified at the Australian Tropical Herbarium and the Queensland Herbarium, Brisbane). Herbarium specimen numbers were assigned to each plant species (Table 4.1), and samples were deposited at ATH. Collected fresh plant materials were washed repeatedly under the running tape water and dried in the oven (40 °C) for one week.

Dried samples of the five medicinal plants (10 g each) were ground into a coarse powder using a nutriBullet grinder (NutriBullet, Australia). The coarse powder of each plant was macerated with ethanol/ or distilled water three times for 20 mins (~one-hour total extraction time), and the fresh solvent was replaced at each 20 min interval. The extracts from three rounds of extraction were combined and filtered with a Whatman filter paper (Whatman Asia Pacific, San Centre, Singapore). Then, the ethanol extract was concentrated with the help of a rotary evaporator (Heidolph, Germany), and the aqueous extracts were frozen in −80°C freezer and subsequently freeze-dried. From the concentrated ethanol extract of each plant, 16 mL of extract was used for the qualitative phytochemical screening tests following methods adapted from Wangchuk et al. (Wangchuk et al., 2011), Iqbal et al. (Iqbal et al., 2017), Shah and Hossain (Shah & Hossain, 2014), and Yeshi et al. (Yeshi et al., 2022).

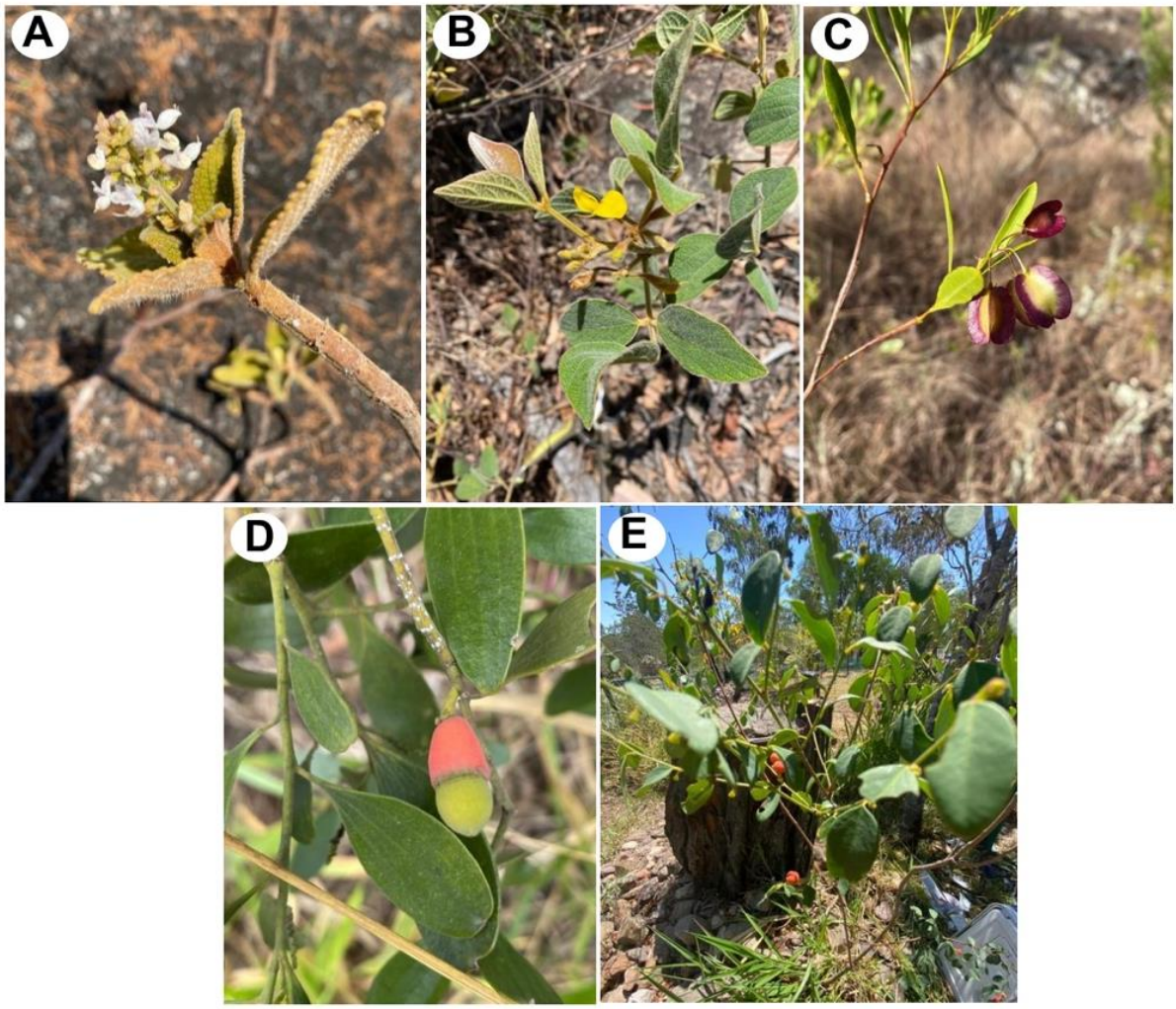

Figure 4.1 Aerial parts of five medicinal plants investigated in this study. A) *Coleus amoenus,* B) *Cajanus reticulatus,* C) *Dodonaea lanceolata,* D) *Exocapos latifolius,* and E) *Breynia oblongifolia*. (Photo courtesy: G. Turpin).

Figure 4.2 A map of Queensland, Australia, showing the collection sites of five medicinal plants. GPS coordinates for *Breynia oblongifolia* (latitude:17.3569; longitude:145.3150); *Cajanus reticulata* (latitude:17.3569; longitude:145.3130); *Dodonaea lanceolata var. subsessifolia* (latitude:17.3569; longitude:145.3580); *Exocarpos latifolius* (latitude:17.3626; longitude: 145.3118); *Coleus amoenus* (latitude:17.3492; longitude:145.3094); Plant coordinates were obtained while collecting the plant samples.

4.3.5. Total Phenolic content (TPC)

The Folin-Ciocalteu (F-C) method was used to determine the total phenolic content (TPC) in crude extracts of the five medicinal plants following the slightly modified methods described by Molole et al. (Molole, Gure, & Abdissa, 2022) and Yeshi et al. (Yeshi et al., 2022). Briefly, 0.5 mL of each plant's crude extract (1 mg/mL) was mixed with 2 mL of a freshly prepared F-C reagent (diluted with distilled water at a 1:10 ratio) in triplicates. After three but not more than eight minutes, 4 mL of sodium carbonate (7.5% w/v in distilled water) was added. Then, the reaction mixture was incubated at room temperature for 30 min. After 30 min incubation, absorbance was recorded at 760 nm. The gallic acid standard calibration curve (0-150 µg/mL concentrations in triplicates) was prepared to calculate the TPC following the same procedure. The coefficient of determination (R^2) of the calibration curve was used to evaluate the linearity of the curve. Finally, the TPC was expressed in terms of milligrams of gallic acid equivalent per gram of dry extract (mg GAE/g) from the equation below (Siddiqui et al., 2017):

$$C = C1 \times \frac{V}{m}$$

Where 'C' = TPC in mg GAE/g extract, 'C_1' = the concentration of gallic acid determined from the calibration curve in mg/mL, 'V' = the volume of the extract in mL, and 'm' = the weight of the dry plant extract in g.

4.3.6. DPPH-free radical scavenging antioxidant activity and determination of EC_{50} value

The capacity of the crude extracts to scavenge DPPH-free radicals was examined using the modified methods from Brand-Williams et al. (Brand-Williams, Cuvelier, & Berset, 1995), Yeshi et al. (Yeshi et al., 2022), and Molole et al. (Molole et al., 2022). Briefly, crude samples or gallic acid standard compound (1 mg/mL) were mixed well with 0.1 mM methanolic DPPH solution 1:2 ratio. The reaction mixture was incubated in the dark at room temperature for 1 hour, and following the incubation, their absorbance was measured at 517 nm. Methanol with DPPH was used as a negative control, and methanol as a blank. The percentage of scavenging activity was calculated from their absorbance readings using the formula:

$$\text{Percentage of DPPH free radical scavenged (\%)} = \frac{Ac - As}{Ac} \times 100\%$$

where A_c is the absorbance of the control and A_s is the absorbance of the sample/gallic acid.

To determine EC_{50} values (half maximal effective concentration), the dose-response DPPH-free radical scavenging activity was determined at various concentrations (0-150 µg/mL) for each sample following the same method described above. The dose-response curve was plotted with the obtained data, and EC_{50} values were determined by interpolating the standard curve (asymmetric sigmoidal, five-parameter logistic equation, 5PL), where 'X' is concentration using a GraphPad prism (v.10.0).

4.3.7. In-vitro anti-inflammatory activity and quantification of cytokines

4.3.7.1. Human peripheral blood mononuclear cells (PBMCs) assay culture condition and sample treatment

The *in-vitro* anti-inflammatory activity was determined using a human PBMCs assay. Blood from healthy donors was supplied by the Australian Red Cross Lifeblood (two donors) was used to separate PBMCs using the Ficoll-Paque PLUS (GE Health) density gradient (STEMCELL technologies). The PBMCs were cryopreserved in Foetal Bovine Serum (FBS, filtered heat-inactivated) (Corning #35-076-CV) containing 10% dimethyl sulfoxide (DMSO, Sigma-Aldrich).

On the day of the experiment, PBMCs were thawed and washed with R-10 media (RPMI-1640 (Gibco), containing 10% heat-inactivated FBS, 100 U/mL Penicillin, and 100 µg/mL Streptomycin (Gibco). For the assay, 1×10^6 cells in 100 µL of R-10 media were seeded per well in the 96-well U-bottom culture plates (Falcon®, Corning, NY, USA). For stimulated conditions, PBMCs were

stimulated with lipopolysaccharide (LPS, 10 ng/mL) (Sigma-Aldrich) and incubated inside the incubator (at 37 °C in a 5% CO_2) for 2 h. After 2 h of LPS stimulation, PBMCs in respective treatment wells were treated in triplicates with the aqueous crude extracts of five medicinal plants (100 µg/mL in cell culture media with 0.5% DMSO). The R-10 media with DMSO was used as the negative control. Culture plates were incubated overnight at 37 °C in a 5% CO_2 incubator. Following overnight incubation, plates were centrifuged (1500 rpm at 4 °C for 5 min), and the culture supernatants were collected for cytokine analysis. The remaining cells in the culture plate were stained and analysed further to analyse the effect of tested aqueous crude extracts on cell viability, as described below.

4.3.7.2. Quantification of pro-inflammatory cytokines

Culture supernatants were analysed (in triplicates for each treatment and control group) using a customised human 13-plex LEGENDplex™ multi-analyte flow assay kit (BioLegend®, USA) to quantify the cytokines released (pg/mL) by the LPS-stimulated PBMCs. This assay kit allows simultaneous detection of 13 human pro-inflammatory cytokines/chemokines, including interleukin (IL)-1β, interferon alpha (IFN-α), IFN-γ, tumor necrosis factor (TNF), monocyte chemoattractant protein-1 (MCP-1), IL-6, IL-8 (CXCL8), IL-10, IL-12p70, IL-17A, IL-18, IL-23, and IL-33 with the help of a flow cytometer. Flow cytometry data files were analysed in a cloud-based software platform from the BioLegend® website (San Diego, CA, USA). The concentrations of cytokines obtained in picograms per millilitres (pg/mL) were further normalised to the LPS-stimulated control (100%) and expressed as mean ± standard error (SEM).

4.3.8. Data analysis

All data were expressed as the mean ± SEM and one sample t-test was performed to determine the statistical significance in GraphPad Prism (v.10.0, San Diego, CA, USA).

4.4. Results and discussion

4.4.1. Ethnopharmacological uses of five medicinal plants

Mbabaram people use five medicinal plants to treat wounds, sores, and toothache (Table 4.1). While only the leaves of some medicinal plants were used, stems, wood, and roots were also used for other plants. For example, leaves, stems, and roots of *Dodonaea lanceolata var. subsessilifolia* J.G.West are used, leaves, and stems of *Breynia oblongifolia* (Müll.Arg.) Müll.Arg. are used, and for *Coleus amoenus* P.I.Forst only leaves are used. Lukhoba et al. (Lukhoba et al., 2006) indicated that 20 other species of *Coleus* are used for t reating wounds and sores in other parts of the world, including India, Brazil, and Africa. *Breynia* species are also traditionally used for medicinal purposes in China, Philippines, and Indonesia (Arif et al., 2022; He et al., 2019).

Table 4.1 Ethnopharmacological uses of medicinal plants included for phytochemical and biological activity analysis in this study.

Collection Number	Plant species and family	Common name	Parts used	Ethnopharmacological uses	Location
MB6	*Breynia oblongifolia* (Müll.Arg.) Müll.Arg. (Phyllanthaceae)	Coffee Bush	Leaves	Sores	Bischoff Mill Rd, N of Watsonville, Qld
MB5	*Cajanus reticulatus* (Aiton) F.Muell. (Leguminosae)	Cajanus	Whole plant	Toothache	Bischoff Mill Rd, N of Watsonville, Qld
MB8	*Dodonaea lanceolata var. subsessilifolia* J.G.West (Sapindaceae)	Hop Bush	Leaves, stems, roots	Wounds	Creek W of Silver Valley Road, km W of Herberton, Qld
MB2	*Exocarpos latifolius* R.Br. (Santalaceae)	Broad-leaved Cherry	Leaves, stems, bark	Sores	Bischoff Mill Rd, N of Watsonville, Qld
MB3	*Coleus amoenus* P.I.Forst. (Lamiaceae)	Coleus	Leaves, stem	Wounds	Bischoff Mill Rd, N of Watsonville, Qld

4.4.2. Major groups of phytochemicals of five medicinal plants

Ethanolic crude extracts from the five medicinal plants were analysed for major phytochemical classes, including alkaloids, flavonoids, tannins, terpenoids, glycosides, steroids, anthraquinones, and saponins. All tested positive for flavonoids, and all except *Breynia oblongifolia* and *Coleus amoenus* tested positive for saponins. Only *Cajanus reticulatus* and *Exocarpos latifolius* tested positive for alkaloids, and none tested positive for anthraquinones (Table 4.2). Of the five plants, *Dodonaea lanceolata var. subsessilifolia* showed positive results for the widest range of phytochemical classes (Table 4.2). Prior to this study, little was known of the phytochemistry of these five medicinal plant species. At the genus level, *Coleus* is known to contain mostly diterpenoids and phenolic compounds (Abdel-Mogib et al., 2002; Lukhoba et al., 2006). More than 90 compounds have been isolated from the genus *Breynia,* and glycosides are the major constituents (Arif et al., 2022). However, *Breynia oblongifolia* tested negative for glycosides in this study.

4.4.3. Total phenolics content (TPC)

The total phenolic content (TPC) of the crude extracts was determined by the Folin-Ciocalteu method and expressed in mg of gallic acid equivalent per gram of dried crude extract (GAE/g extract). The water and ethanol extracts of the leaves were prepared, and, for some plants, of stems and roots as well. Of the ethanol extracts, the highest TPC was found in *Breynia oblongifolia* leaf (128.36±14.09 GAE/g extract), followed by *Coleus amoenus* root and stem with 119.67±3.03 and 94.23±10.16 GAE/g extract, respectively. Of the aqueous extracts, the *C. amoenus* root extract showed the highest TPC (99.88±4.47 GAE/g extract), followed by *Dodonaea lanceolata var. subsessilifolia* stem extract and *Exocarpos latifolius* root extract with 48.96±3.70 and 46.66±11.96 GAE/g extract, respectively (Figure 4.3). Overall, *Coleus amoenus* had the highest TPC for the ethanol and aqueous extracts. After determining the qualitative and quantitative phytochemical contents, crude extracts were examined for their antioxidant activity using the DPPH-free radical scavenging assay.

Table 4.2 The screening of crude extracts from five medicinal plants for the presence of major classes of phytochemicals.

Plant species	Sample tested	Alkaloids	Flavonoids	Tannins	Terpenoids	Cardiac glycosides	Anthraquinone glycosides	Steroids	Saponins
Breynia oblongifolia	Leaf	-	+	-	-	-	-	-	-
Cajanus reticulatus	Whole	+	+	-	+	-	+	+	+
Dodonaea lanceolata var. subsessilifolia	Leaf	-	+	+	+	+	-	-	+
	Stem	-	+	+	+	-	-	-	+
	Root	-	+	+	+	-	-	-	+
Exocarpos latifolius	Leaf	+	+	-	-	+	-	-	-
	Stem	+	-	-	-	-	+	-	-
	Bark	+	-	-	-	-	-	+	+
Coleus amoenus	Stem	-	+	-	-	-	-	+	-
	Root	-	-	-	-	+	-	+	-

Symbols: – indicates negative test for the phytochemical class tested; + indicates positive test for the phytochemical class tested.

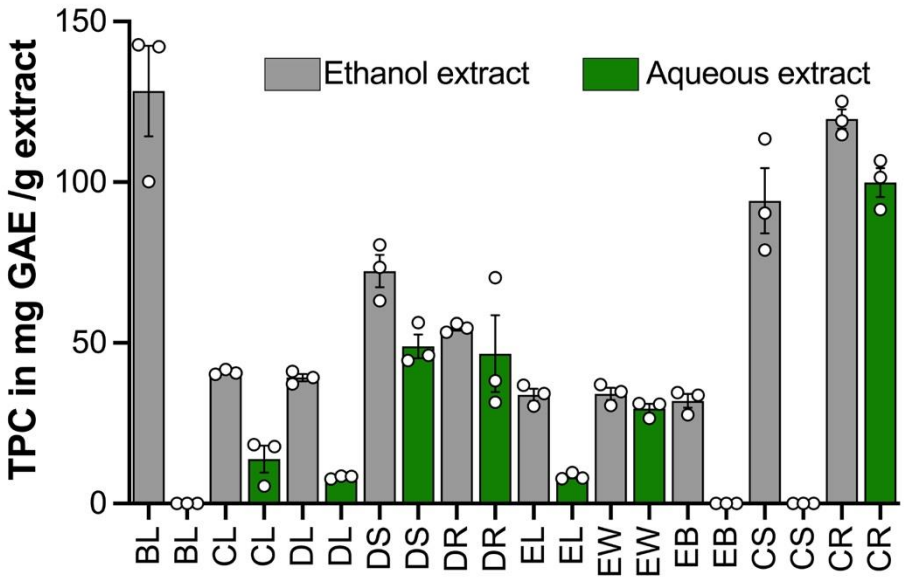

Figure 4.3 TPC of various parts of five medicinal plants extracted using ethanol (grey bar) and water (green bar). TPC is expressed in mg gallic acid equivalent (GAE)/g extract and shown as mean±SEM (n=3). BL-*Breynia oblongifolia var. subsessilifolia* leaf; CL-*Cajanus reticulatus* leaf; DL-*Dodonaea lanceolata var. subsessilifolia* leaf; DS-*Dodonaea lanceolata var. subsessilifolia* stem; EL-*Exocarpus latifolius* leaf; EW-*Exocarpos latifolius* wood; EB-*Exocarpus latifolius* bark; CS-*Coleus amoenus* stem; CR-*Coleus amoenus* root.

4.4.4. DPPH-free radical scavenging activity and EC_{50} values

The antioxidant activity of the crude extracts (ethanol and aqueous extracts) obtained from different parts of five plants was investigated by determining the half-maximal effective concentration (EC_{50}) values from the dose-response curves of a percentage (%) of DPPH-free radical scavenged by the crude extracts (Figure 4.4). Based on the EC_{50} values (Table 4.3), *Cajanus reticulatus* showed the best DPPH-free radical scavenging activity, followed by *Breynia oblongifolia*, *Coleus amoenus*, and *Exocarpos latifolius*. In general, the ethanol crude extracts showed better antioxidant activity than the aqueous extracts (Table 4.3). Except for *Exocarpos* and *Dodonaea* species, the EC_{50} values of the ethanol extracts were better than those of aqueous extracts, indicating higher antioxidant activity. However, the Mbabaram Aboriginal community uses water extracts of these medicinal plants to treat wounds and sores; thus, we further examined the aqueous extracts of the leaf and stem of plants for their effect on cell viability and anti-inflammatory activities.

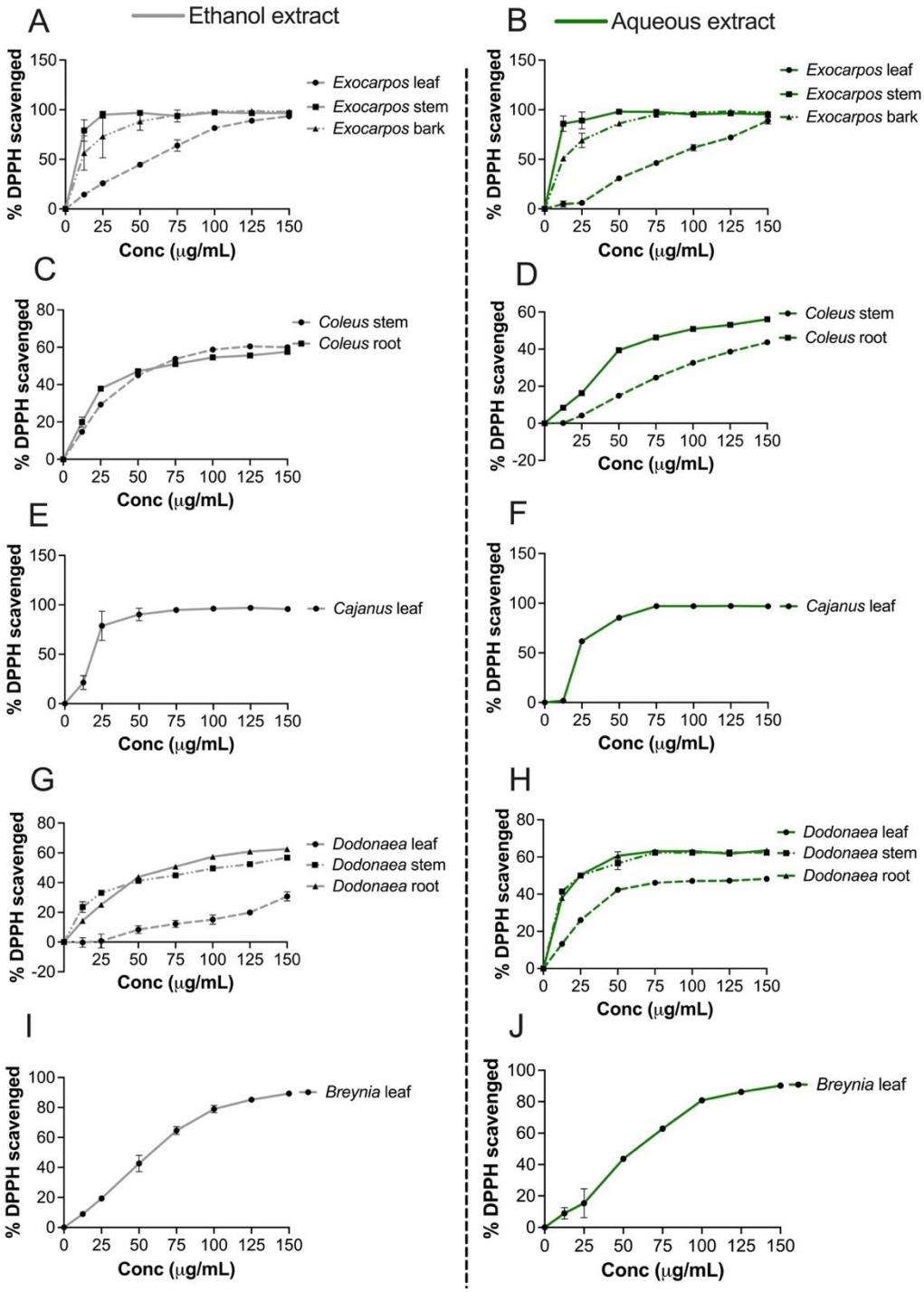

Figure 4.4 DPPH radical scavenging effects of different parts of five medicinal plants extracted using ethanol and water. Results were expressed as mean ±SEM of triplicates (n = 3). BL-*Breynia oblongifolia* var. *subsessilifolia* leaf; CL-*Cajanus reticulatus* leaf; DL-*Dodonaea lanceolata var. subsessilifolia* leaf; DS-*Dodonaea lanceolata* var. *subsessilifolia* stem; EL-*Exocarpos latifolius* leaf; EW-*Exocarpus latifolius* wood; EB-*Exocarpos latifolius* bark; CS-*Coleus amoenus* stem; CR-*Coleus amoenus* root.

Table 4.3 EC_{50} values of DPPH-free radical scavenging activity of ethanol and aqueous crude extracts from five medicinal plant species.

Plant species	Parts used	Crude extracts	EC_{50} value (µg/mL)
Breynia oblongifolia	Leaf	Ethanol extract	53.96
		Aqueous extract	56.46
Exocarpos latifolius	Leaf	Ethanol extract	51.99
		Aqueous extract	232.80
	Stem	Ethanol extract	7.905
		Aqueous extract	0.024
	Bark	Ethanol extract	8.84
		Aqueous extract	11.21
Coleus amoenus	Stem	Ethanol extract	35.61
		Aqueous extract	67.98
	Root	Ethanol extract	30.10
		Aqueous extract	143.40
Cajanus reticulatus	Leaf	Ethanol extract	16.31
		Aqueous extract	20.45
Dodonaea lanceolata var. *subsessilifolia*	Leaf	Ethanol extract	17.98
		Aqueous extract	297.4
	Stem	Ethanol extract	17.60
		Aqueous extract	34.79
	Root	Ethanol extract	85.33
		Aqueous extract	172.30
Gallic acid			0.634

Gallic acid was used as standard antioxidant compound.

4.4.5. Effect of crude extracts on cell viability

Before the PBMCs culture supernatant was analysed for pro-inflammatory cytokines, cells were first analysed for viability by determining the percentage of live/dead cells resulting from crude extract treatment. The effect of selected aqueous crude extracts on cell viability was determined by staining cells with a live/dead viability dye, as described in the methods section. The crude extract is considered toxic if the percentage of dead cells is ≥50% (Yeshi et al., 2022). None of the tested crude extracts affected the cell viability (Figure 4.5), as the percentage of live cells was more than 99% in all treatment groups. Thus, the culture supernatant was further analysed to determine the quantity of pro-

inflammatory cytokines/chemokines released by the LPS-stimulated PBMCs after treatment with the aqueous crude extracts of five medicinal plants.

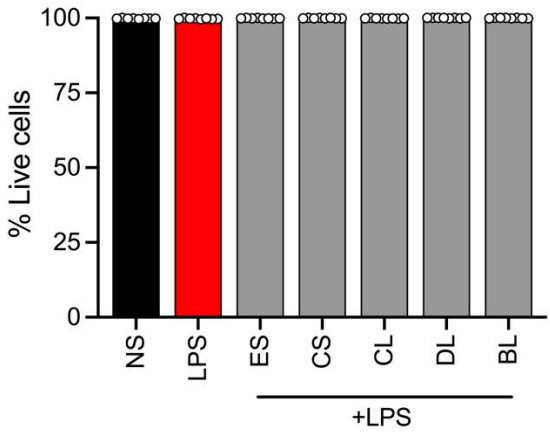

Figure 4.5 The effect of aqueous crude extracts (100 µg/mL) from five medicinal plants (ES-*Exocarpos latifolius* stem; CS-*Coleus amoenus* stem; CL-*Cajanus reticulatus* leaf; DL-*Dodonaea lanceolata* var. *subsessilifolia* leaf; BL-*Breynia oblongifolia* leaf) on the viability of LPS-stimulated PBMCs after the overnight incubation. The bar plots represent the mean % live cells (±SEM) from two independent experiments conducted in triplicates (n= 2 donors).

4.4.6. Anti-inflammatory activity of aqueous crude extracts

The anti-inflammatory activities of the aqueous crude extracts were measured after treating LPS-stimulated PBMCs with each crude extract at a concentration of 100 µg/mL post overnight incubation. Of the crude extracts tested, *Breynia oblongifolia* leaf showed the best anti-inflammatory activity by significantly reducing the levels of four pro-inflammatory cytokines, namely IL-1β, IL-6, IL-8, and TNF, in both PBMC donors (Figure 4.6A-H). Interleukin (IL)-1β is considered a key pro-inflammatory cytokine implicated in the inflammatory processes observed in patients with inflammatory bowel disease (IBD), with heightened IL-1β levels often correlating with increased disease severity (Coccia et al., 2012; Ligumsky et al., 1990). *Cajanus reticulatus* leaf also significantly reduced IL-1β (Figure 4.6A), but the suppression was statistically significant in the second donor (Figure 4.6E). Although IL-1β alone might not be sufficient to incite inflammation, its interaction with other pro-inflammatory cytokines, such as IL-6 and TNF, has been shown to exacerbate IBD-related inflammation (Mao et al., 2018). *Coleus amoenus* stem extract also significantly reduced IL-1β, IL-6, and IL-8 in the first PBMCs donor (Figure 4.6B-D) but significantly suppressed only TNF in the second donor (Figure 4.6F). Interleukin (IL)-6 is pleiotropic and plays a multifaceted role in mediating host defence, immunity, disease pathogenesis, and inflammation (Friedrich, Pohin, & Powrie, 2019; Narazaki & Kishimoto, 2018). Additionally, IL-8, belonging to the CXC family of chemokines, attracts and activates neutrophils

at infection sites, initiating the pathogenic cascade seen in IBD (Weng, Walker, & Sanderson, 2007). *Dodonaea lanceolata var. subsessilifolia* extract also significantly suppressed IL-1β in one of the PBMC donors (Figure 4.6E).

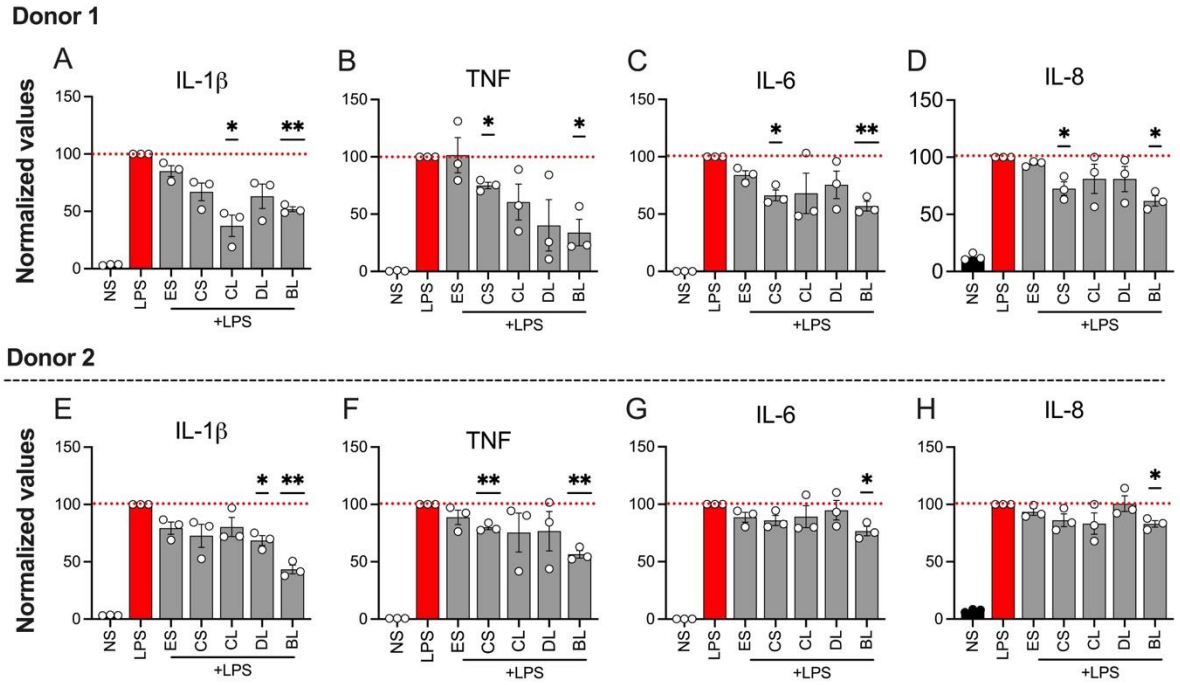

Figure 4.6 The effects of aqueous crude extracts (100 µg/mL) from five medicinal plants (ES-*Exocarpos latifolius* stem; CS-*Coleus amoenus* stem; CL-*Cajanus reticulatus* leaf; DL-*Dodonaea lanceolata* var. *subsessilifolia* leaf; BL-*Breynia oblongifolia* leaf) on the secretion of pro-inflammatory cytokines by lipopolysaccharide (LPS, 10 ng/mL) stimulated-PBMCs incubated overnight. The levels of secreted cytokines (IL-1β, TNF, IL-6, and IL-8) were determined by flow cytometry and are shown as % of stimulated concentrations of the LPS (100%, red dotted line) for each donor. Bar plots represent the mean normalized cytokine expression of each treatment group (±SEM) performed in triplicates (n=3). Statistical significance was determined by a one-sample t-test comparing the LPS-stimulated group to each treatment. $^{**}p \leq 0.0021$, $^{*}p \leq 0.0332$, and no p value = > 0.05.

4.5. Conclusion

Globally, medicinal plants and traditional medicine (TM) remain important in managing and preventing many chronic diseases. While many Indigenous Australians have lost much of their knowledge, traditional owners such as the Mbabaram Aboriginal community are actively recording and documenting medicinal plant knowledge to reinvigorate back into their community. This study evaluated five medicinal plants used by the Mbabaram Aboriginal community near the Atherton Tablelands in Far-North Queensland for their phytochemical contents, antioxidant, and anti-inflammatory properties.

All five species tested positive for flavonoids. All species except *Breynia oblongifolia* and *Coleus amoenus* tested positive for saponins. Only *Cajanus reticulatus* and *Exocarpos latifolius* tested positive for alkaloids, and none tested positive for anthraquinones. The highest TPC in the ethanol extracts was from *Breynia oblongifolia* leaf, followed by *Coleus amoenus* root and stem. For the aqueous extracts, *C. amoenus* root extract had the highest TPC, followed by that of *Dodonaea lanceolata* var. *subsessilifolia* stem extract and *Exocarpos latifolius* root extract. *Cajanus reticulatus* extract showed the best DPPH-free radical scavenging activity, followed by those of *Breynia oblongifolia*, *Coleus amoenus*, and *Exocarpos latifolius*.

None of the tested crude extracts affected cell viability. Of the five aqueous crude extracts tested, that from *Breynia oblongifolia* leaf showed the best anti-inflammatory activity by significantly reducing the levels of four pro-inflammatory cytokines, namely IL-1β, IL-6, IL-8, and TNF. *Coleus amoenus* stem extract also significantly reduced IL-6, IL-8, and TNF cytokines. *Cajanus reticulatus* leaf extract also significantly reduced IL-1β. Based on the antioxidant and anti-inflammatory activities, *Cajanus reticulatus, Breynia oblongifolia*, and *Coleus amoenus* are worth considering for further investigation, including isolation and characterisation of pure compounds, and may yield potent drug leads for developing a treatment for inflammation and inflammatory-related diseases such as IBD.

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Chapter 5. Overall Summary and future directions

5.1. Overview

This research aimed to provide an overview of the knowledge of medicinal plants used by Aboriginal and Torres Strait Islander people in Queensland, Australia, and to investigate and test, using laboratory assays, the properties of medicinal plants used by two Aboriginal nations in Queensland: Iningai and Mbabaram. Prior to this study, the biocultural knowledge of these two nations was least documented. This research has helped preserve Aboriginal culture and traditional medical knowledge for future generations and provided results that may potentially result in the commercialisation of this knowledge for the benefit of the knowledge owners.

5.2. Medicinal plant knowledge in Queensland

In Chapter 2, the literature review on Queensland medicinal plants was conducted; it was found that while many medicinal plants were recorded from Aboriginal communities in the Northern Territory, New South Wales, South Australia, and Western Australia, little has been reported on Aboriginal medicinal plants in Queensland. This review identified 135 species of Queensland Aboriginal medicinal plants, including those used by the two communities selected for this study. These medicinal plants belong to 53 families (Myrtaceae representing the highest) and 103 genera. Many of these medicinal plants were native to Australia and used as bush food by Aboriginal peoples. Most of the widely used plants were trees whose leaves are used to treat as many as 62 different types of ailments. Skin sores, wounds, cuts, and infections were the most treated ailments. More than 48 medicinal plants were reported to be in use by Iningai and Mbabaram Aboriginal communities. In this study, more than 20 medicinal plants used by these two communities have been botanically identified and eight species have been investigated for their major classes of phytochemicals and the antioxidant and anti-inflammatory activities of their crude extracts—content and biological properties.

5.3. Iningai medicinal plants

In Chapter 3, eight plants out of 30 medicinal plants identified from Iningai community forest, were selected for investigation of major phytochemical contents, antioxidant properties, and anti-inflammatory properties. Phytochemical studies of the crude extracts focused on three major classes of phytochemicals: alkaloids, flavonoids, and terpenoids. When their antioxidant capacities were determined, *V. macrocalyx* showed the best result with IC_{50} value of 37.37 ± 1.01 μg/mL, followed by *P. angustifolium* (48.33 ± 1.07 μg/mL), TB75 (119.90 ± 1.11 μg/mL) and *S. lanceolatum* (134.30 ± 2.49 μg/mL). *D. tenuifolia* showed the weakest antioxidant activity with an IC_{50} value of 206.50 ± 2.44 μg/mL. The crude leaf extract from *Pittosporum angustifolium* caused the highest cell death (73.97% dead cells) in the cell viability test, indicating potential toxicity to human cells which is interesting in the context of its use for treating cancers by the Aboriginal people in Central Queensland. Seven plant extracts significantly inhibited the pro-inflammatory cytokines TNF, IFN-γ, MCP-1, and IL-23 as

determined by LPS-activated human PBMCs assay. The three most active crude leaf extracts were TB75, *G. australe,* and TB79. *Dodonaea tenuifolium* extract showed the weakest anti-inflammatory capacity. This study generated data validating eight medicinal plants' traditional uses.

5.4. Mbabaram medicinal plants

In Chapter 4, eight medicinal plants used by Mbabaram community for treating inflammation and inflammatory-related conditions were selected to be tested. The test for major groups of phytochemicals from five different plant parts produced mixed results. *Breynia oblongifolia* performed poorly, with only a moderate concentration of flavonoids found in the leaves and no other phytochemicals detected. The rest of the plants produced some good results, and a detailed discussion of phytochemical and anti-inflammatory screenings was provided. The total phenolic content (TPC) in crude extracts was determined by the Folin-Ciocalteu method described in the methods section and expressed in mg of gallic acid equivalent (GAE)/gram of dried crude extract. *Dodonaea lanceolata* showed the highest phenolic content, followed by *C. reticulatus* and *E. latifolius*.

The effect of aqueous crude extracts on cell viability was determined by staining cells with a live/dead viability dye (LIVE/DEAD™ fixable near-IR dead cell stain kit following the manufacturer's instructions as mentioned in the methods. There was no toxic effect on cell viability by any of the crude extracts, and therefore, anti-inflammatory testing of these extracts was undertaken. In the anti-inflammatory test, the aqueous crude extract from *B. oblongifolia* leaves significantly suppressed all four pro-inflammatory cytokines, showing the best anti-inflammatory activity. *Exocarpus latifolius* did not show significant suppression of any of the pro-inflammatory cytokines.

5.5. Final words

Before colonisation, Aboriginal people lived on the Australian continent for thousands of years, acquiring intimate knowledge to survive and live sustainably in harmony with nature. While the assimilation pressure from other cultures and traditions that came with Europeans and other immigrants was significant, the biocultural knowledge of bush food and bush medicine survived and is still practiced today by many Australian Aboriginal peoples, especially in rural and regional areas. However, bush medicine practices have been dwindling over the past century due to a diminishing population, the loss of language and culture, lack of interest by the younger generations, inaccessibility to the land that occurred after the arrival of Europeans, globalisation, marginalisation, environmental pressures, and economic and health inequity (Sutherland, 2003). Additionally, knowledge of medicinal plants by the Elders, knowledge holders, and practitioners is disappearing faster than that of the plants themselves (Anyinam, 1995). It is now urgent that Indigenous People’s Traditional Knowledge be recorded and preserved.

Therefore, there is an immediate need to document Aboriginal biocultural knowledge, preserve it, and add value to this knowledge through scientific studies. There are more than 500 Aboriginal tribes

in Australia, and their biocultural knowledge is distinct as the environment may influence the people they live in. As a result, a diverse range of flora and fauna are being used in bush medicine, opening huge opportunities for scientific studies, commercialisation, and biodiscovery projects. There is an urgent need to fund these crucial documentation and scientific exploration studies.

In 2009, Traditional Owners in North Queensland identified that an ethnobotany research and resource centre could support them in the conservation, management, use and communication of their Traditional Ecological Knowledge. The Tropical Indigenous Ethnobotany Centre (TIEC) - the first Indigenous-driven ethnobotanical research centre in Australia - was established to meet that need. TIEC is a partnership between Traditional Owners and a range of government and non-government research agencies, including the CSIRO, Queensland Department of Environment, Science and Innovation, and James Cook University. Indigenous led and governed, TIEC has been working closely with many Aboriginal communities in Queensland and elsewhere. Its success speaks to suggests TIEC could serve as a model for Indigenous led biodiscovery science.

This project is one of the first of its kind where an Indigenous researcher (e.g., myself), Indigenous communities, other academics, and the universities work together on medicinal plants that interest the communities and would help bring healing to patients and the Aboriginal communities. From the studies discussed here, further research on the most promising plant species is recommended to isolate and identify antioxidant and anti-inflammatory lead compounds for developing novel therapeutics, especially as antioxidant food products and anti-inflammatory agents. For both Mbabaram and Iningai people and researchers, ongoing collaboration must continue by building trust and strengthening existing working relationships in future co-designed cross-cultural or two-way approaches. The protection of Indigenous Cultural and Intellectual Property is also important. For traditional custodians, learning and reinvigorating IBK into the community brings a sense of pride and self-esteem in their culture and customs. It can also inspire economic aspirations by developing bush medicine, bush food products, and other botanicals.

5.6. References

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