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Phytochemical Composition and Antioxidant and Anti-Inflammatory Activities of Iningai Aboriginal Medicinal Plants From Central Queensland, Australia

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ABSTRACT

Indigenous Australians possess vast ethnopharmacological knowledge of native flora and have been using it for millennia. In a collaborative initiative to document and scientifically validate this knowledge, the Tropical Indigenous Ethnobotany Centre, Australian Tropical Herbarium and the James Cook University have been working closely with traditional custodians from the Iningai community near Barcaldine, Queensland. This study aimed to evaluate crude leaf extracts from eight medicinal plant species traditionally used by the Iningai people, focusing on their phytochemical profile, antioxidant potential, cytotoxicity and anti-inflammatory activity. Phytochemical screening confirmed the presence of alkaloids, phenolics, flavonoids and terpenoids. Antioxidant activity, assessed via the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay, showed moderate to strong activity, with IC_{50} values ranging from 37.37 ± 1.01 μg/mL to 206.50 ± 2.44 μg/mL. Cell viability assay using human peripheral blood mononuclear cells (PBMCs) showed that *Pittosporum angustifolium* exhibited the highest cytotoxicity, resulting 73.97% cell death, suggesting potential toxicity to human cells. Anti-inflammatory activity was evaluated in lipopolysaccharide (LPS)-stimulated PBMCs. Seven of the eight plant extracts significantly suppressed the release of proinflammatory cytokines, including tumour necrosis factor (TNF), interferon-gamma (IFN-γ), monocyte chemoattractant protein-1 (MCP-1) and interleukin (IL)-23. Overall, this study provides scientific validation for the traditional use of these eight medicinal plants by the Iningai people. The identification of key phytochemicals, antioxidant potential and anti-inflammatory properties supports their ethnopharmacological relevance. Further investigation is warranted to isolate and characterise the active compounds from the most promising species for potential development into novel therapeutic agents.

Karma Yeshi and Phurpa Wangchuk contributed equally as senior authors.

1 | Introduction

Medicinal plants and traditional medicine (TM) play an important role in the management and prevention of many chronic diseases [1], with about 50,000–70,000 plant species recorded as having medicinal value [2]. More than 170 member states of the World Health Organization (WHO) use various forms of TMs for treating many lifestyle-related chronic diseases [2–4]. Those who practice various forms of TM constitute about 80% of the developing world's population [2]. Recently, the WHO has emphasised the importance of TMs and held the first-ever WHO TM global summit in conjunction with the G20 Health Ministerial Meeting in 2023 in Gujarat, India [5]. 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 [6, 7].

Australian Indigenous people have inhabited the Australian continent for at least 65,000 years [8] and currently constitute 3.3% of the Australian population [9, 10]. They connect deeply with the local flora and fauna, using them for medicine, food, clothing, shelter, tools and weapons [3, 11]. Their ethnomedicinal knowledge is still vibrant, although frequencies of use may differ amongst Aboriginal clan groups across Australia [12]. So far, about 1511 medicinal plants have been recorded in Australia [13], and a recent review by Turpin et al. [3] 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 [3]. Moreover, in our review [4] 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, and it is situated in the desert uplands bioregion (Figure 1(a)). Bioregions are relatively large defined areas based on common climate, geology, landform, native vegetation and species information [15]. The desert upland bioregion has abundant Eucalypt woodlands, *Spinifex* understorey and *Acacia* woodlands growing on sandstone ranges and sand plains [16] (Figure 1(b)). Iningai country spans 50,504 km^2 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.

Recent research has shown that 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 [17]. The collaborative approach (cross-cultural or two-way) of IBK working with modern science has proven to be one of the promising biodiscovery strategies [4, 18, 19], with more than 25% of currently available prescription drugs sourced from natural products associated with IBK [20, 21]. Thus, Iningai medicinal plants used to treat inflammation and related diseases may offer a new avenue for developing natural antioxidant supplements or anti-inflammatory drugs. Therefore, this study aims to (i) to evaluate the phytochemical composition of selected medicinal plants used by the Iningai Aboriginal community, (ii) to assess the antioxidant and anti-inflammatory properties of selected plant crude extracts and (iii) to support the validation and protection of IBK through collaborative research, while identifying promising plant species and extracts for further lead compound isolation, and development of evidence-based herbal or therapeutic products.

2 | Materials and Methods

2.1 | 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.

2.2 | Chemicals and Reagents

The chemicals and reagents used in this study were all of AR grade. 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).

2.3 | Instruments

To determine the 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 LSRFortessa X20 (BD Biosciences), and the data (mean fluorescence intensity [MFI]) obtained were exported in the flow cytometry standard (FCS) format for further analysis.

2.4 | 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 Herbariorum code: BRI). Herbarium specimen numbers were assigned to each plant species (Table 1), and voucher specimens were deposited at the Australian Tropical Herbarium (Cairns). Fresh leaves were washed under running tap water and dried in an oven (40°C) for one week.

2.5 | Crude Extraction

For each plant species, 10 g of dried leaves was 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 min (min) to obtain the optimum extract. Fresh solvent was added every 20

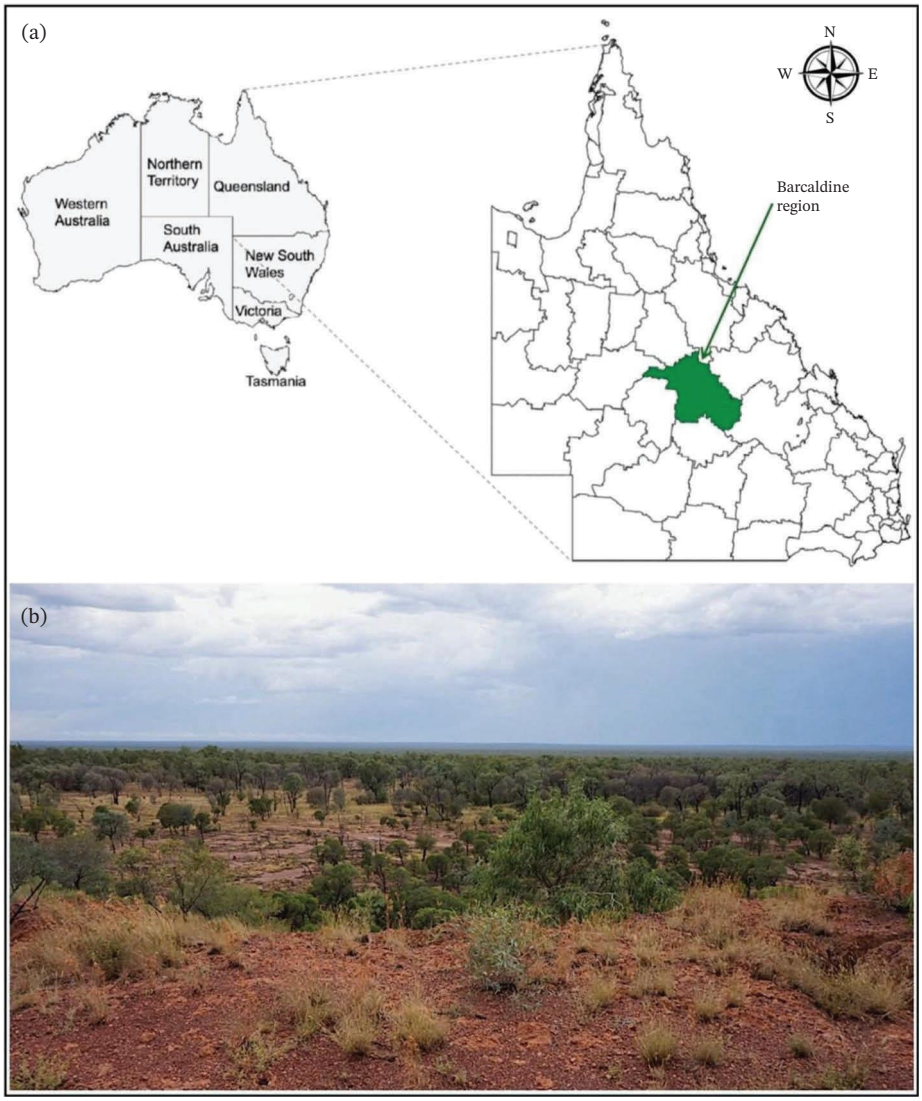

FIGURE 1 | (a) A map of Australia showing the state of Queensland with the shaded green area representing the Barcaldine region of the Iningai Aboriginal community—labels and locations are added from a map of local government areas in QLD [14]. (b) A typical open woodland community on sandy soil found in the Barcaldine region (photo courtesy: G. Turpin).

min (thrice with a total extraction time of 1 h). The three-cycle extract was pooled and filtered using a Stericup 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.

2.6 | Analysis for Major Classes of Phytochemicals

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 2) using a method adapted from Wangchuk et al. [25], Iqbal et al. [26], Yeshi et al. [27] and Shah and Hossain [28]. Briefly, qualitative phytochemical analysis was conducted as follows.

2.6.1 | 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.

2.6.2 | 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 steroids was confirmed by forming a brown ring at the junction and the upper green layer.

2.6.3 | 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.

2.6.4 | 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.

TABLE 1 | Eight medicinal plant species with their specimen voucher number and ethnopharmacological uses [3, 22].

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 and nail fungus, and relieving insect bites.
Pittosporum angustifolium G. Lodd. (Pittosporaceae)	BRI AQ1034549	Leaf	Leaf, fruit	Extract from leaf and fruit is used as poultice or decoction for healing bruises, muscle aches, eczema and other forms of pruritus; leaf infusion is used against cough and cold, cramps, eczema and skin diseases; infusion is used for 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.

TABLE 2 | Qualitative screening for the major phytochemical classes.

Plant species	Sample tested	Alkaloids	Flavonoids	Steroids			Tannins	Saponins	Glycosides	
				Salkowski 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	+	+	+	+		+	−	−	−

Note: + = present; − = absent. All tests were performed following methods (with slight modifications) described by Molole et al. [23] and Adusei et al. [24].

2.6.5 | 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.

2.6.6 | 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.

2.6.7 | 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.

2.7 | Determination of TPC

2.7.1 | Determination of Maximum Absorption Wavelength (λ_{max}) and Optimum Reaction Time

The maximum absorption wavelength (λ_{max}) and optimum reaction time were determined following the methods (with slight modifications) described by Molole et al. [23] and Adusei et al. [24] before evaluating the samples for the TPC. 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 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 400–900 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.

2.7.2 | TPC

After obtaining λ_{max} and the optimum reaction time, the TPC in the crude extract of leaves from eight medicinal plants was determined following the F–C method described by Molole et al. [23], Yeshi et al. [27] and Adusei et al. [24] with slight modifications. The TPC was determined in triplicate 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 triplicate. 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, the 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 [29]:

$$C = C_1 \times \frac{V}{m}, \quad (1)$$

where 'C' is the TPC in mg GAE/g extract, 'C_1' 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).

2.8 | Pharmacological Tests

2.8.1 | DPPH Free Radical Scavenging Activity

The potential to scavenge/reduce the DPPH free radical by the leaf crude extracts of eight medicinal plants was determined following the method described by Brand-Williams [30], Yeshi et al. [27] and Molole et al. [23] with some modifications. A solution of 0.1 mM DPPH was prepared in a 25-mL volumetric flask by dissolving 0.01 g of DPPH in methanol and further diluted to obtain 10, 20, 30, 40, 50 and 60 μM concentrations of DPPH solutions. Then, 200 μL of DPPH from each concentration was transferred to a 96-well flat-bottom plate (in triplicate) and scanned over a 400–600 nm wavelength range against the blank (methanol). The absorbance values obtained were used to determine the λ_{max} and the relationship between absorbance and concentrations of the standards.

2.8.2 | Determination of Half Maximal Inhibitory Concentration (IC_{50})

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 twofold 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 1 hour. After 1 hour of incubation, the absorbance was measured at the previously determined λ_{max} (517 nm). Finally, the percentage of DPPH free radical scavenged in the reaction mixture was calculated using the equation below:

$$\text{percentage}(\varphi)\text{of DPPH scavenged} = \frac{A_0 - A_s}{A_0} \times 100, \quad (2)$$

where A_0 is the absorbance of the DPPH solution without antioxidants and A_s is the absorbance of the sample [31]. The obtained % of DPPH free 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 the concentration.

2.8.3 | Effect of Crude Extracts on Cell Viability and Their Anti-Inflammatory Activity

2.8.3.1 | Human PBMCs Culture and Sample Treatment. PBMCs from a healthy donor (donor ID: BC970050, date of frozen: 20201105) were isolated from buffy coats (supplied by the Australian Red Cross Lifeblood) using the Ficoll-Paque PLUS (GE Health) density gradient method described by the STEMCELL Technologies. PBMCs were then cryopreserved using vapour phase nitrogen in filtered heat-inactivated foetal bovine

serum (FBS) (Corning #35-076-CV) with 10% dimethyl sulfoxide (DMSO, Sigma-Aldrich) until used. On the day of the experiment, PBMCs were thawed in a water bath (37°C) and washed with prewarmed sterile R-10 media (RPMI-1640, Gibco), containing 10% heat-inactivated FBS, 100 U/mL penicillin and 100 μg/mL streptomycin (Gibco). Subsequently cells were counted with the help of a CASY cell counter (Roche Innovatis, Germany) and adjusted cell densities to approximately 1×10^6 cells in 100 μL of R-10 media to be seeded per well in sterile 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) and R-10 media with 0.5% DMSO were used as positive and negative controls, respectively. 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.

2.8.3.2 | Effect of Crude Extracts on Cell Viability. Before quantifying proinflammatory cytokines in the PBMC culture soup, PBMCs were analysed for cell health/viability (cytotoxicity) [27]. 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 the 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 were determined using a FlowJo Version 10.8.1 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 nontoxic crude extracts was further analysed for proinflammatory cytokines/chemokines profile.

2.8.3.3 | Quantification of Proinflammatory Cytokines and Chemokines. Proinflammatory cytokines/chemokines in the PBMC culture supernatant were quantified (in pg/mL) using a customised LEGENDplex multianalyte 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 proinflammatory 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) were further analysed using cloud-based software from BioLegend (San Diego, CA, USA), accessible from this link: https://legendplex.qognit.com/user/login?next%3d;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.

2.9 | Data Analysis

All data were expressed as the mean ± SD (standard deviation). 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 an unpaired t test ($p < 0.05$).

3 | Results

3.1 | 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 1) from the Barcaldine region by engaging the Iningai community and evaluated their phytopharmaceutical properties using various approaches. These species are TB75, TB79, *Pittosporum angustifolium, Gossypium australe*, *Velleia macrocalyx*, *Petalostigma pubescens* and *Santalum lanceolatum* (Figure 2).

3.2 | Qualitative Screening for Major Classes of Phytochemicals in Crude Extracts

Plants generally produce diverse classes of phytochemicals, and most possess interesting biological activities [32]. 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.

Of the eight medicinal plants tested, *D. tenuifolia* tested positive for all six major classes of phytochemicals, namely, alkaloid, flavonoid, tannin, steroids, saponin and glycoside (Table 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, steroids and tannin). *V. macrocalyx* showed the least positive results, with only a weak presence of flavonoids, steroids and saponins. Crude extracts of *S. lanceolatum* and *G. australe* showed a strong presence of steroids. 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 alkaloids, with a strong positive result for TB79.

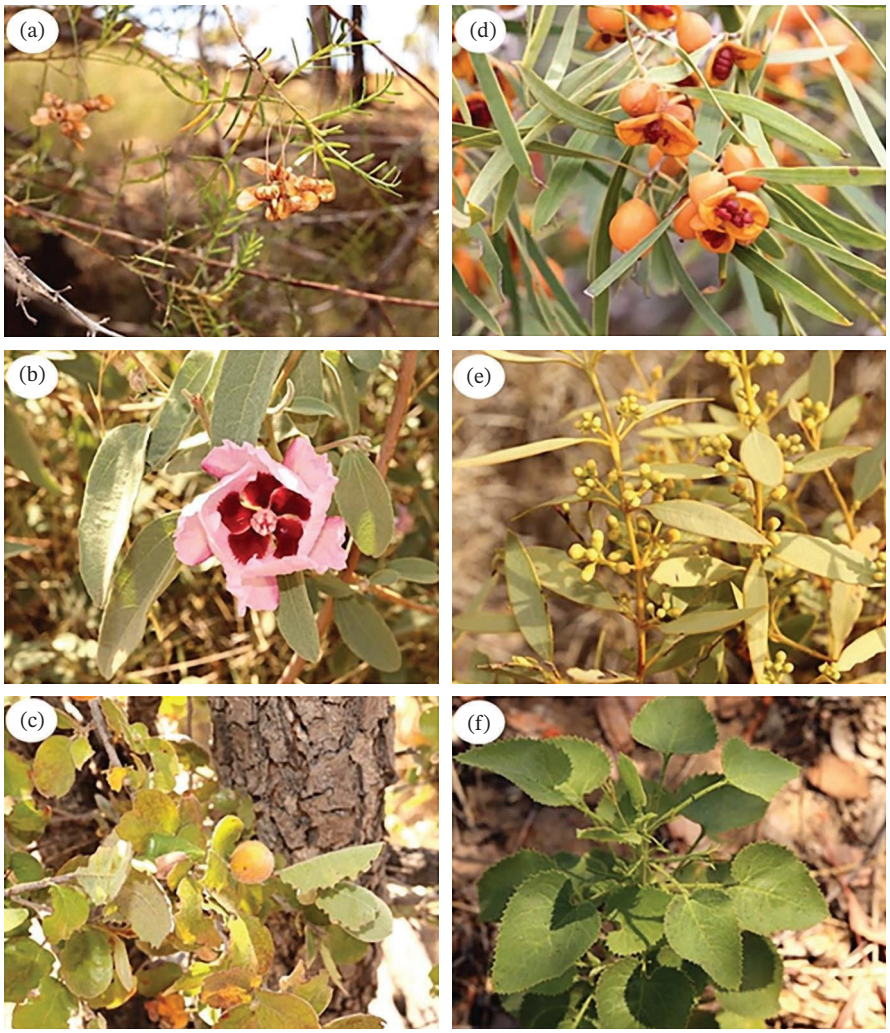

FIGURE 2 | Medicinal plant species included in the study with their aerial parts: (a) *Dodoneae tenuifolia*; (b) *Gossypium australe*; (c) *Petalostigma pubescens*; (d) *Pittosporum angustifolium;* (e) *Santalum lanceolatum*; (f) *Velleia macrocalyx.* Photographs of two species, which have been coded as TB75 and TB79, were excluded due to intellectual property (IP) sensitivity (photo courtesy: G. Turpin).

3.3 | TPC and Antioxidant Activity of Crude Extracts

3.3.1 | TPC

The F–C method is a widely used assay for quantifying total (poly)phenols in food [33]. 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 [34–36]. 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 [37, 38]. 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(a)). The maximum absorption (λ_{max}) lies between 700 and 800 nm, as shown in Figure 3(a). Moreover, there were insignificant variations (coefficient of variation, CV, $\leq 3.67\%$) in the absorbance values between 700 and 800 nm, as shown in Table 3. Thus, to determine the TPC, a wavelength (λ_{max}) of 760 nm was chosen in accordance with previous study [27] and other studies [23, 38, 39].

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 and then became steady or dropped progressively, as shown in Figure 3(b). For all samples, including the gallic acid, the optimum response was observed between 30 and 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 [23, 37, 38]. Thus, λ_{max} of 760 nm and an optimum reaction time of 30 min were used to determine the TPC in the crude leaf extracts of eight medicinal plants.

To quantify the 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. The TPC was measured at two different concentrations (500 and 1000 μg/mL). The results were

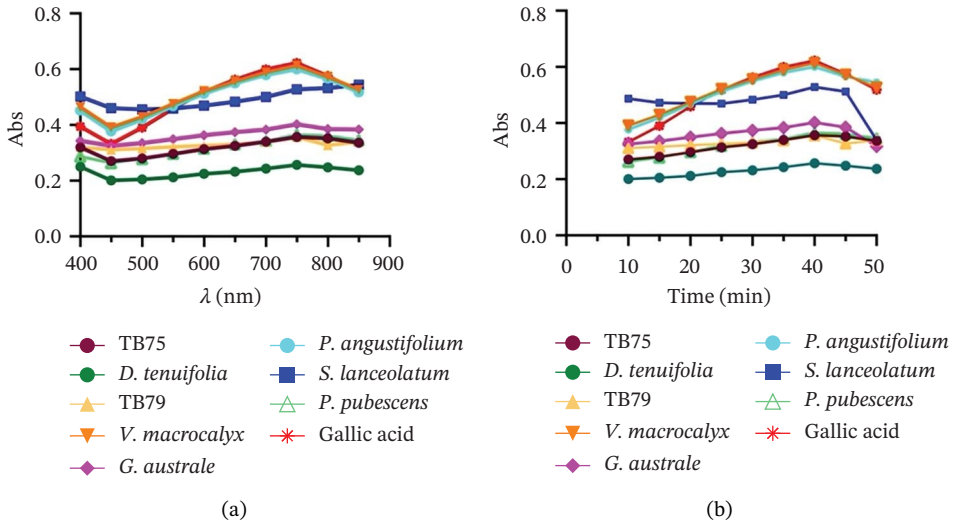

FIGURE 3 | Maximum absorbance (λ_{max}) and optimum reaction time for total phenolic content: (a) absorption spectra (400–850 nm) of the product of the reaction of the Folin–Ciocalteu (F–C) reagent with crude leaf extracts of eight medicinal plant species (400 μg/mL) and gallic acid (100 μg/mL); (b) change in absorbance with a reaction time of the F–C reagent with crude leaf extracts of eight medicinal plant species and gallic acid.

expressed as mg gallic acid equivalent (GAE)/g dry crude extract, as shown in Figure 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 TPC 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 4).

3.3.2 | DPPH Free Radical Scavenging Activity

The 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 free radical is mostly determined spectrophotometrically by measuring the absorbance at a specific

TABLE 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. (μg/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	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	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

Note: Coefficient of variance (CV, %) was calculated by dividing the standard deviation (σ) by the mean (μ) of absorbance values of each sample between 740 and 770 nm wavelength multiplied by 100% (CV = $\sigma/\mu \times 100\varrho$) [23].

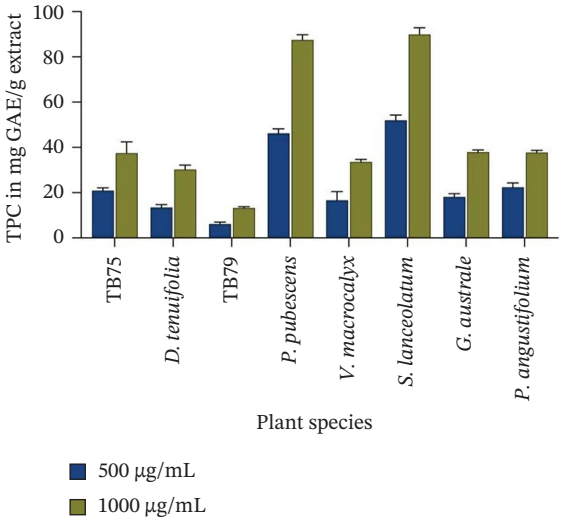

FIGURE 4 | Total phenolic content (TPC) of crude leaf extracts from eight medicinal plant species at two concentrations (500 and 1000 μg/mL). Data were expressed as mean ± SD [n (instrumental × sample replicate) = 9].

wavelength [23, 40]. 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 5(a), the maximum absorption was at 517 nm wavelength (λ_{max}). There was also a linear relationship between the DPPH free radical concentrations and their respective absorbance at 517 nm (Figure 5(b)).

In this study, the antioxidant activity of crude leaf extracts was evaluated by measuring their absorbance at 517 nm in line with other studies [30, 41] at various concentrations between 50 and 400 μg/mL. Gallic acid was used as a reference compound. The DPPH free radical scavenging effect of all crude extracts increased as their concentration was increased (Figure 6). The DPPH free 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 throughout the concentrations (Figure 6).

The current study also determined the inhibitory concentration that scavenged 50% of the initial DPPH free radical concentration (IC_{50} values). The antioxidant capacity of the substance is indirectly proportional to its IC_{50} value [30]. That means the substance with a lower IC_{50} value has better antioxidant activity. Amongst 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) (Table 4). *D. tenuifolia* had the highest IC_{50} value of 206.50 ± 2.44 μg/mL (Table 4).

3.3.3 | 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 free radical scavenging activity (IC_{50} values) (Figure 7). There was a significant correlation (p value = ** < 0.0027) between TPC and antioxidant activity of crude leaf extracts from eight medicinal plant species.

3.4 | Cell Viability and Anti-Inflammatory Activity of Crude Leaf Extracts

3.4.1 | 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

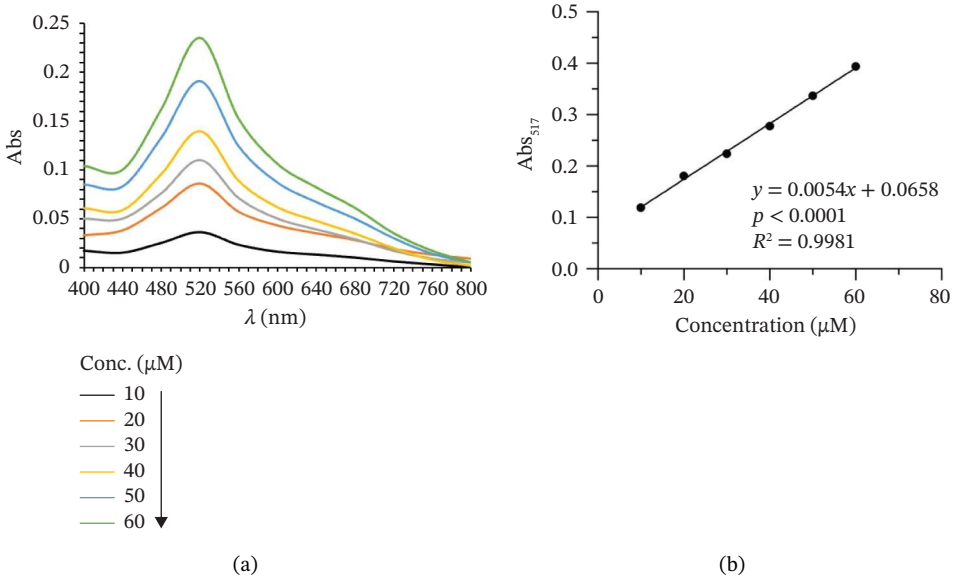

FIGURE 5 | (a) Absorption spectra (400–800 nm) for solutions containing 10, 20, 30, 40, 50 and 60 μM DPPH free radical and (b) the linear relationship between different concentrations of DPPH free radical and their absorbance measured at 517 nm.

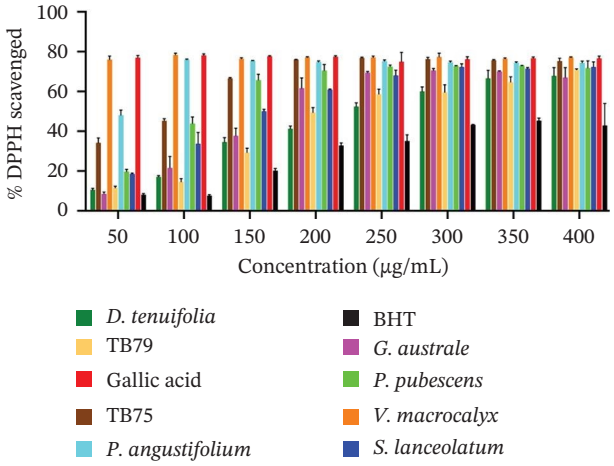

FIGURE 6 | The 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging effect of methanolic leaf crude extracts from eight medicinal plant species, gallic acid and butylated hydroxytoluene (BHT). The results were expressed as mean ± SD of three means ($n = 3$).

cells < 37%) (Figure 8) when compared to the stimulated (21.2%) and unstimulated (19.0%) controls. Therefore, the culture supernatants from these seven species were further analysed to quantify proinflammatory cytokines.

3.4.2 | Anti-Inflammatory Activity

The inhibitory effect of crude leaf extracts (100 μg/mL) from seven species on 13 proinflammatory 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 proinflammatory cytokines and chemokines tested (IL-1*β*, 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 9) significantly reduced the production of IFN-*γ* (Figure 9(a)), TNF (Figure 9(b)), MCP-1 (Figure 9(c)) and IL-23 (Figure 9(d)). Except for the crude extract of *D. tenuifolia*, the remaining plant species significantly suppressed at least four proinflammatory cytokines. Dexamethasone (Dexa) was used as the positive control drug. Based on the significant suppression (*p* values) of four cytokines

TABLE 4 | The IC_{50} values for the antioxidant activity of eight medicinal plant species and standard antioxidants.

Samples	IC_{50} (μg/mL)
TB75	119.90 ± 1.11
D. tenuifolia	206.50 ± 2.44
TB79	176.30 ± 2.26
P. pubescens	102.30 ± 2.35
V. macrocalyx	37.37 ± 1.01
S. lanceolatum	134.30 ± 2.49
G. australe	149.90 ± 3.59
P. angustifolium	48.33 ± 1.07
Gallic acid	218.90 ± 1.83
BHT	174.90 ± 4.11

Note: IC_{50} values were obtained from the GraphPad four-parameter logistic (4PL) regression model.

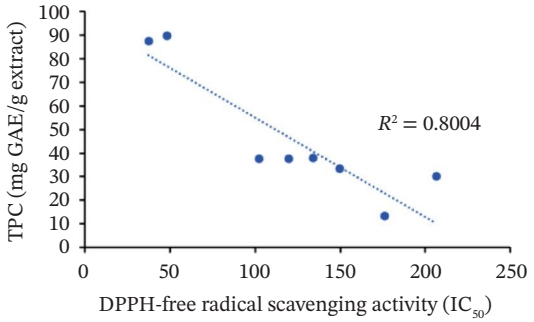

FIGURE 7 | A Pearson correlation scatter plot showing the positive relationship between the total phenolic content (TPC) and DPPH free radical scavenging activity (IC_{50}).

(Figure 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 9). *D. tenuifolium* was the least active of all seven species tested.

4 | Discussion

Many medicinal plants and other native plants and herbs used by the Aboriginal people of Australia have exhibited antioxidant and anti-inflammatory potentials [4, 22, 27, 42, 43]. The antioxidant potential of medicinal plants is mainly attributed to polyphenols, including phenolic acids, flavonoids, stilbenes and lignans [44–46]. These antioxidant compounds are capable of preventing or minimising the generation of free radicals in living cells [47–49], as excessive free radicals are toxic and cause damage to important biomolecules, such as DNA, lipids and proteins [49, 50]. 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 [51–53] and

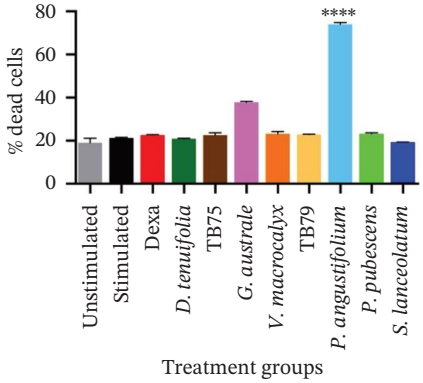

FIGURE 8 | The percentage of dead cells in lipopolysaccharide (LPS)-stimulated human PBMCs treated with the methanolic leaf crude extracts (overnight) obtained from eight medicinal plant species at a 100 μg/mL concentration. Data were expressed as mean ± SD ($n = 3$). Statistical significance was determined by the 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 nontoxic to the cells [27].

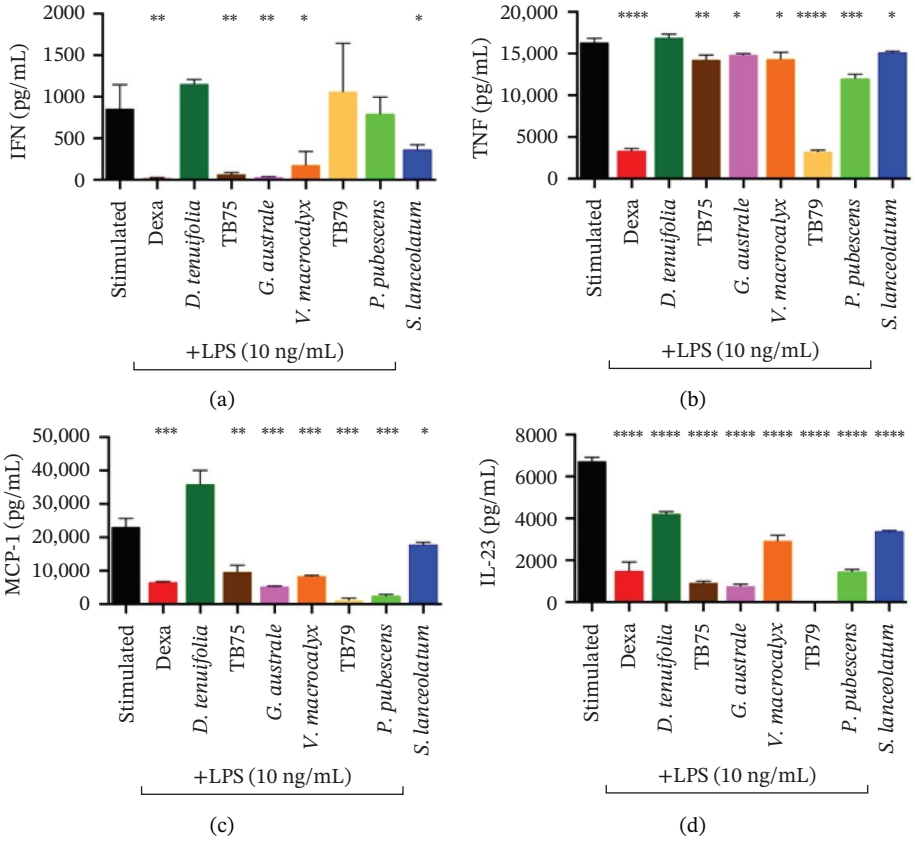

FIGURE 9 | Inhibitory effects of methanolic crude leaf extracts (100 μg/mL) obtained from seven medicinal plant species on the secretion of proinflammatory cytokines: (a) interferon-gamma (IFN-γ), (b) tumour necrosis factor (TNF), (c) monocyte chemoattractant protein-1 (MCP-1) and (d) interleukin 23 (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 the unpaired t test using GraphPad Prism (v.10.2.2), where $^{*}p = 0.0332$, $^{**}p = 0.0021$, $^{***}p = 0.0002$, $^{****}p < 0.0001$. TB75, *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.

multiple diseases, including neurodegenerative diseases [54, 55], cancer [56], diabetes [57, 58], arthritis [59, 60], atherosclerosis [61] and fast ageing [62, 63]. 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 [64, 65].

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 [66–68]. For instance, ether is recommended to extract alkaloids and chloroform for flavonoids and terpenoids [69, 70], and alcohols are mostly preferred for extracting polar secondary metabolites [71]. 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 [72]. The saponin test was positive for five of the eight species; *P. angustifolium* and *S. lanceolatum* tested negative. Although a few studies have reported saponins from seeds of *Pittosporum* species [73–75], in our study, *P. angustifolium* tested negative for saponins and steroids. The amount and distribution of saponins amongst plants and within tissues and organs of individual plants greatly vary based on their functions/maturation process and seasonal fluctuations [76], mostly synthesised in the underground organs [77–79]. Thus, the qualitative screening of results of such phytochemicals may be affected by the parts of plant used for the test and other factors, such as collection season and plant maturity.

Based on the qualitative phytochemical screening results, we next searched for the literature on these eight medicinal plants and reviewed whether they had been explored for their biodiscovery potential. Out of eight species, only *P. 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* [80–82]. Additionally, 20 secondary metabolites have been identified from leaves and stems by gas chromatography-mass spectrometry (GC-MS/MS)

[83]. Some of these compounds were tested for antimicrobial and DNA-topoisomerase I inhibitory activity [84]; 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 free radical, as the antioxidant potential of medicinal plants is mainly attributed to the presence of polyphenols [44–46]. They showed a percentage DPPH free radical scavenging capacity ranging from 66.97% to 77.09% and IC_{50} values between 37.37 ± 1.01 and $206.5 \pm 2.44\ \mu g/mL$. Based on the IC_{50} values, *V. macrocalyx* (Goodeniaceae) and *P. angustifolium* (Pittosporaceae) showed the best DPPH free radical scavenging activity with IC_{50} values of 37.37 ± 1.01 and $48.33 \pm 1.07\ \mu g/mL$, respectively. However, *V. macrocalyx* had a very low TPC, while *S. lanceolatum* showed the highest TPC but only moderate antioxidant activity, suggesting that nonphenolic compounds or certain structural features of phenolics present in these plants may be responsible for their potent free radical scavenging property. Thus, the TPC is a useful but not definitive predictor of antioxidant capacity. A significant positive correlation ($^{**}p < 0.0027$) was observed between DPPH IC_{50} values and TPC. Moreover, five polyphenolic compounds, including rutin and isoquercitrin, were also isolated from the chloroform extract of leaves of *P. angustifolium* [85], which could be responsible for the scavenging effect, as many studies [86–88] have reported excellent DPPH free radical scavenging effect by these two compounds.

However, the TPC was highest in the leaf crude extracts of *P. pubescens* and *S. lanceolatum,* which showed higher IC_{50} values for DPPH free radical scavenging assay with 102.30 ± 2.35 and $134.30 \pm 2.49\ \mu g/mL$, respectively, than the two medicinal plants showing the highest antioxidant activity. Such variations in the antioxidant activity amongst the plant species may be due to their different phenolic profiles and their nature of interaction with DPPH free radical. Antioxidant phenolics mostly quench DPPH free radical by transferring electrons or donating hydrogen atoms [89, 90], and phenolic compounds with more hydroxyl groups possess better scavenging capacity [91]. 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. [92] compared the DPPH free 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 have rigorous detoxification strategies before using any medicinal plants [4], 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 [73–75]. Recently, Bäcker et al. (2024) [75] have 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 and $34.1 \pm 2.3\ \mu M$. 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, this plant should be used with caution for consumption or treatment, including prior detoxification.

Antioxidants can suppress the expression of transcription factors that induce the secretion of proinflammatory cytokines and chemokines [93–95]. When crude extracts (except for *P. angustifolium*) were tested for anti-inflammatory activity using the LPS-stimulated human PBMCs assay, the crude extracts from *V. microcalyx,* TB79 and TB75 most significantly suppressed the release of proinflammatory cytokines, namely TNF, IFN-γ, MCP-1 and IL-23. TNF is a key cytokine that orchestrates inflammatory responses by directly triggering inflammatory gene expression or indirectly inducing cellular apoptosis [96, 97]. Excessive expression of TNF is associated with several inflammatory-related diseases, including inflammatory bowel diseases (IBDs), rheumatoid arthritis and ankylosing spondylitis [98, 99]. 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 [96, 97]. Many studies have reported the inhibition of TNF by crude extracts from medicinal plants [100–102]. 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 [103]. All crude extracts also significantly suppressed interleukin (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 [104, 105]. 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 IBDs, which currently have no cure.

5 | Conclusions and Future Perspectives

As an initial collaborative work towards scientifically validating the IBK of the Iningai Aboriginal community in central Queensland, this study evaluated the crude extracts from eight medicinal plants for the 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 free radical, with IC_{50} values ranging from $37.37 \pm 1.01\ \mu g/mL$ to $206.50 \pm 2.44\ \mu g/mL$. *V. macrocalyx* exhibited the highest antioxidant activity ($IC_{50} = 37.37 \pm 1.01\ \mu g/$

mL), followed by *P. angustifolium* (48.33 ± 1.07 μg/mL) and TB75 (119.90 ± 1.11 μg/mL). In the human PBMC 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 proinflammatory 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 IBDs. For these kinds of studies, establishing standardised extraction protocols, phytochemical profiles and bioactivity benchmarks would enhance reproducibility and regulatory acceptance. Such standardisation could facilitate the development of reliable, evidence-based herbal products suitable for broader clinical and commercial applications. The results from the current study highlight strong potential for the targeted isolation and characterisation of antioxidant and anti-inflammatory lead compounds for the development of novel therapeutics. Future studies should also focus on comprehensive metabolite profiling of selected bioactive crude extracts to identify marker compounds responsible for the observed bioactivity and cytotoxicity.

The study benefited researchers and traditional custodians by establishing collaboration, partnerships, engagement and relationship ties through a research codesign process. The immediate benefits to the Iningai people include reinvigorating knowledge, supporting the passing of IBK from elders to the younger generation, engaging with researchers to discover scientific value in their IBK and, thus, protecting Iningai intellectual property.

Author Contributions

Gerry Turpin collected and identified plant samples, performed the experiments, analysed and interpreted the data and wrote the original manuscript draft. Darren Crayn conceptualised and supervised the study and reviewed and edited the manuscript. Karma Yeshi performed data curation, formal analysis and validation, and reviewed and edited the manuscript. Suzanne Thompson conceptualised the study and reviewed and edited the manuscript. Phurpa Wangchuk conceptualised and supervised the study, acquired funding and reviewed and edited the manuscript.

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Disclosure

The views expressed herein are those of the authors and are not necessarily those of the Australian Government or Australian Research Council.

Ethics Statement

The human ethics approval (H8523) and the plant collection permit (BIBC20200417-2) were obtained from James Cook University Ethics Committee and the Department of Environment and Science, Queensland government, respectively. Informed consent was obtained from Iningai Aboriginal Community as per the human ethics approval (H8523).

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

All data are included in the manuscript.

References

1. WHO, *WHO Global Report on Traditional and Complementary Medicine 2019* (Geneva, 2019).

2. P. Wangchuk, "Therapeutic Applications of Natural Products in Herbal Medicines, Biodiscovery Programs, and Biomedicine," *Journal of Biologically Active Products From Nature* 8, no. 1 (2018): 1–20, https://doi.org/10.1080/22311866.2018.1426495.

3. G. Turpin, E. Ritmejeryte, J. Jamie, D. Crayn, and P. Wangchuk, "Aboriginal Medicinal Plants of Queensland: Ethnopharmacological Uses, Species Diversity, and Biodiscovery Pathways," *Journal of Ethnobiology and Ethnomedicine* 18, no. 1 (2022): 54, https://doi.org/10.1186/s13002-022-00552-6.

4. K. Yeshi, G. Turpin, T. Jamtsho, and P. Wangchuk, "Indigenous Uses, Phytochemical Analysis, and Anti-inflammatory Properties of Australian Tropical Medicinal Plants," *Molecules* 27, no. 12 (2022): 3849, https://doi.org/10.3390/molecules27123849.

5. Who, "The First WHO Traditional Medicine Global Summit" (2023).

6. Who, *WHO Global Report on Traditional and Complementary Medicine 2019. Geneva* (World Health Organization, 2019).

7. F. Panzironi, *Hand in Hand: Report on Aboriginal Tradtional Medicine* (Adelaide: A angu Ngangka i Tjutaku Aboriginal Corporation, 2013).

8. C. Clarkson, Z. Jacobs, B. Marwick, et al., "Human Occupation of Northern Australia by 65,000 Years ago," *Nature* 547, no. 7663 (2017): 306–310, https://doi.org/10.1038/nature22968.

9. Australian Bureau of Sttistics, "Cultural Identification. National Aboriginal and Torres Strait Islander Health Survey TableBuilder" (2019).

10. *Australian Bureau of Sttistics. Estimates and Projections, Aboriginal and Torres Strait Islander Australians, 2006 to 2031. ABS Cat. No. 3238.0*.

11. P. A. Clarke, *Aboriginal People and Their Plants* (Dural Delivery Centre, 2007).

12. S. J. Oliver, "The Role of Traditional Medicine Practice in Primary Health Care Within Aboriginal Australia: a Review of the Literature," *Journal of Ethnobiology and Ethnomedicine* 9, no. 1 (2013): 46, https://doi.org/10.1186/1746-4269-9-46.

13. J. Packer, J. Gaikwad, D. Harrington, S. Ranganathan, J. Jamie, and S. Vemulpad, "Medicinal Plants of New South Wales, Australia," in *Medicinal Plants, Genetic Resources, Chromosome Engineering, and Crop Improvement*, 6 (Boca Raton: CRC Press, 2012).

14. Dsdilgp, *Local Government Areas and Council Chamber Locations* (Queensland: Infrastructure, Local Government and Planning (DSDILGP), 2021).

15. G. J. Williamson, L. Christidis, J. Norman, B. W. Brook, B. Mackey, and D. M. J. S. Bowman, "The Use of Australian Bioregions as Spatial Units of Analysis to Explore Relationships Between Climate and Songbird Diversity," *Pacific Conservation Biology* 17, no. 4 (2011): 354–360, https://doi.org/10.1071/pc110354.

16. Q. Herbarium, *Regional Ecosystem Description Database (REDD). Version 13 2023, Department of Environmental Science (DES): Brisbane*.

17. B. S. Simpson, V. Bulone, S. J. Semple, G. W. Booker, R. A. McKinnon, and P. Weinstein, "Arid Awakening: New Opportunities for Australian Plant Natural Product Research," *The Rangeland Journal* 38, no. 5 (2016): 467–478, https://doi.org/10.1071/rj16004.

18. E. J. Ens, P. Pert, P. A. Clarke, et al., "Indigenous Biocultural Knowledge in Ecosystem Science and Management: Review and Insight from Australia," *Biological Conservation* 181 (2015): 133–149, https://doi.org/10.1016/j.biocon.2014.11.008.

19. E. Ens, M. L. Scott, Y. M. Rangers, C. Moritz, and R. Pirzl, "Putting Indigenous Conservation Policy into Practice Delivers Biodiversity and Cultural Benefits," *Biodiversity & Conservation* 25, no. 14 (2016): 2889–2906, https://doi.org/10.1007/s10531-016-1207-6.

20. P. Wangchuk and T. Tobgay, "Contributions of Medicinal Plants to the Gross National Happiness and Biodiscovery in Bhutan," *Journal of Ethnobiology and Ethnomedicine* 11, no. 1 (2015): 48, https://doi.org/10.1186/s13002-015-0035-1.

21. S. M. K. Rates, "Plants as Source of Drugs," *Toxicon* 39, no. 5 (2001): 603–613, https://doi.org/10.1016/s0041-0101(00)00154-9.

22. J. S. Mani, J. B. Johnson, H. Hosking, et al., "Antioxidative and Therapeutic Potential of Selected Australian Plants: a Review," *Journal of Ethnopharmacology* 268 (2021): 113580, https://doi.org/10.1016/j.jep.2020.113580.

23. G. J. Molole, A. Gure, and N. Abdissa, "Determination of Total Phenolic Content and Antioxidant Activity of Commiphora mollis (Oliv.) Engl. Resin," *BMC Chemistry* 16, no. 1 (2022): 48, https://doi.org/10.1186/s13065-022-00841-x.

24. S. Adusei, J. K. Otchere, P. Oteng, R. Q. Mensah, and E. Tei-Mensah, "Phytochemical Analysis, Antioxidant and Metal Chelating Capacity of Tetrapleura tetraptera," *Heliyon* 5, no. 11 (2019): e02762, https://doi.org/10.1016/j.heliyon.2019.e02762.

25. P. Wangchuk, P. A. Keller, S. G. Pyne, et al., "Evaluation of an Ethnopharmacologically Selected Bhutanese Medicinal Plants for Their Major Classes of Phytochemicals and Biological Activities," *Journal of Ethnopharmacology* 137, no. 1 (2011): 730–742, https://doi.org/10.1016/j.jep.2011.06.032.

26. Z. Iqbal, M. S. Iqbal, and K. Mishra, "Screening of Antioxidant Property in Medicinal Plants Belonging to the Family Apocynaceae," *Screening* 10 (2017): 12.

27. K. Yeshi, R. Ruscher, K. Miles, D. Crayn, M. Liddell, and P. Wangchuk, "Antioxidant and Anti-inflammatory Activities of Endemic Plants of the Australian Wet Tropics," *Plants* 11, no. 19 (2022): 2519, https://doi.org/10.3390/plants11192519.

28. M. D. Shah and M. A. Hossain, "Total Flavonoids Content and Biochemical Screening of the Leaves of Tropical Endemic Medicinal Plant Merremia borneensis," *Arabian Journal of Chemistry* 7, no. 6 (2014): 1034–1038, https://doi.org/10.1016/j.arabjc.2010.12.033.

29. N. Siddiqui, A. Rauf, A. Latif, and Z. Mahmood, "Spectrophotometric Determination of the Total Phenolic Content, Spectral and Fluorescence Study of the Herbal Unani Drug Gul-e-Zoofa (Nepeta bracteata Benth)," *Journal of Taibah University Medical Sciences* 12, no. 4 (2017): 360–363, https://doi.org/10.1016/j.jtumed.2016.11.006.

30. W. Brand-Williams, M. E. Cuvelier, and C. Berset, "Use of a Free Radical Method to Evaluate Antioxidant Activity," *Technology* 28, no. 1 (1995): 25–30, https://doi.org/10.1016/s0023-6438(95)80008-5.

31. A. Fadda, M. Serra, M. G. Molinu, E. Azara, A. Barberis, and D. Sanna, "Reaction Time and DPPH Concentration Influence Antioxidant Activity and Kinetic Parameters of Bioactive Molecules and Plant Extracts in the Reaction with the DPPH Radical," *Journal of Food Composition and Analysis* 35, no. 2 (2014): 112–119, https://doi.org/10.1016/j.jfca.2014.06.006.

32. A. Geyid, D. Abebe, A. Debella, et al., "Screening of Some Medicinal Plants of Ethiopia for Their Anti-microbial Properties and Chemical Profiles," *Journal of Ethnopharmacology* 97, no. 3 (2005): 421–427, https://doi.org/10.1016/j.jep.2004.08.021.

33. M. Pérez, I. Dominguez-López, and R. M. Lamuela-Raventós, "The Chemistry Behind the Folin–Ciocalteu Method for the Estimation of (Poly)Phenol Content in Food: Total Phenolic Intake in a Mediterranean Dietary Pattern," *Journal of Agricultural and Food Chemistry* 71, no. 46 (2023): 17543–17553.

34. V. L. Singleton and J. A. Rossi Jr, "Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents," *American Journal of Enology and Viticulture* 16, no. 3 (1965): 144–158, https://doi.org/10.5344/ajev.1965.16.3.144.

35. D. O. Rosbash, "Preparation of Folin-ciocalteu's Phenol Reagent," *American Journal of Clinical Pathology* 19, no. 33_ts) (1949): 297, https://doi.org/10.1093/ajcp/19.3_ts.297.

36. E. A. Ainsworth and K. M. Gillespie, "Estimation of Total Phenolic Content and Other Oxidation Substrates in Plant Tissues Using Folin–Ciocalteu Reagent," *Nature Protocols* 2, no. 4 (2007): 875–877, https://doi.org/10.1038/nprot.2007.102.

37. M. A. M. Galvão, A. O. de Arruda, I. C. F. Bezerra, M. R. A. Ferreira, and L. A. L. Soares, "Evaluation of the Folin–Ciocalteu Method and Quantification of Total Tannins in Stem Barks and Pods from Libidibia ferrea Mart. Ex Tul," *Brazilian Archives of Biology and Technology* 61 (2018): 1–20.

38. A. Blainski, G. C. Lopes, and J. C. P. de Mello, "Application and Analysis of the Folin Ciocalteu Method for the Determination of the Total Phenolic Content from Limonium brasiliense L," *Molecules* 18, no. 6 (2013): 6852–6865, https://doi.org/10.3390/molecules18066852.

39. V. L. Singleton, R. Orthofer, and R. M. Lamuela-Raventos, "Analysis of Total Phenols and Other Oxidation Substrates and Antioxidants by Means of Folin-Ciocalteu Reagent," *American Journal of Enology and Viticulture* 25, no. 119 (1974): 152–178.

40. R. Amorati and L. Valgimigli, "Advantages and Limitations of Common Testing Methods for Antioxidants," *Free Radical Research* 49, no. 5 (2015): 633–649, https://doi.org/10.3109/10715762.2014.996146.

41. K. Yeshi, P. Yangdon, S. Kashyap, and P. Wangchuk, "Antioxidant Activity and the Polyphenolic and Flavonoid Contents of Five High Altitude Medicinal Plants Used in Bhutanese Sowa Rigpa Medicine," *JBAPN* 7, no. 1 (2017): 18–26, https://doi.org/10.1080/22311866.2017.1287593.

42. A. Ali, J. J. Cottrell, and F. R. Dunshea, "Characterization, Antioxidant Potential, and Pharmacokinetics Properties of Phenolic Compounds from Native Australian Herbs and Fruits," *Plants* 12, no. 5 (2023): 993, https://doi.org/10.3390/plants12050993.

43. K. Akter, E. C. Barnes, J. J. Brophy, et al., "Phytochemical Profile and Antibacterial and Antioxidant Activities of Medicinal Plants Used by Aboriginal People of New South Wales, Australia," *Evidence-Based Complementary and Alternative Medicine* 2016, no. 1 (2016): 4683059, https://doi.org/10.1155/2016/4683059.

44. C. Manach, A. Scalbert, C. Morand, C. Remesy, and L. Jimenez, "Polyphenols: Food Sources and Bioavailability," *American Journal Clinical Nutrition* 79, no. 5 (2004): 727–747, https://doi.org/10.1093/ajcn/79.5.727.

45. K. B. Pandey and S. I. Rizvi, "Plant Polyphenols as Dietary Antioxidants in Human Health and Disease," *Oxidative Medicine and Cellular Longevity* 2, no. 5 (2009): 270–278, https://doi.org/10.4161/oxim.2.5.9498.

46. A. Oreopoulou, D. Tsimogiannis, and V. Oreopoulou, "Chapter 15-Extraction of Polyphenols from Aromatic and Medicinal Plants: an

Overview of the Methods and the Effect of Extraction Parameters," in *Polyphenols in Plants*, 2nd ed., ed. R. R. Watson (Academic Press, 2019), 243–259.

47. Y. Cai, Q. Luo, M. Sun, and H. Corke, "Antioxidant Activity and Phenolic Compounds of 112 Traditional Chinese Medicinal Plants Associated with Anticancer," *Life Sciences* 74, no. 17 (2004): 2157–2184, https://doi.org/10.1016/j.lfs.2003.09.047.

48. S. Dragland, H. Senoo, K. Wake, K. Holte, and R. Blomhoff, "Several Culinary and Medicinal Herbs Are Important Sources of Dietary Antioxidants," *Journal of Nutrition* 133, no. 5 (2003): 1286–1290, https://doi.org/10.1093/jn/133.5.1286.

49. P. Diaz, S. C. Jeong, S. Lee, C. Khoo, and S. R. Koyyalamudi, "Antioxidant and Anti-inflammatory Activities of Selected Medicinal Plants and Fungi Containing Phenolic and Flavonoid Compounds," *Chinese Medicine* 7, no. 1 (2012): 26, https://doi.org/10.1186/1749-8546-7-26.

50. L. Zhang, A. S. Ravipati, S. R. Koyyalamudi, et al., "Antioxidant and Anti-inflammatory Activities of Selected Medicinal Plants Containing Phenolic and Flavonoid Compounds," *Journal of Agriculture Food Chemistry* 59, no. 23 (2011): 12361–12367, https://doi.org/10.1021/jf203146e.

51. E. J. Ramos-González, O. K. Bitzer-Quintero, G. Ortiz, J. J. Hernández-Cruz, and L. J. Ramírez-Jirano, "Relationship Between Inflammation and Oxidative Stress and Its Effect on Multiple Sclerosis," *Neurologia* 39, no. 3 (2024): 292–301.

52. S. K. Biswas, "Does the Interdependence Between Oxidative Stress and Inflammation Explain the Antioxidant Paradox?" *Oxidative Medicine and Cellular Longevity* 2016, no. 1 (2016): 5698931, https://doi.org/10.1155/2016/5698931.

53. T. Hussain, B. Tan, Y. Yin, F. Blachier, M. C. Tossou, and N. Rahu, "Oxidative Stress and Inflammation: what Polyphenols Can Do for Us?" *Oxidative Medicine and Cellular Longevity* 2016, no. 1 (2016): 7432797, https://doi.org/10.1155/2016/7432797.

54. K. A. Wojtunik-Kulesza, A. Oniszczuk, T. Oniszczuk, and M. Waksmundzka-Hajnos, "The Influence of Common Free Radicals and Antioxidants on Development of Alzheimer's Disease," *Biomedicine & Pharmacotherapy* 78 (2016): 39–49, https://doi.org/10.1016/j.biopha.2015.12.024.

55. X. Chen, C. Guo, and J. Kong, "Oxidative Stress in Neurodegenerative Diseases," *Neural Regeneration Research* 7, no. 5 (2012): 376–385, https://doi.org/10.3969/j.issn.1673-5374.2012.05.009.

56. M. Valko, C. Rhodes, J. Moncol, M. Izakovic, and M. Mazur, "Free Radicals, Metals and Antioxidants in Oxidative stress-induced Cancer," *Chemico-Biological Interactions* 160, no. 1 (2006): 1–40, https://doi.org/10.1016/j.cbi.2005.12.009.

57. F. Giacco and M. Brownlee, "Oxidative Stress and Diabetic Complications," *Circulation Research* 107, no. 9 (2010): 1058–1070, https://doi.org/10.1161/circresaha.110.223545.

58. J. S. Bhatti, A. Sehrawat, J. Mishra, et al., "Oxidative Stress in the Pathophysiology of Type 2 Diabetes and Related Complications: Current Therapeutics Strategies and Future Perspectives," *Free Radical Biology and Medicine* 184 (2022): 114–134, https://doi.org/10.1016/j.freeradbiomed.2022.03.019.

59. K. Hadjigogos, "The Role of Free Radicals in the Pathogenesis of Rheumatoid Arthritis," *Panminerva Medica* 45, no. 1 (2003): 7–13.

60. O. M. Zahan, O. Serban, C. Gherman, and D. Fodor, "The Evaluation of Oxidative Stress in Osteoarthritis," *Medicine Pharmalogy Reports* 93, no. 1 (2020): 12–22, https://doi.org/10.15386/mpr-1422.

61. P. Rajendran, N. Nandakumar, T. Rengarajan, et al., "Antioxidants and Human Diseases," *Clinica Chimica Acta* 436 (2014): 332–347, https://doi.org/10.1016/j.cca.2014.06.004.

62. N. Getoff, "Anti-Aging and Aging Factors in Life. the Role of Free Radicals," *Radiation Physics and Chemistry* 76, no. 10 (2007): 1577–1586, https://doi.org/10.1016/j.radphyschem.2007.01.002.

63. E. Maldonado, S. Morales-Pison, F. Urbina, and A. Solari, "Aging Hallmarks and the Role of Oxidative Stress," *Antioxidants* 12, no. 3 (2023): 651, https://doi.org/10.3390/antiox12030651.

64. S. C. Lourenço, M. Moldão-Martins, and V. D. Alves, "Antioxidants of Natural Plant Origins: from Sources to Food Industry Applications," *Molecules* 24, no. 22 (2019).

65. J. Pokorný, "Are Natural Antioxidants Better and Safer Than Synthetic Antioxidants?" *European Journal of Lipid Science and Technology* 109, no. 6 (2007): 629–642.

66. S. B. Mahato and S. Sen, "Advances in Triterpenoid Research, 1990–1994," *Phytochemistry* 44, no. 7 (1997): 1185–1236, https://doi.org/10.1016/s0031-9422(96)00639-5.

67. O. Odebiyi and E. Sofowora, "Phytochemical Screening of Nigerian Medicinal Plants II," *Lloydia* 41, no. 3 (1978): 234–246.

68. A. R. Abubakar and M. Haque, "Preparation of Medicinal Plants: Basic Extraction and Fractionation Procedures for Experimental Purposes," *Journal of Pharmacy and BioAllied Sciences* 12, no. 1 (2020): 1–10, https://doi.org/10.4103/jpbs.jpbs_175_19.

69. P. Tiwari, B. Kumar, M. Kaur, G. Kaur, and H. Kaur, "Phytochemical Screening and Extraction: a Review," *Internationale Pharmaceutica Sciencia* 1, no. 1 (2011): 98–106.

70. A. Pandey and S. Tripathi, "Concept of Standardization, Extraction and Pre Phytochemical Screening Strategies for Herbal Drug," *Journal of Pharmacognosy and Phytochemistry* 2, no. 5 (2014): 115–119.

71. K. Das, R. Tiwari, and D. Shrivastava, "Techniques for Evaluation of Medicinal Plant Products as Antimicrobial Agent: Current Methods and Future Trends," *Journal of Medicinal Plants Research* 4, no. 2 (2010): 104–111.

72. P. Kerr, "Phytochemical Investigations of Species of Semi-arid Australian Plants: a Neglected Area of Research," *Pharmacognosy Communications* 2, no. 1 (2012): 3–4, https://doi.org/10.5530/pc.2012.1.2.

73. I. D'Acquarica, M. C. Di Giovanni, F. Gasparrini, et al., "Isolation and Structure Elucidation of Four New Triterpenoid Estersaponins from Fruits of Pittosporum tobira AIT," *Tetrahedron* 58, no. 51 (2002): 10127–10136, https://doi.org/10.1016/s0040-4020(02)01364-9.

74. Y. Seo, J. M. Berger, J. Hoch, et al., "A New Triterpene Saponin from Pittosporum viridiflorum from the Madagascar Rainforest," *Journal of Natural Products* 65, no. 1 (2002): 65–68, https://doi.org/10.1021/np010327t.

75. C. Backer, K. Jenett-Siems, K. Siems, M. Wurster, A. Bodtke, and U. Lindequist, "Cytotoxic Saponins from the Seeds of Pittosporum angustifolium," *Zeitschrift fuer Naturforschung, C: Journal of Biosciences* 69, no. 5-6 (2014): 191–198, https://doi.org/10.5560/znc.2014-0011.

76. T. Moses, K. K. Papadopoulou, and A. Osbourn, "Metabolic and Functional Diversity of Saponins, Biosynthetic Intermediates and Semi-synthetic Derivatives," *Critical Reviews in Biochemistry and Molecular Biology* 49, no. 6 (2014): 439–462, https://doi.org/10.3109/10409238.2014.953628.

77. J. D. Park, D. K. Rhee, and Y. H. Lee, "Biological Activities and Chemistry of Saponins from Panax ginseng CA Meyer," *Phytochemistry Reviews* 4, no. 2-3 (2005): 159–175, https://doi.org/10.1007/s11101-005-2835-8.

78. H. Seki, K. Ohyama, S. Sawai, et al., "Licorice β-amyrin 11-oxidase, a Cytochrome P450 with a Key Role in the Biosynthesis of the Triterpene Sweetener Glycyrrhizin," *Proceedings of the National Academy of Sciences* 105, no. 37 (2008): 14204–14209, https://doi.org/10.1073/pnas.0803876105.

79. H. M. Teng, M. F. Fang, X. Cai, and Z. H. Hu, "Localization and Dynamic Change of Saponin in Vegetative Organs of Polygala tenuifolia," *Journal of Integrative Plant Biology* 51, no. 6 (2009): 529–536, https://doi.org/10.1111/j.1744-7909.2009.00830.x.

80. C. Backer, K. Jenett-Siems, K. Siems, et al., "Triterpene Glycosides from the Leaves of Pittosporum angustifolium," *Planta Medica* 79, no. 15 (2013): 1461–1469, https://doi.org/10.1055/s-0033-1350806.

81. C. Bäcker, K. Jenett-Siems, K. Siems, et al., "Taraxastane-Type Triterpene Saponins Isolated from Pittosporum angustifolium Lodd," *Zeitschrift für Naturforschung B* 70, no. 6 (2015): 403–408.

82. C. Bäcker, K. Jenett-Siems, K. Siems, et al., "New Mono- and Bisdesmosidic Triterpene Glycosides from Pittosporum angustifolium Lodd," *Zeitschrift für Naturforschung B* 69, no. 9-10 (2014): 1026–1044.

83. J. Mani, J. Johnson, H. Hosking, et al., "Bioassay-Guided Fractionation of Pittosporum angustifolium and Terminalia ferdinandiana with Liquid Chromatography Mass Spectroscopy and Gas Chromatography Mass Spectroscopy Exploratory Study," *Plants* 13, no. 6 (2024): 807, https://doi.org/10.3390/plants13060807.

84. C. Backer, M. N. Drwal, R. Preissner, and U. Lindequist, "Inhibition of DNA-Topoisomerase I by Acylated Triterpene Saponins from Pittosporum angustifolium Lodd," *Nature Production Bioprospective* 6, no. 2 (2016): 141–147, https://doi.org/10.1007/s13659-016-0087-5.

85. C. Bäcker, K. Jenett-Siems, A. Bodtke, and U. Lindequist, "Polyphenolic Compounds from the Leaves of Pittosporum angustifolium," *Biochemical Systematics and Ecology* 55 (2014): 101–103.

86. S. L. Patil, S. H. Mallaiah, and R. K. Patil, "Antioxidative and Radioprotective Potential of Rutin and Quercetin in Swiss Albino Mice Exposed to Gamma Radiation," *Journal of Medical Physics* 38, no. 2 (2013): 87–92, https://doi.org/10.4103/0971-6203.111321.

87. J. Yang, J. Guo, and J. Yuan, "In Vitro Antioxidant Properties of Rutin," *LWT-Food Science and Technology* 41, no. 6 (2008): 1060–1066, https://doi.org/10.1016/j.lwt.2007.06.010.

88. X. Li, Q. Jiang, T. Wang, J. Liu, and D. Chen, "Comparison of the Antioxidant Effects of Quercitrin and Isoquercitrin: Understanding the Role of the 6″-OH Group," *Molecules* 21, no. 9 (2016): 1246, https://doi.org/10.3390/molecules21091246.

89. B. Matthäus, "Antioxidant Activity of Extracts Obtained from Residues of Different Oilseeds," *Journal of Agricultural and Food Chemistry* 50, no. 12 (2002): 3444–3452.

90. M. Yamauchi, Y. Kitamura, H. Nagano, J. Kawatsu, and H. Gotoh, "DPPH Measurements and Structure—Activity Relationship Studies on the Antioxidant Capacity of Phenols," *Antioxidants* 13, no. 3 (2024): 309, https://doi.org/10.3390/antiox13030309.

91. S. Mathew, T. E. Abraham, and Z. A. Zakaria, "Reactivity of Phenolic Compounds Towards Free Radicals Under in Vitro Conditions," *Journal of Food Science Technology* 52, no. 9 (2015): 5790–5798, https://doi.org/10.1007/s13197-014-1704-0.

92. T. Joshi, P. R. Deepa, and P. K. Sharma, "Effect of Different Proportions of Phenolics on Antioxidant Potential: Pointers for Bioactive Synergy/Antagonism in Foods and Nutraceuticals," *Processing Natural Academic Science India Section B Biology Science* 92, no. 4 (2022): 939–946, https://doi.org/10.1007/s40011-022-01396-6.

93. N. Yahfoufi, N. Alsadi, M. Jambi, and C. Matar, "The Immunomodulatory and Anti-inflammatory Role of Polyphenols," *Nutrients* 10, no. 11 (2018): 1618, https://doi.org/10.3390/nu10111618.

94. C. A. Ferreira, D. Ni, Z. T. Rosenkrans, and W. Cai, "Scavenging of Reactive Oxygen and Nitrogen Species with Nanomaterials," *Nano Research* 11, no. 10 (2018): 4955–4984, https://doi.org/10.1007/s12274-018-2092-y.

95. L. S. Borquaye, M. K. Laryea, E. N. Gasu, et al., "Anti-Inflammatory and Antioxidant Activities of Extracts of Reissantia indica, Cissus cornifolia and Grosseria Vignei," *Cogent Biology* 6, no. 1 (2020): 1785755, https://doi.org/10.1080/23312025.2020.1785755.

96. G. van Loo and M. J. M. Bertrand, "Death by TNF: a Road to Inflammation," *Nature Reviews Immunology* 23, no. 5 (2023): 289–303, https://doi.org/10.1038/s41577-022-00792-3.

97. D. I. Jang, A. H. Lee, H. Y. Shin, et al., "The Role of Tumor Necrosis Factor Alpha (TNF-α) in Autoimmune Disease and Current TNF-α Inhibitors in Therapeutics," *International Journal of Molecular Sciences* 22, no. 5 (2021): 2719, https://doi.org/10.3390/ijms22052719.

98. J. R. Bradley, "TNF-mediated Inflammatory Disease," *The Journal of Pathology* 214, no. 2 (2008): 149–160, https://doi.org/10.1002/path.2287.

99. C. Popa, M. G. Netea, P. L. C. M. van Riel, J. W. M. van der Meer, and A. F. H. Stalenhoef, "The Role of TNF-α in Chronic Inflammatory Conditions, Intermediary Metabolism, and Cardiovascular Risk," *Journal of Lipid Research* 48, no. 4 (2007): 751–762, https://doi.org/10.1194/jlr.r600021-jlr200.

100. H. Ye, Y. Wang, A. Bennett Jenson, and J. Yan, "Identification of Inflammatory Factor TNFα Inhibitor from Medicinal Herbs," *Experimental and Molecular Pathology* 100, no. 2 (2016): 307–311, https://doi.org/10.1016/j.yexmp.2015.12.014.

101. P. R. Campana, D. S. Mansur, G. S. Gusman, D. Ferreira, M. M. Teixeira, and F. C. Braga, "Anti-TNF-α Activity of Brazilian Medicinal Plants and Compounds from Ouratea semiserrata," *Phytotherapy Research* 29, no. 10 (2015): 1509–1515, https://doi.org/10.1002/ptr.5401.

102. S. Arora, P. Rushiya, K. Tirpude, et al., "Exploring the Phytoconstituents Targeting TNF-α as Potential Lead Compounds to Treat Inflammatory Diseases: an in-silico Approach," *Digital Chinese Medicine* 5, no. 3 (2022): 264–275, https://doi.org/10.1016/j.dcmed.2022.10.003.

103. S. H. Lee, J. Y. Kwon, S.-Y. Kim, K. Jung, and M.-L. Cho, "Interferon-Gamma Regulates Inflammatory Cell Death by Targeting Necroptosis in Experimental Autoimmune Arthritis," *Scientific Reports* 7, no. 1 (2017): 10133, https://doi.org/10.1038/s41598-017-09767-0.

104. B. Verstockt, A. Salas, B. E. Sands, et al., "IL-12 and IL-23 Pathway Inhibition in Inflammatory Bowel Disease," *Nature Reviews Gastroenterology & Hepatology* 20, no. 7 (2023): 433–446, https://doi.org/10.1038/s41575-023-00768-1.

105. E. N. Benveniste, "Cytokines," in *Encyclopedia of the Neurological Sciences*, 2nd ed., ed. M. J. Aminoff and R. B. Daroff (Oxford: Academic Press, 2014), 921–925.