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Australian Native Lemongrass (*Cymbopogon ambiguus* A. Camus): An Underestimated Herbal Plant

Yuntao Zhou¹, Saleha Akter^{1,*} , Anh Dao Thi Phan¹ , Eshetu Mulisa Bobasa¹, Maral Seididamyeh¹ , Dharini Sivakumar^{1,2}  and Yasmina Sultanbawa^{1,*} 

- ¹ ARC Industrial Transformation Training Centre for Uniquely Australian Foods, Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, Indooroopilly, QLD 4068, Australia; yuntao.zhou@uq.net.au (Y.Z.); a.phan1@uq.edu.au (A.D.T.P.); e.bobasa@uq.edu.au (E.M.B.); s.maral@uq.edu.au (M.S.); d.sivakumar@uq.edu.au (D.S.)
- ² Phytochemical Food Network, Department of Crop Sciences, Tshwane University of Technology, Pretoria 0183, South Africa
- * Correspondence: saleha.akter@uq.edu.au (S.A.); y.sultanbawa@uq.edu.au (Y.S.); Tel.: +61-7-3443-2474 (S.A.); +61-7-3443-2471 (Y.S.)

Abstract: Lemongrass (genus *Cymbopogon*) is commonly used in foods, beverages, cosmetics, pharmaceuticals, and material science. *Cymbopogon ambiguus* A. Camus, the Australian Native Lemongrass, is a lesser-known member of the genus *Cymbopogon*, and research on this plant is scarce. Australian Indigenous people use the stalks and leaves of *C. ambiguus* as teas. Dried chopped leaves are also used as herbs in cooking. The aim of this study was to determine the proximate composition and bioactive properties of Australian native lemongrass (*C. ambiguus*). Antimicrobial capacity was carried out using the well diffusion method, antioxidant capacity by the FRAP method, and antidiabetic capacity by using the α -glucosidase inhibitory activity assay. The results obtained in the current study were compared with previously published literature on lemongrass (*C. citratus*). The results showed that *C. ambiguus* has lower fat and protein content and lower antioxidant and antimicrobial capacities than *C. citratus*, but it is very rich in fibre (67.55%) and has strong α -glucosidase inhibitory capacity. The total phenolic and total flavonoid content determined in the aqueous extract of *C. ambiguus* are also notable. The results of the present study showed that Australian native lemongrass has promising bioactive potential to be used as an alternative native herbal tea.

Keywords: native aromatic grass; antioxidant properties; antimicrobial properties; ascorbic acid; citral; antidiabetic activity; phytochemicals



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1. Introduction

Cymbopogon ambiguus A. Camus (Australian native aromatic grass) (Figure 1), is an understudied and widely distributed Australian native herb. The plant is also named Marr by the Nyul Nyul people. *C. ambiguus* grows abundantly throughout warmer Australian climates in Central Australia, Western Australia, the Northern Territory and Northern Queensland of Australia. Figure 1 presents the distribution of *C. ambiguus* in Australia. Its leaves release a strong, lemony aroma when crushed. Stalks and leaves of *C. ambiguus* are traditionally used as teas by Australian Indigenous people. Also, as a medical herb, native Australians use the leaves and roots of *C. ambiguus* in combination with hot water as a steam inhalation remedy for colds and tightness in the chest. Other usages include the treatment of chest infections, muscle cramps and headaches [1]. *C. ambiguus* belongs to the genus *Cymbopogon*, subfamily *Panicoideae* of the family *Poaceae*. The *Poaceae* family has several important meanings to humans, in addition to buckwheat (*Polygonaceae*) and quinoa (*Amaranthaceae*), almost all foods for humans and livestock are *Poaceae*, such as wheat, corn, barley, sorghum, etc. [2]. The genus *Cymbopogon* is called differently around the world, including lemongrass, barbed wire grass, silky heads, citronella grass, cha de

Dartigalongue, fever grass, tanglad, serai, hierba Luisa, or gavati chahapati [3]. There are more than 140 cultivated species, 52 of which are grown in Africa, 42 in India, 2 in North America, 4 in Europe, 6 in South America and Australia, and the rest in South Asia [4]. The discovered *Cymbopogon* contains 144 species, including *Cymbopogon nardus* (L.) Rendle (*C. nardus*), *Cymbopogon citratus* (DC.) Stapf (*C. citratus*), *Cymbopogon giganteus* Chiov (*C. giganteus*), *Cymbopogon flexuosus* (Nees ex Steud.) W. Watson (*C. flexuosus*), *Cymbopogon martini* (Roxb.) W. Watson (*C. martinii*), etc. [5].

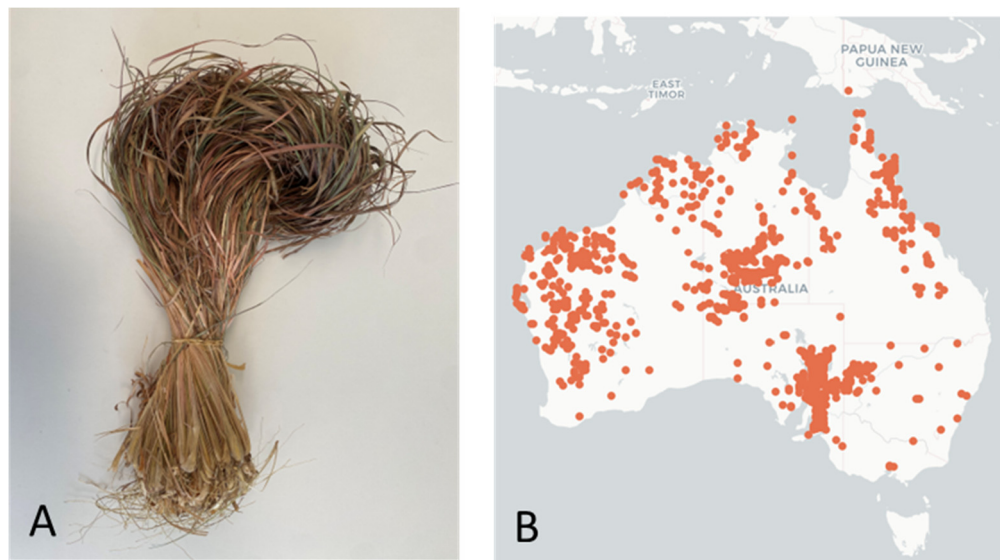


Figure 1. (A) The dried Australian native lemongrass (*Cymbopogon ambiguus* A. Camus) and (B) distribution of *Cymbopogon ambiguus* A. Camus in Australia. The red dots indicate the distribution of *C. ambiguus* in Australia. (Image collected from Atlas of Living Australia website. <https://bie.ala.org.au/species/https://id.biodiversity.org.au/node/apni/2901878> (accessed on 12 May 2023).

Genus *Cymbopogon* has attracted the attention of researchers because of its high economic value and strong environmental adaptability. It has been found that lemongrass has antioxidant and free radical scavenging capabilities and the potential to be used as an excellent antioxidant in the food industry [6]. Irfan et al. found that the total phenolic content in different solvent extracts of *C. citratus* from Islamabad, Pakistan, ranged from 50–60 mg GAE/g, with 50% ethanol extract showing the highest values (61.2 mg GAE/g) [7]. Other species of *Cymbopogon* mentioned in the literature showed a basically lower total phenolic content than *C. citratus*, such as the 50% methanol extract of *C. flexuosus* from Vietnam, which showed a total phenolic content of 8.36 mg GAE/g [8]; the essential oil of *C. martinii* from Colombia contains 13.6 mg GAE/g of phenolic compounds [7]. Rao et al. (2009) reported the ability of the hydroalcoholic extract of *C. citratus* to inhibit different free radicals [9]. A report from Saudi Arabia showed that the 50% ethanol extract of *C. citratus* showed higher scavenging ability than the water extract in terms of DPPH free radical scavenging ability [10]. Bhatnagar reported in 2020 that *C. flexuosus* showed a high DPPH radical scavenging capacity (78.19%) at a concentration of 150 µg/mL [11]. From these results, it can be concluded that *C. citratus* is basically the strongest in the *Cymbopogon* family in terms of antioxidant capacity.

Except for the antioxidant capacity, the *Cymbopogon* genus has also been found to have antimicrobial and anticancer capacities [12–19]. Because of the multi-drug resistance of microorganisms to antibiotics, research on natural antibacterial agents has attracted widespread attention in academic circles [20]. In 2020, Subramaniam et al. determined the antibacterial capacity of *C. citratus* using agar diffusion assay. Thirteen Gram-positive and 9 Gram-negative bacteria were used to test the essential oil and methanal extracts of

C. citratus leaves and roots [21]. The results indicated that the essential oil of *C. citratus* showed a strong inhibition zone against Gram-positive bacteria (from 20–40 mm) and showed a moderate inhibition zone against Gram-negative bacteria (from 10–15 mm). The methanol extracts showed a moderate inhibition zone against Gram-positive and negative bacteria, both from 10–15 mm [21]. Existing reports also prove that other members of the *Cymbopogon* family, such as *C. giganteus*, *C. flexuosus*, *C. martinii*, etc., have different degrees of inhibitory effects on different bacteria [22–24]. Lemongrass has also been found to have anticancer properties [10,16,17,25–27]. As can be seen, the genus *Cymbopogon* is a kind of plant with sufficient nutritional and functional potential, so the aim of this project is to access the nutritional and functional properties of Australian native lemongrass (*Cymbopogon ambiguus* A. Camus). Additionally, contrast these properties with normal lemongrass (*Cymbopogon citratus*) and estimate whether *C. ambiguus* can benefit the small and medium-sized indigenous food industries. Therefore, the present study has been designed to investigate the nutritional composition, total phenolic contents, total flavonoid contents, and antioxidant activity by using the ferric-reducing antioxidant power of plasma (FRAP) assay. The citral content and the bioactive properties, such as antimicrobial and antidiabetic properties, have also been investigated in the current study.

2. Materials and Methods

2.1. Sample and Extract Preparation

The dried Australian native lemongrass (*Cymbopogon ambiguus* A. Camus) was collected from the Indigenous partners (Marion Dann and Bruno Dann) from Twin Lakes Cultural Park, Western Australia. Approximately 300 g of sample (including leaves, stems, and roots) was shredded to obtain fragments with a length between 2 and 5 cm and was freeze-dried, a part of which was separately coarsely and finely ground and stored separately at $-30\text{ }^{\circ}\text{C}$ for further analysis. Altogether, three sets of freeze-dried samples were obtained, such as short fragments, fine powder, and coarsely grounded particles. All three sets of samples were extracted with water and 80% aqueous acidified methanol (for 100 mL solvent = 80 mL methanol (100%) + 19.8 mL RO water + 0.2 mL conc. HCl) at a pH of 1.76. They were used in the study and were named LGW (Australian native lemongrass short fragments aqueous extracts), LGM (Australian native lemongrass short fragments 80% aqueous acidified methanolic extracts), LGPW (Australian native lemongrass powder aqueous extracts), LGPM (Australian native lemongrass powder 80% aqueous acidified methanolic extracts), LMGW (Australian native lemongrass coarsely ground particles aqueous extracts), and LMGM (Australian native lemongrass coarsely ground particles methanolic extracts).

2.2. Chemicals, Reagents and Tested Microorganisms

Iron tripyridyl triazine complexes, sodium phosphate dibasic heptahydrate, sodium phosphate monobasic monohydrate, metaphosphoric acid, gallic acid, ferrous sulphate, dimethyl sulfoxide (DMSO), acetic acid, formic acid, citral (geranial and neral mixture) purity $\geq 96\%$, quercetin, hydrochloric acid, p-nitrophenyl- α -D-glucopyranoside (pNPG), α -glucosidase, acarbose, organic solvents (HPLC-grade), Kjeltabs Cu 3.5 g (catalyst), diethyl ether, and other reagents used throughout the study were of analytical grade and were supplied by Merck Life Science (Sydney, NSW, Australia).

Staphylococcus aureus (NCTC 6571) and *Escherichia coli* (NCTC 9001) were collected from the National Collection of Type Cultures (NCTC, Health Protection Agency Center for Infection, London, UK) and *Candida albicans* (ATCC 10231) from the American Type Culture Collection (ATCC, In Vitro Technologies Pty, Ltd., Noble Park, Melbourne, VIC, Australia).

2.3. Nutritional Composition Analysis

The moisture content and dry matter were measured following the AOAC method 934.01 and the ash analysis according to the AOAC method 942.05 [28]. The protein content was determined using the Kjeldahl method following the AOAC method 990.03 [29]. The

fat content analysis was performed using a standard Soxhlet extraction method following the AOAC method 991.36 [30]. The dietary fiber content was detected following AOAC Method 991.43 and AACC Method 32-07.01 [31].

2.4. Determination of Total Phenolic Content (TPC) and Total Flavonoid Content (TFC)

Short fragments and finely grounded samples were further extracted using different solvents and used in the current study. The water and 80% aqueous acidified methanol extracts required for the determination of total phenolic content were prepared by following the methods previously described with some modifications [32,33]. The total phenolic content (TPC) was determined using a Folin–Ciocalteu assay following the method reported previously [34] using a micro-plate absorbance reader, Varioskan LUX (Thermo Fisher Scientific, Singapore), monitored at 700 nm. An external calibration curve of gallic acid (21–105 mg/L) was prepared to quantify TPC in both extracts. TPC was expressed as mg gallic acid equivalents (GAE) per gram samples in dry weight.

Determination of total flavonoid content (TFC) was performed following a previously published method [35]. TFC was measured using a spectrophotometric method employing a micro-plate absorbance reader, Varioskan LUX (Thermo Fisher Scientific, Singapore), at a wavelength of 415 nm. The extract was used as a corresponding blank to eliminate the influence of the interferences from the extract itself. An external standard curve of quercetin was prepared using six different concentrations (12.5, 25, 50, 75, 100, and 150 mg/L). Results were expressed as milligrams of quercetin equivalent per gram dry weight (mg QE/gDW).

2.5. Determination of Ferric-Reducing Antioxidant Power of Plasma (FRAP Assay)

The total antioxidant capacity of the extracts was determined using the FRAP assay [36]. An external calibration curve of ferrous sulfate (ranged 0.1–1 mmole/L) was used to quantify the ferric-reducing antioxidant power. The absorbance was measured at 593 nm using a Varioskan LUX micro-plate reader. The analysis was conducted in triplicate, and the results were expressed as micromoles of Fe^{2+} per gram of sample on a dry weight basis ($\mu\text{mole Fe}^{2+}/\text{g DW}$).

2.6. Determination of Citral Content

Water and methanol extracts of Australian native lemongrass were used to determine the citral content. The HPLC-PDA method was used to determine citral content in extracts using the instrumental method described previously [37]. A reverse-phase Waters[®] HSS-T3 column (150 mm \times 2.1 mm i.d.; 1.8 μm , Waters, Sydney, NSW, Australia), maintained at 25 °C, was used to separate the compounds with mobile phases consisting of 0.1% formic acid in Milli-Q water (mobile phase A) and 0.1% formic acid in acetonitrile (mobile phase B). The gradient program applied at the flow rate of 0.2 mL/min was as follows: 5% B—1 min, 70% B—4.3 min, isocratic elution at 70% B until 8 min, 95% B—10 min, isocratic at 95% B until 12 min, and ramped back to the original condition at 5% B for 4 min before the next injection. The injection volume was 2 μL . The sample extracts were scanned at a UV wavelength ranging from 200 to 400 nm, and the absorbance value of citral at 233 nm (maxima absorbance) was extracted for data analysis using Chromeleon CDS ver 7.2 software (Thermo Fisher Scientific, Brisbane, QLD, Australia). External calibration curves of citral standard, consisting of 2 isomers: neral and geranial, were prepared in methanol at different concentrations ranging from 21.49 to 1074.5 mg/L. The linear equations obtained for the first peak assigned to neral citral ($y = 0.2309x - 0.5152$, $r^2 = 0.9986$) and the second peak, geranial citral ($y = 0.1872x - 0.5809$, $r^2 = 0.9987$) were used to quantify the level of neral citral and geranial citral in the sample extracts, respectively. In addition, the total citral level of the extracts was also calculated as the sum of the amount of neral and geranial.

2.7. Determination of Antimicrobial Assay

Agar well-diffusion assay was used for screening the antimicrobial activity of the samples [38] against selected food-related microorganisms, including *Staphylococcus aureus* (NCTC 6571), *Escherichia coli* (NCTC 9001), and *Candida albicans* (ATCC 10231). The prepared extracts described in Section 2.4 were evaporated under nitrogen flow, which was followed by freeze-drying ($-50\text{ }^{\circ}\text{C}$, 0.04 mbar). The extract solutions (100 mg/mL) were prepared in 20% aqueous dimethyl sulfoxide (DMSO). Streptomycin (20 $\mu\text{g/mL}$) was used as the positive control for *S. aureus* and *E. coli* and 20% DMSO as a negative control. A digital caliper ($\pm 0.01\text{ mm}$) was used to measure the diameter (mm) of the inhibition zones and subtracted from the well diameter. The criteria used to determine the inhibitory area are $<8\text{ mm}$ not sensitive, 9–14 mm sensitive, 15–19 mm very sensitive, and $>20\text{ mm}$ extremely sensitive [39]. All experiments were carried out in triplicate, and results are expressed as the mean \pm standard deviation (SD).

2.8. Determination of Antidiabetic Capacity

The antidiabetic capacity of samples was measured by the inhibition of α -glucosidase activity following the method described previously [40]. The α -glucosidase activity was determined by measuring the yellow-coloured para-nitrophenol released from p-nitrophenyl- α -D-glucopyranoside (pNPG) at 405 nm using a micro-plate reader Varioskan LUX (Thermo Fisher Scientific, Singapore). An external acarbose standard solution was prepared using six different concentrations (1, 0.5, 0.25, 0.125, 0.0625, and 0.03125 mg/mL) as a positive control/standard. The analysis was conducted in triplicate, and the results were expressed as IC_{50} values (mg/mL). The IC_{50} value refers to the concentration of the extract or standard acarbose that is required to inhibit 50 percent of the enzyme in the reaction mixture. The lower the IC_{50} value, the higher the enzymatic inhibition, and if the IC_{50} value is higher, the corresponding enzymatic inhibition capacity is lower.

2.9. Statistical Analyses

Statistical analysis of the data and the graphs were created using GraphPad Prism version 9 (San Diego, CA, USA). One-way ANOVA was performed, and values ≤ 0.05 were considered significant.

3. Results

3.1. Nutritional Composition

The nutritional composition of the Australian native lemongrass (*C. ambiguus*) has been presented in Table 1. It can be found that the moisture content, ash content, fat content, and protein content of *C. ambiguus* are all below 10%, and only the fiber content value reaches a staggering 67.55%. Published literature on the proximate composition of *C. citratus* reported that the moisture content (%) ranged from 1.67–13.00 and the ash content (%) ranged from 4.83–7.63 [7,41–43]. The moisture and ash content of Australian native lemongrass from the current study is comparable to the published reports. Published literature on *C. citratus* reported that fat content (%) ranged from 2.23–6.67, protein content (%) ranged from 3.82–22.59, and fibre content (%) ranged from 20.61–37.53 [7,41–43]. Compared to the current study results, it can be stated that the fat content of Australian native lemongrass is similar to or higher than the published reports, while the protein content is lower than the published reports. However, the fibre content is very high compared to the published literature.

Table 1. Nutritional composition of Australian native lemongrass *C. ambiguus*.

Proximate Composition	Australian Native Lemongrass <i>C. ambiguus</i>
Moisture (%)	4.74 \pm 0.10
Ash (%)	4.32 \pm 0.22
Fat (%)	6.79 \pm 0.01
Protein (%)	3.30 \pm 0.07
Fibre (%)	67.55 \pm 0.10

Data are presented as mean \pm SD of three replicates.

3.2. Phenolic Content and Antioxidant Properties

3.2.1. Total Phenolic Content

The total phenolic content of extracts of LGW, LGM, LGPW, and LGPM is presented in Figure 2. The LGW (1.50 ± 0.17 mg GAE/g DW) and LGM (1.55 ± 0.27 mg GAE/g DW) showed similar total phenolic contents. The TPC of LGPW (5.96 ± 0.25 mg GAE/g DW) is lower than LGPM (8.17 ± 0.25 mg GAE/g DW). In both extraction solvents, the TPC content of the powders was higher than that of the lemongrass fragments. The extraction solvents did not affect the total phenolic content of the fragments. However, organic solvent increased the TPC contents in the powder. The aqueous extract of *C. citratus* contained a total phenolic content of 32.1 mg GAE/g DW [44], which is much higher than the TPC content of LGPW (5.96 ± 0.25 mg GAE/g DW) obtained from the current study. Moreover, 50% acidified methanol extract of *C. citratus* has been reported to contain TPC of 3.02 to 2.12 mg GAE/100 g DW, and the TPC content of methanol extract of *C. citratus* is 132.5 mg GAE/g [43,45].

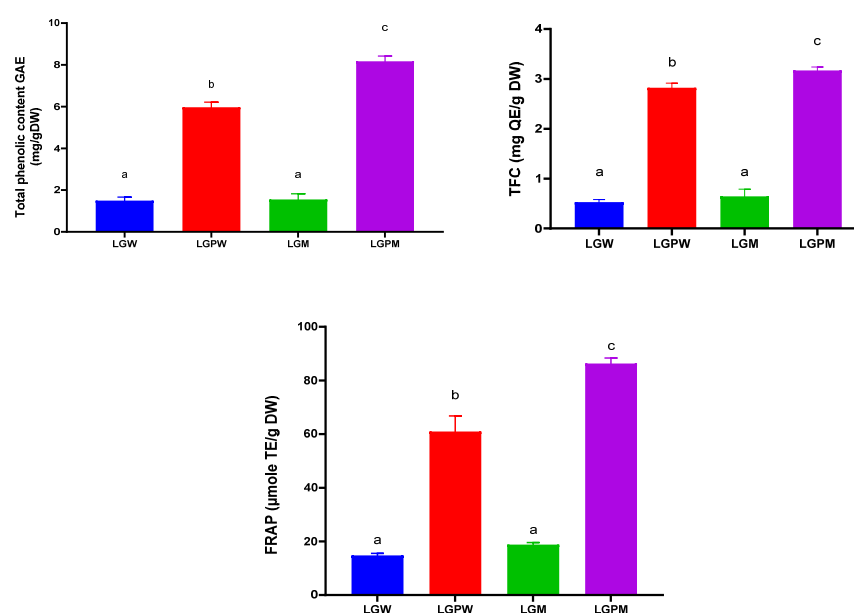


Figure 2. Total phenolic content, total flavonoid content, and ferric-reducing antioxidant power of plasma (FRAP) of the aqueous and 80% aqueous acidified methanolic extracts of *C. ambiguus* short fragments and powder. Data are presented as mean \pm SD ($n = 3$); different letters are significantly different ($p \leq 0.05$). LGW—Australian native lemongrass short fragments aqueous extracts, LGM—Australian native lemongrass short fragments 80% aqueous acidified methanolic extracts, LGPW—Australian native lemongrass powder aqueous extracts, and LGPM—Australian native lemongrass powder 80% aqueous acidified methanolic extracts.

3.2.2. Total Flavonoid Content (TFC)

Figure 2 shows the total flavonoid content of LGW, LGM, LGPW, and LGPM. The LGW (0.53 ± 0.05 mg QE/g DW) and LGM (0.64 ± 0.14 mg QE/g DW) showed similar total flavonoid content. The TFC of LGPW is 2.82 ± 0.09 mg GAE/g DW, while the TPC of LGPM is 3.17 ± 0.07 mg GAE/g DW. The TFC content in the powder extracts (extract from finely grounded integrated lemongrass) was also higher than that of the fragments' extracts (extract from lemongrass cut into pieces), similar to the TPC content. For total flavonoids, the water extract of *C. citratus* was reported to contain TFC of 14.6 mg QE/g DW [44], and the 80% aqueous acidified methanol extract of *C. citratus* contained 18 ± 0.25 to 86 ± 3.00 mg QE/g DW [46]. However, another study reported that an 80% aqueous acidified methanol extract of *C. citratus* contained 0.046 ± 0.003 mg QE/g DW of TFC [47]. Considering the variations of the results among the published literature, it can be inferred that *C. ambiguus* is a potential source of bioactive compounds (phenolics and flavonoids),

and the results obtained from the current study also provide useful information on the potential use of this native lemongrass for health promotion.

3.2.3. Antioxidant Properties—Ferric-Reducing Antioxidant Power of Plasma (FRAP)

The antioxidant activity (FRAP) of LGW, LGM, LGPW, and LGPM is presented in Figure 2. The LGW and LGPW showed ferric-reducing capacities of 14.81 ± 0.74 and $60.95 \pm 5.84 \mu\text{mole Fe}^{2+}/\text{g DW}$, respectively, while the LGM and LGPM show 18.80 ± 0.74 and $86.33 \pm 2.06 \mu\text{mole Fe}^{2+}/\text{g DW}$ of ferric-reducing capacity. It is evident from the data that the powder extracts (LGPW and LGPM) have higher TPC, TFC, total citral, and FRAP activity compared to leaves (LGW and LGM). In addition to that, LGPM exhibited higher TPC, TFC, total citral, and FRAP activity than LGPW. The total phenolic content showed an extremely strong correlation with the ferric-reducing antioxidant power of plasma ($r^2 = 99.9\%$), and the total flavonoid content also showed a high correlation with the ferric-reducing antioxidant power of plasma ($r^2 = 97.9\%$). The correlation between the total citral content and the ferric-reducing antioxidant power of plasma ($r^2 = 85.7\%$) was moderate. The results indicated that the phenolic compounds in the tested samples contributed to the antioxidant capacity of the samples. In terms of the antioxidant capacity, compared to the FRAP activity of LGPM ($86.33 \pm 2.06 \mu\text{mole Fe}^{2+}/\text{g DW}$) from the current study, a study published by Ng et al. in 2020 performed FRAP tests on different parts of *C. citratus* tissues with results ranging from 1.3–5.06 mmol $\text{Fe}^{2+}/100 \text{ g DW}$ [48]. From the results, it can be seen that the antioxidant capacity of *C. ambiguus* is weaker than that of *C. citratus*.

3.3. Citral Content

Citral is a mixture of two stereoisomeric monoterpene aldehydes, geranial and neral. Citral appears as a light-yellow liquid that is insoluble in water while emitting a strong lemon aroma [49]. The citral content of LMGW, LMGW, and LGPM is shown in Figure 3. The neral citral accounts for 40.29%, while the geranial citral accounts for 59.71%. It can be seen from Figure 3 that, compared to the methanol extract, the citral content of the water extract is much lower ($52.64 \pm 0.01 \text{ mg}/100 \text{ g DW}$ of neral and $70.78 \pm 0.11 \text{ mg}/100 \text{ g DW}$ of geranial), which may be because citral, as an organic compound, is easily soluble in organic solvents and not soluble in water [49]. Among them, the lemongrass coarsely ground methanol extract showed the highest content of citral, which was $701.73 \pm 7.01 \text{ mg}/100 \text{ g DW}$ (Neral) and $833.83 \pm 4.39 \text{ mg}/100 \text{ g DW}$ (Geranial), while the lemongrass powder methanol extract accounted for $396.42 \pm 2.74 \text{ mg}/100 \text{ g DW}$ (Neral) and $637.23 \pm 2.17 \text{ mg}/100 \text{ g DW}$ (Geranial). Except for these three types of extraction, LGW and LGPW have also been used to test the total citral content. However, the levels were too low; therefore, they were not presented in Figure 3, which were $1.59 \pm 0.01 \text{ mg}/100 \text{ g DW}$ for LGW and $6.28 \pm 0.58 \text{ mg}/100 \text{ g DW}$ for LGPW.

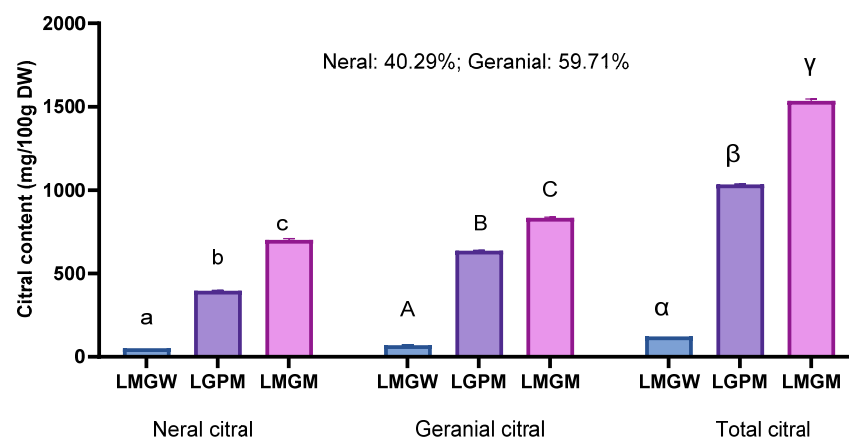


Figure 3. Citral content of the aqueous and methanolic extracts of *C. ambiguus* coarsely grounded fragments, compared with methanolic extract of Australian native lemongrass powder; data are

presented as mean \pm SD (n = 3); data with different letters are significantly different ($p \leq 0.05$). LMGW—Australian native lemongrass coarsely ground particles aqueous extracts, LGPM—Australian native lemongrass powder 80% aqueous acidified methanolic extracts, and LMGM—Australian native lemongrass coarsely ground particles methanolic extracts.

3.4. Antimicrobial Assay

The antimicrobial capacity of LGW, LGM, LGPW, and LGPM is shown in Table 2. LGW and LGPW showed no inhibitory activity against *S. aureus*, *E. coli*, or *C. albicans*, while none of the four extracts showed inhibitory activity against *C. albicans*. LGM showed low inhibitory activity against *S. aureus* and *E. coli*, which was significantly less than streptomycin. It is worth mentioning that, whether it is *S. aureus* or *E. coli*, LGM showed a stronger inhibitory effect than LGPM. It may indicate that the particle size affects the extraction of bioactive compounds, and grinding can release the readily available volatile compounds that might be on the surface of leaves. However, further histochemical studies of the leaves are required to better understand the effect of particle size as well as the strength of the antimicrobial properties using different solvents for extraction.

Table 2. Inhibition diameters of different extracts of *C. ambiguus* against selected microorganisms.

Microorganism	Zone of Inhibition (mm)				
	LGW	LPW	LGM	LGPM	Streptomycin
<i>Staphylococcus aureus</i>	-	-	2.97 \pm 0.33 ^a	2.02 \pm 0.08 ^b	11.58 \pm 0.33 ^c
<i>Escherichia coli</i>	-	-	8.64 \pm 0.57 ^a	4.70 \pm 1.14 ^b	16.09 \pm 0.06 ^c
<i>Candida albicans</i>	-	-	-	-	-

(-) means no inhibition; different letters in the same row mean significant differences ($p \leq 0.05$) in the inhibition diameter produced by the extract of *C. ambiguus* at a dose of 100 mg/mL. (Mean and standard error in mm). LGW—Australian native lemongrass short fragments aqueous extracts, LGM—Australian native lemongrass short fragments 80% aqueous acidified methanolic extracts, LGPW—Australian native lemongrass powder aqueous extracts, and LGPM—Australian native lemongrass powder 80% aqueous acidified methanolic extracts.

From the antimicrobial test results, LGW, LGPW, LGM, and LGPM have no inhibitory ability against *C. albicans*, but LGM and LGPM have shown a mild inhibitory ability against *S. aureus* and *E. coli*, and the inhibitory activity towards *E. coli* is higher than that of *S. aureus*. Being a Gram-negative bacteria, generally, *E. coli* is more resistant to extract treatment due to having more complex cell membranes [50]. As the native lemongrass extract was able to inhibit the growth of *E. coli*, further research on the antimicrobial potential of the extract by including more Gram-negative bacteria and determining the minimum inhibitory concentration (MIC) would provide valuable information on the antimicrobial potential of this plant. There are reports on the inhibitory ability of a methanolic extract of *C. citratus* at a concentration of 100 mg/mL, which had an inhibitory area of 23 \pm 0.523 mm for *E. coli* and 14.5 \pm 0.816 mm for *S. aureus* [51]. However, Bassolé et al. (2011) reported that the inhibition range of *C. citratus* against *S. aureus* was 24.3 \pm 0.4 mm, while the inhibition range of *E. coli* was 15.3 \pm 1.1 mm [52]. This result is more in line with the characteristics of Gram-positive and Gram-negative bacteria. Moreover, Kausar et al. (2017) reported that the extract of *C. citratus* had a moderate inhibitory ability against *C. albicans* at a concentration of 100 mg/mL, and the inhibition range was 6.87 \pm 0.8 mm [53]. It is also reported that the aqueous extract of *C. citratus* has an inhibitory effect on *S. aureus* that exceeds 30 mm, while the inhibitory ability of *E. coli* was 25 mm [54]. The compared results indicated that the Australian native lemongrass (*C. ambiguus*) has antimicrobial potential; however, the activity is lower than that of *C. citratus* in inhibiting different pathogens.

3.5. Antidiabetic Assay

Inhibition of carbohydrate-digesting enzymes in the gastrointestinal tract, such as α -glucosidase, is considered to be one of the most effective strategies for treating diabetes [55]. In the α -glucosidase activity inhibition experiment, a lower IC₅₀ value represents a higher

α -glucosidase inhibitory activity [56]. Table 3 shows the inhibition of α -glucosidase activity by the LGW, LGPW, LGM, and LGPM. All the tested samples showed stronger inhibition on α -glucosidase activity (lower IC_{50} values) than acarbose, which is between 0.16 and 0.27 mg/mL. However, LGW (0.18 ± 0.05 mg/mL), LGPW (0.27 ± 0.14 mg/mL), LGM (0.17 ± 0.04 mg/mL), and LGPM (0.16 ± 0.09 mg/mL) showed stronger inhibition ability than the acarbose (standard). But the results for water extracts did not differ significantly in inhibition activity. However, LGPM extracts showed significantly higher α -glucosidase activity than acarbose (standard). Compared to the study mentioned in Wang et al. in 2022, the inhibition ability of *C. citratus* methanol extract against α -glucosidase was 7.90 ± 0.55 μ g/mL [57], and it seems that the α -glucosidase inhibition ability of *C. ambiguus* is much stronger than that of *C. citratus*. Proença et al. (2022) reported that flavonoids, such as geraldone and luteolin, both showed good α -glucosidase inhibitory ability [58], but the content of flavonoids obtained in the current study did not show any correlation with α -glucosidase inhibition activity ($r^2 = 32.1\%$). However, α -glucosidase can hydrolyze the terminal glycosidic bond to release glucose, and glycosidic bonds are also abundant structures in the flavonoids reported in natural plants [59]. Though the current study is preliminary in nature, future research needs to be designed to characterize the flavonoids and glycosidic bonds in *C. ambiguus* to derive compounds specific to α -glucosidase inhibition.

Table 3. IC_{50} values obtained in α -glucosidase activity inhibition assay.

Samples	IC_{50} α -glucosidase (mg/mL)
Acarbose (standard)	0.38 ± 0.02 ^a
LGW	0.18 ± 0.05 ^{ab}
LGPW	0.27 ± 0.14 ^{ab}
LGM	0.17 ± 0.04 ^{ab}
LGPM	0.16 ± 0.09 ^b

Results are expressed as mean \pm SD (n = 6); different letters in the same row indicate significant differences of different samples' inhibitory activity on α -glucosidase compared to acarbose at the level $p \leq 0.05$. LGW—Australian native lemongrass short fragments aqueous extracts, LGM—Australian native lemongrass short fragments 80% aqueous acidified methanolic extracts, LGPW—Australian native lemongrass powder aqueous extracts, and LGPM—Australian native lemongrass powder 80% aqueous acidified methanolic extracts.

4. Conclusions

Preliminary determinations of the various nutritional and bioactive properties of Australian native lemongrass (*C. ambiguus*) have been reported in the current study. There are differences between the bioactive properties of Australian native lemongrass (*C. ambiguus*) obtained in the current study and the published reports on lemongrass (*C. citratus*). The result of the study suggests that the Australian native lemongrass has low antioxidant and antimicrobial properties compared to the lemongrass (*C. citratus*). However, the dietary fibre and fat content of the native lemongrass are high. In terms of α -glucosidase inhibition, *C. ambiguus* shows higher inhibitory ability than the standard acarbose, irrespective of the nature of the extracting solvents. Indigenous Australians have been using the native lemongrass tea for centuries and believe that the tea promotes health. The results of the current study have also indicated that the water extract and 80% aqueous acidified methanol extracts of Australian native lemongrass (*C. ambiguus*) contain similar levels of phenolic and flavonoid compounds. These results demonstrate the potential of *C. ambiguus* as a beneficial daily drink for humans. At the same time, its high dietary fibre content also reflects the potential inclusion of *C. ambiguus* in natural alternative herbal tea. Further research on the composition of the volatile oil and phytochemical profiling of the leaves will provide a scientific basis for the traditional use of the plant as tea and its potential to be used as an alternative herbal tea for the industry.

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