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Nutritional analysis, volatile composition, antimicrobial and antioxidant properties of Australian green ants (*Oecophylla smaragdina*)

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ABSTRACT

Edible green ants (*Oecophylla smaragdina*) are distributed in Asia, Australia, and the Pacific Islands, and are known for their pharmacological and nutritional applications, yet various properties are to be explored. Nutritional value, volatile compounds, antimicrobial and antioxidant potential were determined in four body regions [ant nest, whole ant, anterior part of the body, and gaster]. Proximate analysis revealed the anterior part have higher protein and fibre content, whilst fat content was higher in the gaster. GC–MS analysis revealed the complexity of ant nest with most compounds being organic acids, alcohols and alkanes. Antimicrobial activity was observed for whole ants (ZOI: 13.3 ± 0.8 mm) and anterior part (ZOI: 11.9 ± 0.5 mm) methanol extracted against *Staphylococcus aureus* (~104 CFU/ml). Whole ants methanol (813 ± 22.6 μg TROLOX eq/g of DW) and water (617.6 ± 59.2 μg TROLOX eq/g of DW) extracted exhibited higher free radical scavenging capacity amongst the four ant body regions. Whole ants water extracted (7 ± 0.4 mg GAE/g of DW) had the highest total phenolic content. In the methanol extracts, the gaster (6.2 ± 0.2 mg GAE/g of DW) exhibited the highest phenolic content. Whole ants exhibit good antioxidant and antimicrobial activity and considerable folate content.

1. Introduction

The world population is expected to reach 9.5 billion by 2050, thereby increasing the global demand for food and balanced diets (Meyer-Rochov, 1975; Kouřimská and Adámková, 2016; Sogari et al., 2019; Kim et al., 2019). Meanwhile, livestock farming is becoming more challenging due to deterioration of the environment (Leip et al., 2015; Kouřimská and Adámková, 2016; Kim et al., 2019); the livestock sector has been reported to produce up to 15% of the greenhouse gases in the environment (Kim et al., 2019; Baldini et al., 2020). Hence, alternative methods are being explored and utilised to meet the nutritional demands of the growing population and utilisation of edible insects is one amongst them. More than 2000 species of insects are being consumed worldwide by different ethnic groups as an essential part of their diets (Ramos-Elorduy, 2009; Kouřimská and Adámková, 2016; Kim et al., 2019). Insects, in general, are good sources of protein and other macro and micro nutrients (Chakravorty et al., 2016; Kouřimská and Adámková, 2016; Kim et al., 2019). Insects also exhibit better feed conversion rates and are considered ideal potential sources of protein in sustainable agriculture systems (Oonincx et al., 2015; Kouřimská and Adámková, 2016; Kim et al., 2019).

In recent years the Food and Agriculture Organization (FAO) has started promoting edible insects as a sustainable dietary alternative for humans (van Huis et al., 2013; Kim et al., 2019). Several reports indicated that by 2023 the edible-insect market might exceed USD 522 million (Han et al., 2017; Kim et al., 2019).

Edible ants (*O. smaragdina* Fabricius 1775), commonly referred to as weaver ants or green ants, are distributed in the tropical woodland

Abbreviations: ZOI, Zone of Inhibition; CFU, Colony forming unit; GAE, Gallic Acid equivalent; DW, Dry weight.

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regions of Southern Asia, Australia and several other Pacific Islands (Lokkers, 1986; Offenberg and Wiwatwitaya, 2010). They feed on a variety of natural vegetation and are harvested directly with their nest in the wild. Green ants (with their nests) are reported to be consumed by different populations across the globe for their inherent taste and flavour (Sribandit et al., 2008). In some parts of India these ants are used for treating a variety of health issues, such as dysentery, fever, etc. (Chakravorty et al., 2016; Kouřimská and Adámková, 2016; Kim et al., 2019). Indonesia and Thailand use these insects in restaurants and for developing pet feed (Offenberg and Wiwatwitaya, 2010; Kouřimská and Adámková, 2016; Kim et al., 2019).

Edible insects are suggested to be good sources of Vitamins B12, folate and C compared with plant and animal sources (Rumpold and Schlüter, 2013; Kim et al., 2019). The gaster extracts of weaver ants (*Oecophylla longinoda*) grown in Africa exhibit anti-microbial properties (Oladunmoye et al., 2018). Similarly, the "Australian bull" ants (*Myrmecia* spp.*)* and the mandibular gland secretions of *Calomyrmex* sp. (Australian Formicine ants - ants belonging to the *Calomyrmex* species are collectively called Australian Formicine ants as they belong to the family Formicidae) are also found to exhibit bactericidal properties (Brough, 1983; Veal et al., 1992; Kim et al., 2019).

Green ants in Australia are distributed in the Northern Territory, Kimberly region in Western Australia, and Gladstone in Queensland (Lokkers, 1986). These ants are consumed by the Australian Indigenous communities as part of their diet for many years (Peerzada et al., 1990). They are also commonly used to control the pests of cashew nuts in Australia (Peng et al., 2001). Green ants are used during the production of gin (Adelaide, South Australia) or added into goat cheese (e.g. "Anthill" commercially produced by the Woodside Cheese Wrights company in Adelaide Hills, South Australia) to bring a characteristic prominent acid flavour.

Despite the uptake and commercialisation of this insect for some food companies, the nutritional profile and other properties of the Australian green ants are yet to be explored. Therefore, the objective of this study was to evaluate the nutritional value (proximate analysis), the volatile composition, folate content as well as the antimicrobial and antioxidant properties of different green ant body regions as a potential source of food ingredients.

2. Materials and methods

2.1. Samples and sample processing

Approximately 1 kg (fresh weight) of green ants which contained part of the nest debris were obtained from a commercial supplier 'Something Wild' (Something wild Australia, Edwardstown, SA 5039, Australia). The supplier sourced the green ants from the *Larrakia* people who collected the ants from the area of Darwin (Northern Territory, Australia). The samples were sorted into four different body regions namely ant nest (includes whole ants with twigs and leaves), whole ants, anterior part of the body (including thorax, head and legs), and ant gaster, using tweezers under a magnifying lens (See Fig. 1). The different ant body regions were then subjected to freeze-drying using a Scan Vac freeze dryer (ScanVac Cool safe 95/55) at −50 °C for 3 days followed by ball milling using a Retsch 400 oscillating mill (Retsch NM400, 42781 Haan, Germany) and subsequent freeze drying again for another 3 days. The freeze-dried samples were subjected to a second round of ball milling and the powdered samples were then stored at −20 °C for all future analyses. All the analyses were carried out in triplicate ($n = 3$).

2.2. Proximate analyses

Proximate and elemental analysis of the powdered samples were carried out by the analytical services unit of the School of Agriculture and Food Science, The University of Queensland (St Lucia, QLD, Australia). The nitrogen (N) and sulphur (S) content in the four different ant body regions were determined by AOAC method 992.15 where crude protein was calculated as N x 6.25. Elemental analysis was carried out following the AOAC method 2015.01. Neutral detergent fibre (NDF) and crude fat content (Soxhlet extraction) were quantified using the AOAC methods 991.43 and 960.39 respectively (AOAC, 2019). Approximately 2 gr of fresh insects were used for each of the analyses.

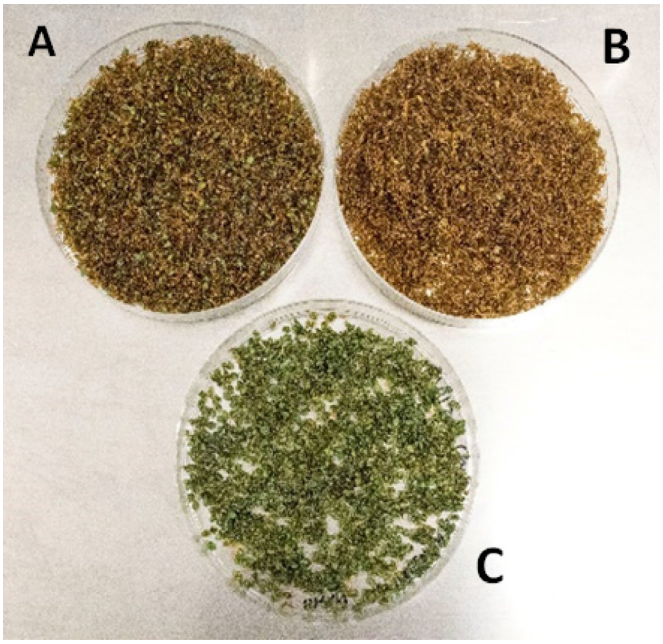

Fig. 1. Sorting of green ant by body regions. A: whole ants; B: anterior part of the body; C: gaster.

2.3. Antimicrobial and antioxidant properties

Both antimicrobial and antioxidant properties were measured following the method described by Akter et al. (2019). The different body regions of the ant were extracted using two solvents: water and methanol. The obtained extracts were then stored at −20C° before analysis. Well diffusion assay was employed for determining the antimicrobial activity of both extracts; which were tested against a gram-positive bacterium- *Staphylococcus aureus* and a gram-negative bacterium- *Escherichia coli* at two different concentrations- 5×10^6 CFU/ml and 5×10^4 CFU/ml. Penicillin streptomycin (1 µg) and 100 µl of 20% aqueous methanol was added as control and solvent control, respectively.

The antioxidant capacity was determined by the DPPH (2,2-diphenyl-1-picryl hydrazyl) radical scavenging assay (Moore and Yu, 2007). TROLOX with varying concentrations (7 - 35 µM) was used as standard. Plates were incubated in the dark and the absorbence was measured at 517 nm using a Tecan plate reader (Tecan Infinite M200, Tecan Trading AG, Mannedorf, Switzerland).

2.4. Total phenolic content

Total phenolic content was measured using the method described by Shingleton and Rossi (1965). Gallic acid with concentrations ranging from 21–105 µg was used as standard. The total phenolic content was determined by measuring the absorbence at 700 nm using a Tecan plate reader (Tecan Infinite M200, Tecan Trading AG, Mannedorf, Switzerland).

2.5. GC-MS analysis - volatile compounds

Volatile compounds in the headspace of the different ant parts were identified, separated and semi-quantified using the GC-MS method de-

Table 1
Proximate composition of all four ant body regions (dry mass).

	AN	WA	AB	G
CP (%)	44.3	51.5	59.1	34.3
NDF (%)	20.1	24	33.8	10.4
CF (%)	11.8	13.8	9.5	18.3
Mg (mg/kg)	680	799	791	708
Ca (mg/kg)	494	614	573	596
P (%)	0.5	0.6	0.7	0.5
K (%)	0.5	0.7	0.6	0.6
Na (%)	0.2	0.2	0.2	0.2
S (%)	0.1	0.2	0.3	0.2
Zn (mg/kg)	198	241	249	198
Fe (mg/kg)	192	214	190	194
Mn (mg/kg)	89	114	97	107
B (mg/kg)	37	45	56	61
Al (mg/kg)	27	31	46	17
Cu (mg/kg)	18	24	18	24

AN: ants nest; WA: whole ants; AB: anterior part of the body; G: gaster; CP: crude protein; NDF: neutral detergent fibre; CF: crude fat.

Table 2
Diameter of Zone of Inhibition of different ant sections extracted with methanol against different concentrations of *S. aureus*.

	Diameter of ZOI (mm) A*	Diameter of ZOI (mm) B*
P/S	53.4	56.2
AN	ND	ND
WA	13.3 ± 0.8[a]	19±0.4[a]
AB	11.9 ± 0.5[a]	15.4 ± 0.2[b]
G	ND	ND

P/S- Penicillin Streptomycin; AN: ants nest; WA: whole ants; AB: anterior part of the body; G: gaster; ND- Not Detected.

* A: Antimicrobial activity against *S. aureus* of concentration 5×10^6 CFU/ml, B: antimicrobial activity against *S. aureus* of concentration 5×10^4 CFU/ml [a,b] Different letters in a column comparing WA and AB indicate significant difference ($P \leq 0.05$). Data is presented as mean and standard deviation from three replicates.

scribed in a previous study (Olarte Mantilla et al., 2020). The mass spectrum for each peak in the obtained chromatogram from each sample was compared with the mass spectrum libraries NIST21 and NIST107 with the aid of GC-MS solutions software (Shimadzu, Kyoto, Japan). Peaks with highest number of matches and SI values >90 were selected by the software and the peak area and retention index were determined.

2.6. Folate content

Proximate and elemental analysis of the powdered samples were carried out by the procedure described in Striegel et al. (2018). To calculate the total folate content, five folate vitamers (PteGlu, H_4Folate, 5-CH_3–H_4Folate, 5-CHO–H_4Folate, 10-CHO-PteGlu) were analysed by stable isotope dilution analysis (SIDA). Of the homogenized samples, 20–25 mg were analysed in triplicate. The internal standards [$^{13}C_5$]-PteGlu, [$^{13}C_5$]-H_4Folate, [$^{13}C_5$]−5-CH_3–H_4Folate and [$^{13}C_5$]−5-CHO–H_4Folate were added to the sample in an amount similar to that expected for the unlabelled analytes in the sample. Further purification was done by solid phase extraction (SPE) before the total folate content was determined by LC-MS/MS (Nexera X2 UHPLC System with a triple quadrupole ion trap mass spectrometer (LCMS-8050), Shimadzu, Kyoto, Japan).

2.7. Statistical analysis

One-way Analysis of Variance (ANOVA) was used ($p<0.05$) to compare the antimicrobial activity, phenolic content, and antioxidant activity amongst the four body regions of the ant using Microsoft Excel (2016). Principal component analysis (PCA) was carried out to identify associations between the different measured variables using XLSTAT (version 2018.6.54124, Addinsoft SARL, Paris, France) using cross validation.

3. Results and discussion

3.1. Proximate composition of different sections of green ants

Table 1 presents the compositional parameters measured in the different ant body regions. The crude protein (CP) content in all four body regions ranged between 34.3% to 59.1% DW. The highest content found was in the anterior part of the body of the ant and the lowest in the gaster. However, the CP values might have been overestimated due to the insects' indigestible nitrogenous components (chitin) of the cuticle not measured in this study. The total NDF fibre content amongst the four different ant body regions ranged between 10.4% to 33.8% DW. Similar to CP, the highest content was observed for the anterior part of the body followed by whole ants, nest and gaster. Other authors have reported that high fibre content found in insects can be associated with the chitin which forms part of the exoskeleton of the insect (Chakravorty et al., 2016; Ghosh et al., 2017; Kouřimská and Adámková, 2016). Crude fat (CF) content also varies with the part of the insect analysed where the gaster has the highest content (18.3% DW) and the whole ant the lowest (9.5% DW) (Helms and Kaspari, 2014; Kouřimská and Adámková, 2016). The compositional results obtained for Australian green ants are in agreement with those results reported by other authors analysing green weaver ants found in India (Chakravorty et al., 2016; Kouřimská and Adámková, 2016) and Thailand (Raksakantong et al., 2010; Kouřimská and Adámková, 2016). The proximate results obtained for the green ants are comparable to those of other commonly consumed insects such as mealworm and house crickets (Kouřimská and Adámková, 2016; Ghosh et al., 2017).

The concentration of macro minerals such as Ca, Mg, Na, and K was found to be higher in the whole ant analysed while the concentration of P and S was found to be higher in the anterior part of the body of the ant (see Table 1). Amongst the micro minerals, the concentration of Fe, Mn, and Cu was found to be higher in whole ant. The concentration of other trace minerals such as Zn, B and Al were found to be higher in the anterior part of the body of the samples analysed. A high concentration of Al content was observed in the set of samples analysed which might have resulted either from the feed or from the soil where the nest is located. It is important to note that tropical soils in Australia tend to be acidic in nature with excessive Al content (Brunner and Sperisen, 2013). Overall, the mineral composition of Australian green ant is comparable to green ants from India (Chakravorty et al., 2016), and similar to the mineral composition of other edible insect species (Raksakantong et al., 2010).

3.2. Antimicrobial assay

Insects from the *Hymenoptera* family are known to contain antimicrobial peptides rich in proline, which are active against a variety of gram positive and gram negative bacteria species (Mylonakis et al., 2016). However, the water extracts obtained from the four different body regions of the green ants did not exhibit any anti-microbial activity (Table 2). It is suggested that the high concentration of water insoluble compounds might explain the inability of the water extract to express any anti-microbial activity (Ngo et al., 2017).

On the other hand, the methanol extracts were found to exhibit antimicrobial activity against the gram-positive bacteria (*Staphylococcus aureus*) at two different concentrations (5×10^6 and 5×10^4 CFU/ml)

Table 3
Antioxidant capacity, total phenolic content and folate vitamer distribution in the different ant body regions.

	Antioxidant capacity		Total phenolic		Folate vitamer			
	WE µg TROLOX eq/g DW	ME µg TROLOX eq/g DW	WE mg GAE/g DW	ME mg GAE/g DW	PteGlu µg/100 g	5-CHO–H4Folate µg/100 g	10-CHO-PteGlu µg/100 g	TF µg/100 g
AN	423.8 ± 74.8[a]	ND	6.1 ± 0.7[a]	ND	84.9 ± 4.3	20.9 ± 1.0	148 ± 0.6	254 ± 3.9
WA	617.6 ± 59.2[b]	813±22.6[a]	7.0 ± 0.5 [b]	5.5 ± 0.4 [a]	36.3 ± 1.1	19.5 ± 1.6	196 ± 12.5	252 ± 13.3
AB	501.6 ± 13.6[c]	797.5 ± 81.4[b]	5.3 ± 0.5 [c]	4.9 ± 0.5 [b]	20.6 ± 1.8	21.7 ± 0.2	158 ± 2.1	201 ± 2.3
G	532.3 ± 100[d]	775.8 ± 55.6[c]	5.8 ± 0.6 [d]	6.2 ± 0.3 [c]	63.9 ± 1.8	15.1 ± 0.6	233 ± 6.7	312 ± 4.7

AN: ants nest; WA: whole ants; AB: anterior part of the body; G: gaster; ND- Not determined. WE: water extract; ME: methanol extract; TF; total folate.
[a,b,c,d] Different letters in a column indicate significant difference ($p \leq 0.05$). Data is represented as mean and standard deviation from three replicates.

Table 4
Aromatic and volatile compounds and their chemical group common to all four ant body regions expressed in percentage.

Compound	Family	AN%	WA%	AB%	G%
2-methyl-butanal	Aldehyde	0.08±0.01	0.08±0.004	0.07±0.005	0.1 ± 0.01
3-methyl- butanal	Aldehyde	0.2 ± 0.04	0.3 ± 0.006	0.2 ± 0.01	0.4 ± 0.03
Decane	Alkane	0.3 ± 0.01	0.2 ± 0.004	0.1 ± 0.001	0.08±0.007
Undecane	Alkane	35.6 ± 1.9	2.9 ± 0.04	4 ± 0.06	5.9 ± 0.4
Dodecane	Alkane	0.8 ± 0.02	0.1 ± 0.003	0.1 ± 0.01	0.1 ± 0.01
1-Hexanol	Alcohol	9.2 ± 0.1	1.5 ± 0.03	12.5 ± 0.1	0.2 ± 0.009
Octanal	Aldehyde	0.03±0.003	0.08±0.001	0.03±0.003	0.07±0.003
Tridecane	Alkane	6.9 ± 1	0.2 ± 0.005	0.6 ± 0.008	1.1 ± 0.1
Nonanal	Aldehyde	0.2 ± 0.006	0.2 ± 0.004	1.6 ± 0.01	0.2 ± 0.02
Formic acid	Acid	44.3 ± 2	93.6 ± 0.1	78.4 ± 0.6	91.2 ± 0.7
Acetamide	Carboxylic acid amides	0.03±0.003	0.2 ± 0.003	0.2 ± 0.01	0.2 ± 0.01
Hexanoic acid	Acid	2.3 ± 0.06	0.7 ± 0.01	2.2 ± 0.03	0.5 ± 0.02

AN: ants nest; WA: whole ants; AB: anterior part of the body; G: gaster. Each data point is the mean of three replications.

(Table 2 and 3). It was observed that the diameter of ZOI increased slightly with decrease in the concentration of the bacteria (Table 2). The diameters of ZOI using the ant methanol extracts were lower than the control (penicillin streptomycin) at both concentrations of bacteria. Other authors have reported that the gaster extracts of green weaver ant samples from Nigeria might exhibit antimicrobial activity against several species of fungi and bacteria including *E. coli* (Oladunmoye et al., 2018). In this study, the methanol extracts from different body regions of the green ant samples did not yield any anti-microbial activity against *Escherichia coli*. No antimicrobial effect was detected using the gaster, irrespective of extraction method.

3.3. Antioxidant activity and total phenolic content

The antioxidant activity of both water and methanol extracts were measured as TROLOX equivalent (Table 3). The antioxidant activity of the methanol extracts was significantly higher than the water extracts. The methanolic and water extracts of the whole ant exhibited the highest antioxidant activity followed by the anterior part of the body and the gaster. Due to laboratory access restrictions, the antioxidant capacity of the ant nest using the methanol extract was not determined. Comparing the water extracts, the highest antioxidant activity was observed for the whole ant followed by the gaster, anterior part of the body and ant nest. Overall, the antioxidant capacity of the green ants was observed to be comparable with other edible insects such as mealworms, beetles and field crickets (Pyo et al., 2020).

Total phenolic content of different ant body regions extracted with methanol and water were quantified in terms of gallic acid equivalent (Table 3). It was observed that the phenolic content of the water extracts was higher than the methanol extracts. Amongst the water extracts, the highest value was observed for the whole ants followed by the ant nest, gaster and the anterior part of the body. The phenolic content in the ant nest might be attributed to the presence of leaves, woody materials and twigs mixed in with the ants . When comparing the methanol extracts, the gaster had the highest gallic acid equivalent value followed by the whole ants and the anterior part of the body. Despite the higher phenolic content, the ants extracted with water exhibited reduced antioxidant activity when compared to that of methanol extracts.

Insects like any other living system, contain bioactive compounds, natural antioxidant enzymes, and several other chemical compounds that can mediate or affect the antioxidant activity of the sample (Oghenesuvwe and Chinwuba, 2019). It is suggested that methanol being an amphiphilic compound might have better favoured the extraction of different bioactive compounds present in the sample matrix. Therefore, samples extracted with methanol will exhibit better antioxidant activity than the water extracts.

3.4. Volatile compounds

Volatile compounds are responsible for the aroma and other properties of the food. The distribution of the volatile compounds found in the four body regions of the ant analysed along with their odour description and family are presented in Table 4. Organic acids were found to be in relatively higher proportions (46.6% - 94.3%) compared to other compounds. Formic acid (44.3% - 93.6%) was the most prevalent organic acid. Green ants belong to the genus *Oecophylla* sub family *Formicidae* where the members of this family are said to be characterised with the prominent presence of formic acid (Peerzada et al., 1990). Alkanes were found to be in higher ratios (3.4% - 43.7%) than alcohols (0.2% - 12.5%), aldehydes (0.2% - 0.8%) and carboxy acid amides (0.03% - 0.2%). Although, the volatile compounds described above were presented in all body regions of the ant analysed, differences were observed amongst the different body regions (Table 5).

The ant nest was complex with the highest number of compounds observed with the majority belonging to the terpenes, alkanes, and alcohol groups. The complexity in this sample was due to the presence of foreign material such as leaves and twigs along with the ants. Certain terpenes such as α-pinene, α-phellandrene, and β-phellandrene were found only in the ant nest. The anterior part of the body had the second highest number of volatile compounds with the vast majority belonging to al-

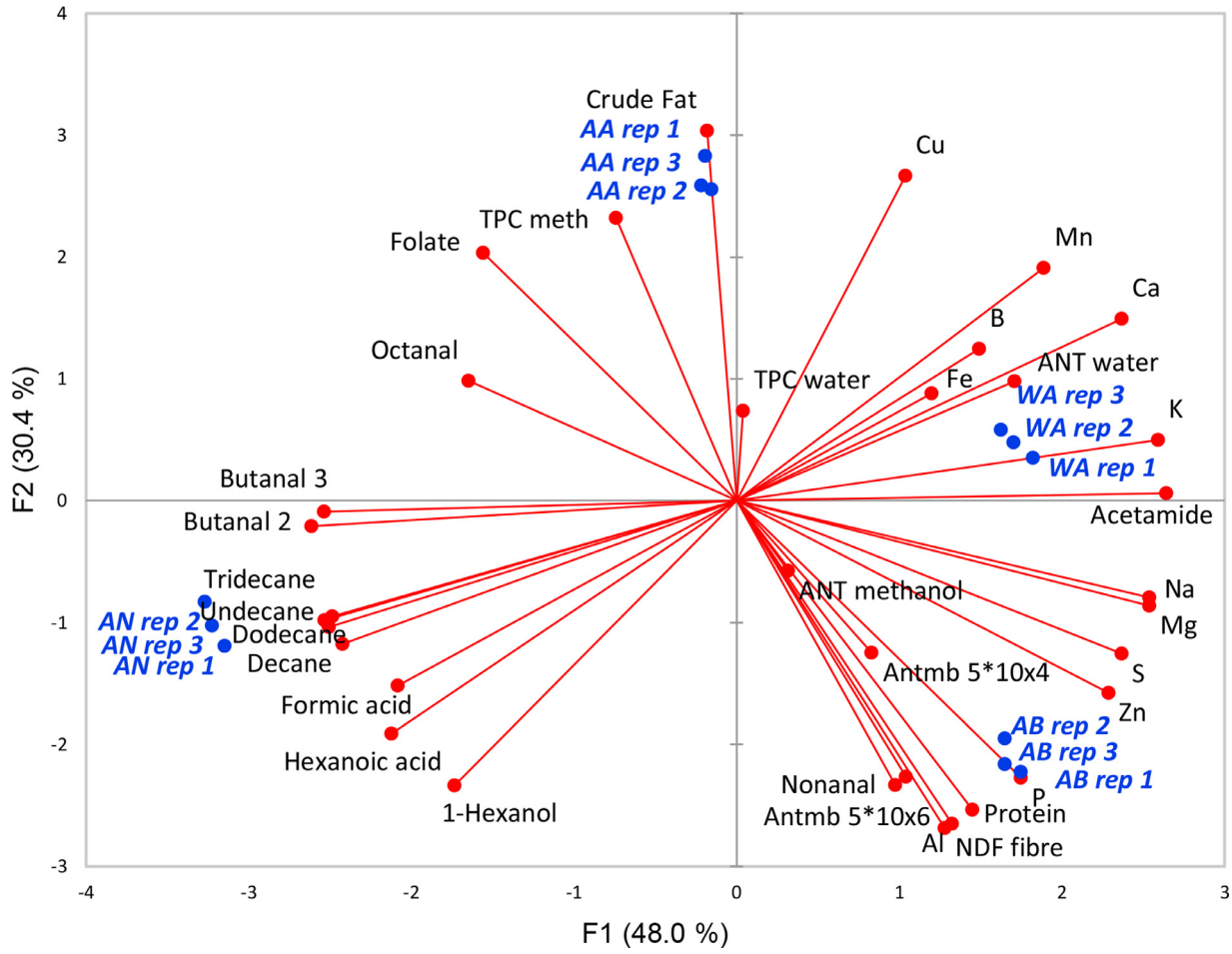

Fig. 2. Principal component analysis biplot (scores and loadings) for all variables in the ant body regions for each replicate. AB: anterior part of the body; WA: whole ants; AN: ant nest; AA: grast, Cu: Copper; Mn; Manganese; B: Boron; Fe: Iron; K: Potassium; Ca: Calcium; Na: Sodium; Mg: Magnesium; S: Sulphur; Zn: Zinc; Al: Aluminium; ANT Methanol: antioxidant activity of methanol extracts; ANT water: antioxidant activity of water extracts; TPC water: total phenolic contents of water extracts; TPC meth: total phenolic content of methanol extracts; Antmb 5 × 10 × 6: antimicrobial activity against 5 × 10^6 CFU/ml of *S. aureus*; Antmb 5 × 10 × 4- antimicrobial activity against 5* 10^4 CFU/ml of *S. aureus*, Butanal 2: 2-methyl-butanal; Butanal 3: 3-methyl-butanal; Decane: Decane; Undecane: Undecane; Dodecane: Dodecane; 1-Hexanol: 1-Hexanol; Octanal: Octanal; Tridecane: Tridecane; Nonanal: Nonanal; Formic acid: Formic acid; Acetamide: Acetamide; Hexanoic acid: Hexanoic acid; Folate: total folate.

Table 5
Aromatic and volatile compounds contribution by chemical groups present in the different ant body regions analysed expressed in percentage.

	AN (%)	WA (%)	AB (%)	G (%)
Acids	40.8	92.3	56.5	90.8
Alcohols	13.9	2.5	26.5	0.2
Alkanes	39.8	3.5	3.4	7.2
Aldehyde	0.5	0.7	0.2	1
Ketone	1.8	0.2	7.1	0.1
Terpenes	3.1	0.4	2	0.4
Fatty Acid esters	0.04	0.05	4.4	–
Phenols	–	0.1	–	–
Carboxylic acid amides	0.03	0.2	0.2	0.2

AN: ants nest; WA: whole ants; AB: anterior part of the body; G: gaster.

cohols, terpenes and fatty acid ester groups. In addition, volatile compounds such as linalool and terpineol were found to be present only in the anterior part of the body of the green ant. Whole ant were characterised with a reduced number of compounds belonging to groups similar to those found in the anterior part of the body. Phenols like methoxy eugenol and 3-allyl-2-methoxyphenol were selectively distributed in the whole ant samples. The gaster section had the lowest number of volatile compounds where the predominant ones belong to the alkanes and aldehydes. Two compounds, guaiol - a sesquiterpene and heneicosane - an acyclic alkane, were only found in the ant gaster and the whole ant, indicating that they might be exclusive to the green ant gaster. Both guaiol and heneicosane have been reported in insects and used as natural insecticides or repellent (Mendki et al., 2000; Liu et al., 2013).

The alkanes reported in the samples are referred to as alarm pheromones and are known to warn the members of the nest in response to different stressors (Fujiwara-Tsujii et al., 2006). This could possibly explain the relatively higher alkane content in these samples. Similarly, insects are attracted to certain plant volatiles including fatty alcohols such as hexanol and exhibit a synergy with the plants which is said to promote sexual attraction in several species of insects (Reddy and Guerrero, 2004). This synergism could account for the alcohol content present in the samples. The compounds belonging to other families such as aldehydes, ketones, terpenes and phenols were present in relatively lower proportions.

3.5. Folate content

The total folate content of the four different ant body regions and the vitamer distribution of the five analysed folates is shown in Table 3. The

highest folate content was determined in the ant gaster, followed by the whole ant and the nest, both with similar contents, and the body. The vitamer content showed an atypical distribution compared to the usually known distribution in plants containing mainly 5-CH_3–H_4-Folate and 5-CHO–H_4-Folate. In all four ant body regions, 10-CHO-PteGlu showed considerably the highest fraction (58–79% of the total folate content). Also, a relatively high content of PteGlu was detected in the nest; as PteGlu is not a natural folate, the high content is possibly attributable to a degradation of other folates. Surprisingly, no H_4-Folate and 5-CH_3–H_4-Folate was found in the body regions. Comparing these results with those of Ayieko et al. (2012), the folate content of black ants (*Carebara vidua*) was reported to be almost twice as high as found in green ants. However, the result of Ayieko et al. (2012) study has to be questioned as these authors used a method for only detecting PteGlu without considering other folate vitamers.

3.6. Assessment of relation of the variable measurements and samples

The data were analysed by means of principal component analysis where the scores and loadings are reported as a biplot (Fig. 2). It should be noted that only the compounds that were found common to all four body regions of the ant were included in the PCA analysis. The PCA plot explained 78.4% of variation where factor 1 (F1) (principal component1) explained 48.0% and F2 (principal component 2) 30.5% of the variability, respectively. Separation between the nest and the other three ant body regions was observed along F1. The main variables or loadings that explain this separation where butanyl-3-methyl, butanol-2-methyl, undecane, decane, dodecane, and tridecane, K, Na, Mg, and acetamide (positive and weighted on whole ant) while butanyl-3-methyl, butanol-2-methyl, undecane, decane, dodecane, and tridecane were found to be driven by the nest. Separation between body and gaster was observed along F2. The main variables or loadings influencing the gaster were folate content, Cu, TPC assay measured in the methanol extracts and CF content while nonanal, CP, NDF and Al were associated with the anterior part of the body.

4. Conclusion

This study reported the nutritional value, antioxidant, antimicrobial potential and volatile compounds available in different body regions of Australian green ant. The nutritional value was found to vary amongst the different body regions [ant nest, whole ant, anterior part of the body, and gaster] of ant; both CP and NDF contents were higher in the anterior part of the body while the gaster had the higher folate and CF content. Whole ant exhibited good antioxidant and antimicrobial activity. The ant nest was found to be complex and was characterised by a high number of volatile compounds such as organic acids, alkanes, alcohols and terpenes.

Further studies should be carried out to quantify other bioactive compounds such as Vitamins C, E and B12. Analyses on allergens and toxins will also be required in order to assure the safety of these samples as an ingredient or food source. Additionally, green ant produced in different regions of Australia and globally should be compared to highlight any potential differences.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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