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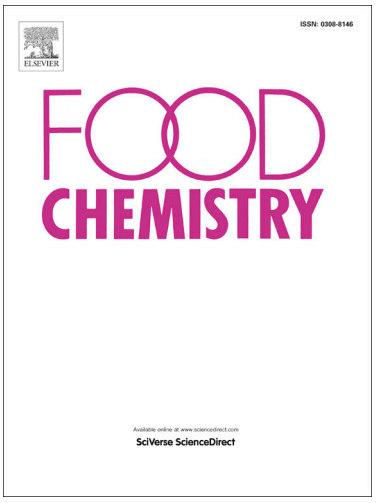

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Soheila Beiranvand, Ashley Williams, Symsia Long, Peter R. Brooks, Fraser D. Russell

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Use of kinetic data to model potential antioxidant activity: Radical Scavenging Capacity of Australian *Eucalyptus* Honeys

Soheila BEIRANVAND,[a,b,d] Ashley WILLIAMS,[a,b] Symsia LONG,[a,b] Peter R. BROOKS,[a,c,d] Fraser D. RUSSELL[a,b,d]

[a] *GeneCology Research Centre, University of the Sunshine Coast, Qld, Australia*

[b] *School of Health and Sport Sciences, University of the Sunshine Coast, Qld, Australia*

[c] *School of Science and Engineering, University of the Sunshine Coast, Qld, Australia*

[d]*CRC for Honey Bee Products Ltd., 128 Yanchep Beach Road, Yanchep, WA, Australia*

Corresponding author:

F.D. Russell

Genecology Research Centre,

School of Health and Sport Sciences,

University of the Sunshine Coast, Maroochydore, QLD 4558, Australia.

Tel: +61 7 5459 4665

Email addresses: Soheila.Beiranvand@research.usc.edu.au;

AshleyWilliams@outlook.com.au; l_s097@student.usc.edu.au; PBrooks@usc.edu.au;

frussell@usc.edu.au

ABSTRACT: Antioxidant activity of honeys may be beneficial in wound healing processes by protecting cells against lipid oxidation. The DPPH assay assesses the efficacy of antioxidant molecules to reduce DPPH$^{\bullet}$ to DPPHH. Studies determining EC_{50} are limited by single time-point determinations of antioxidant effect and can miss vital information about the rate of antioxidant response. Acquisition of kinetic data allows determination of the radical scavenging capacity (RSC) of honeys. The purpose of this study was to determine the RSC of 53 honeys from 16 species of Australian *Eucalyptus* trees and four samples of New Zealand manuka (*Leptospermum scoparium*) honey. Whereas honeys could not be differentiated based on EC_{50} values, significant differences were observed for RSC, supporting collection of kinetic data for honey analysis. The greatest RSC was observed for New Zealand manuka ($4.6\pm0.3\times10^{-5}$ mg.mL^{-1}.min^{-1}), grey ironbark (*E. paniculate*; $3.4\pm0.2\times10^{-5}$ mg.mL^{-1}.min^{-1}) and river red gum honeys (*E. camaldulensis*; $3.2\pm0.2\times10^{-5}$ mg.mL^{-1}.min^{-1}).

KEY WORDS: Honey, radical scavenging capacity, antioxidant activity, *Eucalyptus*

1. Introduction

Eucalyptus and related genera, *Angophora* and *Corymbia* (Myrtaceae) are endemic to Australia and include about 800 species (Bui et al., 2017). A small number of *Eucalyptus* species are also endemic to Indonesia, Timor-Leste and New Guinea, and large tracts of *Eucalyptus* plantation have replaced areas of native forest in countries such as Spain, Portugal, Kenya, Brazil and Chile to service the pulp industry and to facilitate carbon sequestration (Ferreira et al., 2019; Booth, 2013). Flowering species of *Eucalyptus* are a source of nectar for the European honey bee (*Apis mellifera*). The chemical composition of *Eucalyptus* honeys is shaped by the botanical source of the nectar and by proteins that are secreted from bee hypopharyngeal glands into the nectar during honey ripening and proteins that are present in the plant pollen (Machado De-Melo et al., 2017; Lewkowski et al., 2019). Proteins in honey have key roles in carbohydrate metabolism and antimicrobial activity (Lewkowski et al., 2019). Other honey constituents, including phenolic acids, flavonoids and vitamins, contribute to antioxidant activity.

Antioxidant activity has been ascribed to extracts of the fruit, bark and leaf of *Eucalyptus* trees (Vuong et al., 2015), and honey produced by bees and derived from the nectar of *Eucalyptus* flowers (Ciappini & Stoppani, 2014; Valdés-Silverio et al., 2018; Anand et al., 2018; Karabagias et al., 2018). The antioxidant activity of honey protects against lipid oxidation (McKibben & Engeseth, 2002), and this, together with its' antimicrobial and anti-inflammatory activity, is advantageous in the treatment of wounds (Sarkar et al., 2018; Smaropoulos & Cremers, 2020). A limited number of studies have examined antioxidant activity of Australian *Eucalyptus* honeys. Using the model oxidant radical, ($DPPH^{\bullet}$), the reducing activity of an Australian *Eucalyptus* honey (non-disclosed species and geographical origin) was compared to an ascorbic acid standard (Marceau & Yaylayan, 2009). The reducing

activity of Jarrah honey (*E. marginate*) from Western Australia was compared to the water-soluble vitamin E analogue, Trolox (Anand et al., 2018). In both studies, a single concentration of honey was tested at a single reaction time-point. Although single time-point measurements allow titration of the reducing components of honey, they provide no information about the time-dependence of reducing activity (Amorati & Valgimigli, 2015). Karabagias and colleagues (2018) reported the DPPH• reducing activity of four *Eucalyptus* honeys from the Braga and Viseu districts of Portugal (varieties not identified) at steady state in addition to the potency of the honey for DPPH• reduction. However, there are no published reports of the dose-response relationship or time-dependence (kinetics) of reducing activity for Australian *Eucalyptus* honeys. In addition, there have been no studies reporting reducing activity of Australian *Eucalyptus* honeys that originate outside Western Australia. The aim of the present study was to determine the radical scavenging capacity of Australian *Eucalyptus* honeys and to correlate this with a single time-point measurement of antioxidant potency (EC_{50}). We hypothesised that the radical scavenging capacity does not necessarily correlate well with EC_{50}, and that collection of kinetic data is important for assessment of reducing activity over the course of the reaction (the radical scavenging capacity). In this study, DPPH• reducing potency and radical scavenging capacity were determined for 53 monofloral honey samples from 16 different *Eucalyptus* species sourced from eastern (Queensland, New South Wales) and southern (South Australia) states of Australia. Findings are compared to four samples of New Zealand (NZ) manuka (*Leptospermum scoparium*) honey, and a sugar mixture control.

2. Materials and methods

2.1. Physicochemical properties

2.1.1. Honey collection

Unprocessed honeys were obtained from the south-east region of Queensland, Northern Rivers and south-east regions of New South Wales and from coastal South Australia, by Capilano Pty Ltd (Fig. 1). Honey flora source was identified based on local beekeeper knowledge. Honey samples were extracted between October 2016 and March 2019 (Table 1) and stored in sealed containers in the dark at 20°C. Four samples of NZ manuka honey were supplied by Global Proficiency Ltd., New Zealand and used as a comparator. A sugar mixture containing 40.2% D-fructose, 33% D-glucose, 7.5% D-maltose, 1.3% D-sucrose and 18% milliQ water was used as a control for the sugar content of honey samples. Honeys and the sugar control were mixed using a glass rod at 20°C prior to use.

2.1.2. Density and Colour intensity

Honey or sugar mixture was added to a 2.0 mL volumetric flask and the mass of sample was determined. The density of honey or sugar mixture (mass/2) was expressed in g/mL. An equivalent volume of milliQ water was added to the honey or sugar mixture to obtain 1 g/mL solutions. The samples were mixed by vortex, warmed in a 50°C water bath for 5 min, mixed by vortex and allowed to cool to room temperature (20°C). An aliquot of the honey was filtered using a 0.45 µm syringe filter (Millex-HV, Millipore). A 200 µL aliquot of each filtered honey sample was dispensed into a 96 well plate and the plate was read at 450 nm and 720 nm wavelengths. The absorbance at 720 nm was subtracted from the absorbance at 450 nm.

2.1.3. pH determination

Filtered honey or sugar mixture (1 g/mL) was diluted to a final concentration of 10% v/v in milliQ water and equilibrated at 28°C. The pH of solutions was measured using a

CyberScan pH meter (Model 510) with thermometer probe attachment. The pH meter was calibrated using pH 4, 7, and 10 standard buffer solutions (Rowe Scientific Pty Ltd., WA, Australia) at 28°C.

2.1.4. Protein concentration

Protein concentration was determined using the Bradford method (Bradford, 1976). Filtered honey or sugar mixture (1 g/mL; n=3-4) were diluted to a final concentration of 0.3% (v/v) in milliQ water. Bradford reagent (Sigma Aldrich, 20% v/v in milliQ water) was added to the sample in a 1:1 ratio (500 µL sample + 500 µL diluted Bradford reagent). Samples (200 µL) were read in duplicate in an Enspire multimode plate reader (Perkin Elmer, Singapore) at 570 nm wavelength and compared to a protein standard (bovine serum albumin, 0.5-5.0 µg/mL). Absorbance readings for all samples were within the range of standards. Protein concentration was corrected for dilution.

2.1.5. Glucose oxidase activity

Glucose oxidase activity was determined using a glucose oxidase activity assay kit (Abcam), according to manufacturer's instructions. Non-heated, non-filtered honey samples were diluted with milliQ water to a final concentration of 2% (w/v). Samples were placed on ice and mixed using a shaker until dissolved. Samples (50 µL), glucose oxidase standards (0.01-10 mU/mL) and assay buffer were aliquoted into a black 96 well plate with clear bottom. A reaction mixture (50 µL) containing AbRed indicator, glucose, horse radish peroxidase and assay buffer was added to each well and the plate was incubated at 37°C for 10 min. Fluorescence (ex/em 540/590 nm) was read using an Enspire multimode plate reader (Perkin Elmer, Singapore).

2.1.6. %Brix

A digital glucose refractometer (Hanna Instruments, Model HI 96803; range 0-85%) was used to obtain measurements of %Brix for the non-heated sugar mixture and undiluted, non-filtered, non-heated honey samples (n=3). The glucose refractometer was calibrated to zero using milliQ water. A standard 25.0% solution of D-glucose (2.500 g with 7.500 g milliQ water) gave a reading of 25.0% following a 2.0 min incubation of the sample on the refractometer prism. All subsequent readings for honey samples were measured after 2.0 min. The temperature of readings was 20.02±0.05 °C.

2.1.7. % Moisture content

A refractometer (Automatic Temperature Compensation, ATC) was calibrated using a sucrose solution comprising 6.600 g sucrose and 3.400 g millQ water. The sucrose was dissolved overnight on a platform shaker at room temperature in a sealed vial. Percent Brix of the solution was 66.0% and the density was 1.310±0.003 g/mL. Readings for % moisture content of non-heated sugar mixture and undiluted honey that was neither filtered nor heated (n=3), were determined using the refractometer (range, 12.0-27.0%) at 20.02±0.05 °C.

2.1.8. Total phenolic content

An Agilent Reverse Phase-High Performance Liquid Chromatography (RP-HPLC) system, equipped with 1260 Infinity II quaternary pump, autosampler column oven and diode array detector, was used to provide a comparative approximation of the total phenolic acid and flavonoid content of honey samples (0.1 g/mL, prepared in milliQ water). A Synergi Fusion RP (50mm x 4.6mm x 2.5µm), fitted with a C18 guard was used, with flow rate set at 0.5 mL/min, injection volume set at 20 µL and column oven temperature set at 35℃. The binary mobile phase consisted of mobile phase A (MPA) (94.95% H_2O; 5.0% acetonitrile (ACN) and 0.05% formic acid) and mobile phase B (MPB) (99.95% ACN and 0.05% formic acid). The gradient mobile phase program commenced with 100% MPA for 2.0 min, grading to 53:47

MPA: MPB at 20.0min, grading to 20:80 MPA: MPB at 20.5 min, holding isocratic to 21.5 min, and grading back to 100% MPA at 22.0 min and constant to 23.0 min. The detection wavelengths were monitored at 200, 220, 260, 290 and 340nm. The best absorption for phenolics was 260 nm and was used for their analysis.

Agilent OpenLab CDS ChemStation software (Palo Alto, CA, USA) was used for data analysis. The five largest peak areas above a minimum of 20 milli-absorbance units (mAU) were combined and used as an estimate of total phenolic content for each honey sample. Peaks occurring within the first 2.0 min of elution comprised the sugars and were excluded from analysis.

2.2. DPPH reducing activity

Honey samples (1 g/mL, prepared in milliQ water, n=3) were aliquoted into Eppendorf tubes at volumes 3, 10, 30, 50 and 75 µL. Methanol was added to obtain a final volume of 100 µL. Samples were combined with 0.4 mL of 117.5 µM DPPH$^{\bullet}$ (prepared in methanol), vortexed to mix, and incubated in the dark for 30 min at 20°C. Samples were vortexed, dispensed into 96 well plates in duplicate 200 µL aliquots, and plates were read in an Enspire multimode plate reader (Perkin Elmer, Singapore) at 518 nm wavelength. Data were compared to gallic acid standards (1.5-150 µM, in methanol) that were combined with DPPH$^{\bullet}$ solution, as described above. The percentage reducing activity was calculated using Equation 1:

Equation 1: Percentage reducing activity = [(Abs. control – Abs. sample) / Abs. control] x 100

The EC_{50} was calculated as the concentration of honey that produced a half-maximal reducing effect. Data was also expressed as µg gallic acid equivalents/g honey (µg GAE/g honey).

2.2.1. DPPH Radical Scavenging Capacity

Honey samples (0.5 g/mL, prepared in milliQ water, n=3) were mixed by vortex. Nine concentrations were prepared for each sample of honey (10-200 µL and a zero control were

combined with ethanol to a final volume of 375 µL). For kinetic analyses, a high DPPH• concentration relative to the antioxidant is required (Mishra et al., 2012). In the kinetic analysis used in this study, a 5-fold higher concentration of DPPH•, and a 1.5-fold higher maximal concentration of honey was used compared to that used for DPPH reducing activity. DPPH• (375 µL of 988 µM stock concentration, prepared in ethanol) was added to the honey samples and zero control. The samples were mixed by vortex, and aliquoted into a 96-well plate (200 µL; zero control in quadruplicate and singles for each honey concentration). Ethanol was dispensed into all spare wells for purposes of humidification and the plate was covered with a transparent plate lid, which was sealed using tape. The plate was read at 518 nm each minute for 150 min. Ln (% DPPH• remaining) was plotted against time (Fig. 3A-D) and the slope was calculated for each concentration of honey at selected time-points (1 min, 10 min, 30 min, 150 min) to give the rate constant, *K*. The rate constant was plotted against honey concentration (Fig. 3E) and the slope was calculated to give the radical scavenging capacity of the honey.

2.3. Statistics

Data was analysed using IBM SPSS Statistics, Version 26.0 and Microsoft Excel 2016. Equality of variance was determined using the Levene Statistic. Data with equal variance were analysed using one-way ANOVA, with Tukey post hoc analysis. Where equal variance could not be assumed, data were analysed using the Welch test, with Games-Howell post hoc analysis. Comparison of physicochemical properties and DPPH• EC_{50} and radical scavenging capacity of NZ manuka and *Eucalyptus* honeys was analysed using Students t test or Mann-Whitney U test. Normality of distribution was determined using the Shapiro-Wilk W test. Data were expressed as mean±SEM.

3. Results and discussion

3.1 Physicochemical properties of honey samples

Physicochemical properties of the honey samples, including density, pH, absorbance, %Brix, protein, glucose oxidase activity and total phenolic acid content, is reported in Supplement 1 for individual honey samples, and Supplement 2 for honeys of the same botanical source. Supplement 3 contains HPLC chromatograms for each honey variety. An expected strong negative correlation was observed between the percent moisture content, determined using the ATC refractometer and %Brix, determined using the automated glucose refractometer (R^2=0.975, Fig. 2). A moderate positive correlation was observed for absorbance and total protein concentration (R^2=0.492), and a weak positive correlation was detected for absorbance and total phenolic acid content (R^2=0.187; Fig. 2).

3.2 DPPH$^\bullet$ radical scavenging capacity of Australian *Eucalyptus* honeys

2,2-Diphenyl-1-picrylhydrazyl (DPPH$^\bullet$) is a stable free radical that is commonly used to measure the reducing activity of honey samples (Anand et al., 2018; Karabagias et al., 2018; Di Marco et al., 2018; Ciulu et al., 2018). Reduction of DPPH$^\bullet$ (a purple coloured radical) to DPPHH (a yellow coloured reduction product) requires transfer of an electron or hydrogen radical. The change in absorbance at 518 nm is used as an indirect marker of the reducing capacity of the honey. The concentration of honey that decreases absorbance to 50% of its original level (EC_{50}) provides an estimate of the potency of the honey for DPPH$^\bullet$ reduction. A limitation of this method is that it fails to provide temporal information that can be used to distinguish the rate at which different honey samples produce their antioxidant effect. Many studies reporting EC_{50} data for the DPPH$^\bullet$ assay have used a single time-point for reaction (Bobis et al., 2020). Since potency determination does not factor time of reaction, single time-point determinations of EC_{50} may be insensitive to the rate at which the honey samples

produce their effect, with little capacity to differentiate samples that have fast or slow responses. This limitation is addressed by determining the radical scavenging capacity, which is achieved by collecting kinetic antioxidant data (Amorati & Valgimigli, 2015). In this study, we determined the radical scavenging capacity of each honey sample and a sugar control by collecting absorbance readings at one-minute intervals over a reaction lasting 150 min (Table 2). Whilst it is possible to determine the radical scavenging capacity at all times within the reaction, we selected four time-points for analysis: 1 and 10 min (early stages of the reaction), 30 min (a duration that is commonly used for single time-point investigations), and 150 min (a time-point near the end of the reaction, where the plot of Ln(% DPPH remaining) versus honey concentration reaches a plateau). The early time-points are recommended when assessing the rapid antioxidant activity of biological samples (Amorati & Valgimigli, 2018). At 1 min, the radical scavenging activity was most rapid, with similar activity profiles observed across all honey samples. Following 10 min of reaction, river red gum and grey ironbark honeys had 2.8-fold (P=0.049) and 3.2-fold (P=0.049) greater activity than Caley's ironbark, respectively. After 30 min, NZ manuka honey had greater activity than yellow box (2.3-fold, P=0.049), Caley's ironbark (3.8-fold, P=0.024), blue top ironbark (3.5-fold, P=0.016), and mugga ironbark honeys (3.0-fold, P=0.018). After 150 min, the antioxidant activity of grey ironbark honey was superior to white box (1.6-fold, P=0.033; Fig. 3) and blue top ironbark honeys (2.1-fold, P=0.022); activity of river red gum honey was greater than blue top ironbark honey (2.1-fold, P=0.044); and activity of NZ manuka honey was greater than white box (2.2-fold, P=0.007), blue top ironbark (2.9-fold, P=0.003), mugga ironbark (2.9-fold, P=0.008), narrowleaf ironbark (1.9-fold, P=0.012) and hill gum honeys (1.7-fold, P=0.042) (Fig. 3, Table 3).

For purposes of comparison with the kinetic data, we determined the EC_{50} for each honey sample using a commonly used single time-point of reaction (30 min), noting inherent limitations previously identified for this type of assay (Amorati & Valgimigli, 2018). We hypothesised that the radical scavenging capacity of the honey samples doesn't necessarily correlate well with EC_{50}, and that measurement of the former will provide more meaningful information about antioxidant activity over the course of the reaction. Our findings showed only weak-to-moderate correlations between single time-point EC_{50} values and the radical scavenging capacity determined at early (1 min, 10 min), mid (30 min) and late (150 min) stages of the reaction (R^2 values between 0.396 to 0.536; Fig. 2). The findings of the study support the importance of collecting kinetic data in the determination of antioxidant activity at different time points of the reaction.

Analysis of kinetic reactions for antioxidant activity using $DPPH^{\bullet}$ as the model radical has been reported for plant- and insect-derived products, including rosemary extract (Terpinc et al., 2009), honeydew honey (Broznić et al., 2018), tomato waste extract (Savatović et al., 2012) and purple corn extract (Ramos-Escudero et al., 2012). While several studies have investigated the kinetics of temperature effect on honey antioxidant activity (for example, see Molaveisi et al., 2019), the current study is the first to our knowledge to report radical scavenging capacity for honey samples using a kinetic DPPH assay. It is of interest that the radical scavenging capacity was similar for honeys of the same botanical source but different geographical location in Australia. The findings suggest that the botanical origin of the nectar that is collected by bees is a more important determinant of antioxidant activity than the honey's geographical origin.

The antioxidant activity of honeys has been ascribed to their phenolic constituents (Di Marco et al., 2018). In this study, grey iron bark honey, which had high radical scavenging

capacity, was also found to contain the highest total phenolic content of the *Eucalyptus* honeys (mean, 1268 mAU; range, 677-2,538 mAU; Supplement 2). However, only a weak correlation was observed between phenolic content and radical scavenging activity across all *Eucalyptus* honeys (R^2=0.223 at 1 min, 0.230 at 10 min, 0.268 at 30 min and 0.225 at 150 min). Possible explanations for the weak association include the presence of other honey constituents that contribute to radical scavenging capacity or to different distributions of phenolic compounds within honey samples of differing botanical origin. HPLC chromatograms indicate some diversity of phenolic constituents between honey varieties (Supplement 3).

The phenolic composition of Australian and European *Eucalyptus* honeys has been determined previously. Australian mallee (*E. pilligaensis*), yellow box (*E. melliodora*), and river red gum (*E. camaldulensis*) honeys, a Spanish river red gum (*E. camaldulensis*) honey, and *Eucalyptus* honeys from Italy, Spain and Portugal (variety not identified) were reported to contain high levels of luteolin, tricetin and quercetin (Martos et al., 2000a; Martos et al., 2000b). Other flavonoids detected in the *Eucalyptus* honeys included myricetin, quercetin 3-methyl ether, kaempferol, pinobananksin, pinocembrin and chrysin (Martos et al., 2000a; Martos et al., 2000b). The main phenolic compounds present in NZ manuka are phenylacetic acid, phenyllactic acid, 4-methoxyphenyllactic acid, leptosperin, and methyl syringate (Oelschlaegel et al., 2012).

A limitation of using model antioxidant systems is that they don't replicate all features of oxidation that might occur *in vivo*. DPPH$^{\bullet}$ was used in this study because it is an enduring radical that has similarity to peroxyl radicals in both electronic configuration and mechanism of interaction with antioxidant molecules (Amorati & Valgimigli, 2018). While the rate of reaction of DPPH$^{\bullet}$ with antioxidants is considerably slower than the rate of reaction of

endogenous peroxyl radicals with antioxidants, the slower rate of reaction is necessary for analysis of the kinetic antioxidant response.

An immediate assessment of reaction kinetics is required to assess the initial, rapid reactions that occur in the time frame 0-30 sec (Xie et al., 2014). In the current study, this was not possible, with the earliest measurement of absorbance acquired at 1 min.

Beekeeper knowledge of hive location was used to characterise honeys by floral source. Honeys from the same floral origin had similar physicochemical properties and these were often different to honeys sourced from different floral sources. Ideally, pollen morphology would be used to confirm honey variety. However, differentiation of Australian Myrtaceae honeys based on pollen morphology beyond family level is not typically feasible (Thornhill et al., 2012; Sniderman et al., 2018), and we are therefore unable to exclude the possibility that some honey samples were not monofloral.

4. Conclusion

This is the first study to examine the dose-response relationship and radical scavenging capacity of a range of *Eucalyptus* honeys from the eastern and southern states of Australia, with comparison to NZ manuka honeys. The manuka honeys contained a very high concentration of total phenolic compounds and had a correspondingly high radical scavenging capacity. Highest radical scavenging capacity of the *Eucalyptus* honeys was identified for grey ironbark and river red gum honeys. While EC_{50} values did not differ between *Eucalyptus* honey varieties, the honeys could be differentiated by their radical scavenging capacity, supporting the importance of collecting kinetic data for antioxidant activity. Assays that measure EC_{50} at a fixed time may potentially lead to misinterpretation of the contribution of honey samples to reducing activity. Early time-point measurements (1-10 min) have been reported to provide a more accurate assessment of the rank order of efficacy of antioxidants, while late time-

point measurements provide an assessment of the stoichiometry of reaction (Amorati & Valgimigli, 2015; Amorati & Valgimigli, 2018). The determination of antioxidant potential using kinetic analyses is likely to provide improved understandings of which honey samples will be efficacious for protection against lipid oxidation (McKibben et al., 2002). Further exploration of the wound healing potential of grey ironbark and river red gum honeys, which have superior antioxidant activity at 10 min of reaction, is warranted.

CRediT authorship contribution statement

Soheila Beiranvand: Investigation, Validation, Methodology, Writing – original draft. **Ashley Williams:** Investigation, Validation, Methodology, Writing – original draft, Review and editing. **Symsia Long:** Investigation, Methodology, Review and editing. **Peter Brooks:** Investigation, Validation, Supervision, Review and editing, Funding acquisition. **Fraser Russell:** Conceptualization, Investigation, Validation, Supervision, Review and editing, Funding acquisition, Project administration.

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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Fig. 1. Geographical location of hives used in the collection of Australian *Eucalyptus* honeys. Filled square, State or Territory capital city; Filled circle, Hive location.

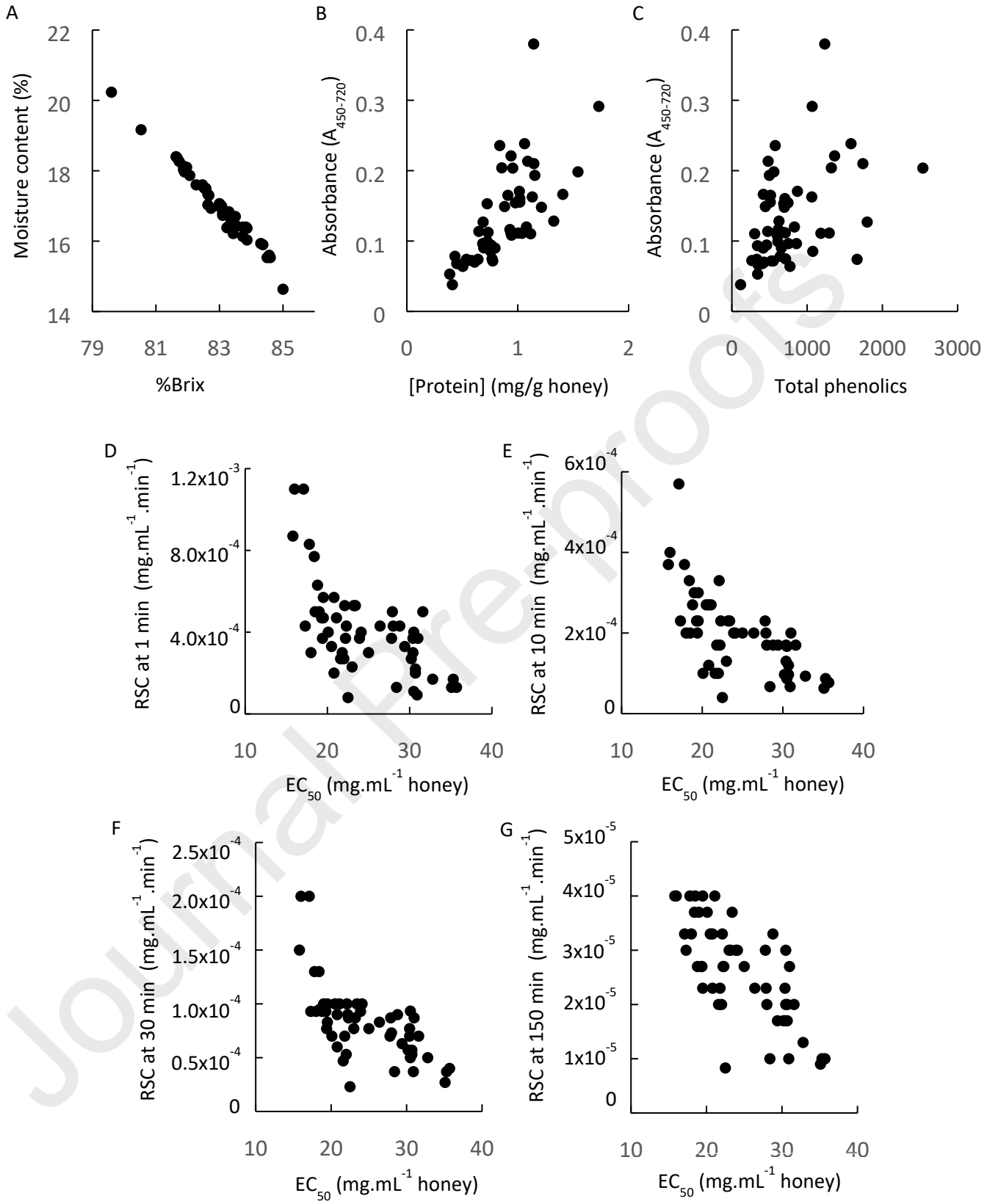

Fig. 2. Correlation between % moisture content and %Brix (A, R^2=0.975), absorbance and protein concentration (B, R^2=0.492), absorbance and total phenolic acid content (C, R^2=0.187), and radical scavenging capacity (RSC) and EC_{50} for DPPH$^{\bullet}$ reduction by Australian *Eucalyptus* honeys at 1 min (D, R^2=0.396), 10 min (E, R^2=0.435), 30 min (F, R^2=0.424) and 150 min (G, R^2=0.536) incubation times.

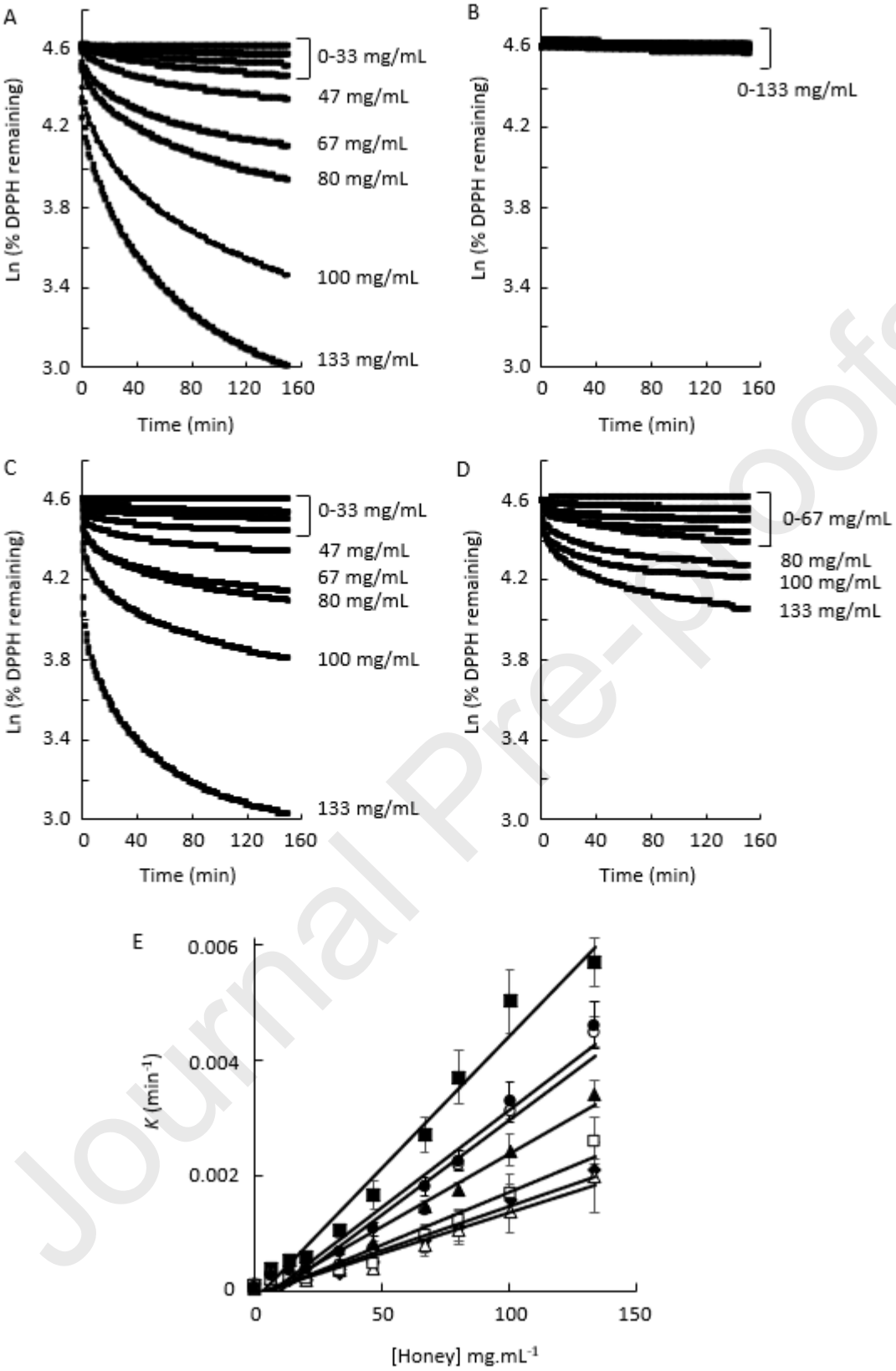

Fig. 3. Kinetics for DPPH• assay. Pseudo first order kinetics for disappearance of DPPH• in an incubation mixture containing 494 µM DPPH• and 0-133 mg/mL New Zealand manuka honey (A; NZM-1), sugar mixture (B), grey ironbark honey (C; GIB-2) and white box honey (D; WB-3). Plot of the equilibrium constant, *K*, against honey concentration for selected honey samples incubated with DPPH• for 150 min (E). The gradient of the lines reports the radical scavenging capacity of the honeys (Tables 2 and 3). NZ manuka (closed square, n=4), grey ironbark (closed circle, n=5), river red gum (open circle, n=4), narrowleaf ironbark (closed triangle, n=4), mugga ironbark (open square, n=5), blue top ironbark (closed diamond, n=5), Caley's ironbark (open triangle, n=3). Values are mean±SEM.

Table 1 Honey samples used in the study

Common name	Genus and Species	Geographical region	Extraction date	Lot number	Sample Identifier
Sugar mixture	NA	NA	NA	NA	Control
NZ manuka	*Leptospermum scoparium*	New Zealand	Not available	Hon-1905.2	NZM-1
		New Zealand	Not available	Hon-1905-3	NZM-2
		New Zealand	Not available	Hon-1905-4	NZM-3
		New Zealand	Not available	Hon-1911-1	NZM-4
River red gum	*Eucalyptus camaldulensis*	Dalby, QLD	12/2017	181841	RRG-1
		Wagga Wagga, NSW	03/2019	194200	RRG-2
		Cootamundra, NSW	01/2018	182160	RRG-3
		Warwick, QLD	12/2017	181338	RRG-4
Yellow box	*Eucalyptus melliodora*	Inverell, NSW	11/2017	180467	YB-1
		Tamworth, NSW	11/2017	180668	YB-2
		Emmaville, NSW	10/2015	189208	YB-3
		Wauchope, NSW	11/2017	181711	YB-4
White box	*Eucalyptus quadrangulata*	Manilla, NSW	07/2017	177735	WB-1
		Glen Innes, NSW	05/2017	177835	WB-2
		Wauchope, NSW	09/2017	178082	WB-3
		Glen Innes, NSW	09/2017	177838	WB-4
Messmate	*Eucalyptus obliqua*	Cooma, NSW	04/2017	177890	M-1
		Torrington, NSW	03/2017	177740	M-2
		Guyra, NSW	05/2016	176342	M-3
		Torrington, NSW	02/2017	183389	M-4
Grey ironbark	*Eucalyptus paniculata*	Imbil, QLD	01/2018	183017	GIB-1
		Warwick, QLD	05/2017	174622	GIB-2
		Grafton, NSW	10/2016	175799	GIB-3
		Grafton, NSW	10/2018	190877	GIB-4
		Gympie, QLD	01/2019	192533	GIB-5
Caley's ironbark	*Eucalyptus caleyi*	Glen Innes, NSW	08/2017	180796	CIB-1
		Inverell, NSW	05/2017	177575	CIB-2

		Bundarra, NSW	05/2017	175650	CIB-3
Bluetop ironbark	*Eucalyptus fibrosa*	Kogan, QLD	09/2017	178322	BTIB-1
		Warwick, QLD	08/2017	177010	BTIB-2
		Cecil Plains, QLD	07/2017	177134	BTIB-3
		Miles, QLD	07/2017	177832	BTIB-4
Mugga ironbark	*Eucalyptus sideroxylon*	Narrabri, NSW	10/2017	179430	MIB-1
		Narrabri, NSW	11/2017	180911	MIB-2
		Wee Waa, NSW	07/2017	176943	MIB-3
		Wee Waa, NSW	10/2018	190881	MIB-4
		Narrabri, NSW	10/2017	179433	MIB-5
Narrowleaf ironbark	*Eucalyptus crebra*	Dinmore, QLD	07/2017	177260	NLIB-1
		Cecil Plains, QLD	07/2017	177135	NLIB-2
		Cecil Plains, QLD	07/2017	177131	NLIB-3
		Warragamba, NSW	09/2018	190878	NLIB-4
Peppermint	*Eucalyptus radiata*	Marulan, NSW	04/2017	175055	P-1
		Tumbarumba, NSW	02/2018	183493	P-2
		Sydney South, NSW	09/2017	182502	P-3
		Tumbarumba, NSW	02/2018	183496	P-4
Coolibah	*Eucalyptus microtheca*	Moree, NSW	01/2017	176944	C-1
		Cooba, NSW	06/2017	194202	C-2
Hill gum (Pink gum)	*Eucalyptus fasciculosa*	Keith, SA	06/2018	188028	HG-1
		Tumut, NSW	11/2017	182506	HG-2
		Gundagai, NSW	11/2017	181449	HG-3
		Warwick, QLD	01/2018	182699	HG-4
		West Wyalong, NSW	11/2017	183144	HG-5
		Cootamundra, NSW	12/2017	181439	HG-6
Grey box	*Eucalyptus microcarpa*	Temora, NSW	09/2018	189129	GB-1
White stringybark	*Eucalyptus globoidea*	Glen Innes, NSW	09/2018	190571	WSB-1
Mallee, SA	*Eucalyptus porosa*	Elliston, SA	10/2018	195991	Mall-1
Mahogany	*Eucalyptus robusta*	Grafton, NSW	12/2018	191906	Mah-1

NA, Not applicable

Table 2 Maximal DPPH effect, EC_{50} and radical scavenging capacity of Australian *Eucalyptus* and NZ manuka honeys, and sugar mixture

Sample Identifier	Maximum effect (%)	EC_{50} mg/mL honey	GAE µg/g honey	Radical Scavenging Capacity $mg.mL^{-1}.min^{-1}$			
				1 min	10 min	30 min	150 min
Sugar mixture	4.4±2.3	NA	NA	0	$2.8±2.6x10^{-7}$	$4.0±2.1x10^{-7}$	$1.8±1.6x10^{-7}$
NZM-1	78.0±1.2	20.2±0.8	0.85±0.04	$8.7±0.7x10^{-4}$	$3.3±0.3x10^{-4}$	$1.7±0.3x10^{-4}$	$5.0±0.0x10^{-5}$
NZM-2	73.5±1.6	15.6±0.2	0.85±0.02	$1.0±0.0x10^{-3}$	$5.0±0.0x10^{-4}$	$2.0±0.0x10^{-4}$	$5.0±0.0x10^{-5}$
NZM-3	68.8±0.7	19.6±0.4	0.75±0.02	$6.0±2.6x10^{-4}$	$3.0±1.0x10^{-4}$	$1.6±0.4x10^{-4}$	$4.0±0.6x10^{-5}$
NZM-4	72.7±0.3	23.6±0.8	0.79±0.02	$6.3±1.4x10^{-4}$	$3.0±0.6x10^{-4}$	$1.3±0.4\ x10^{-4}$	$4.3±0.7x10^{-5}$
RRG-1	72.1±3.2	17.3±0.4	0.62±0.09	$4.3±0.7x10^{-4}$	$2.3±0.3x10^{-4}$	$9.3±0.7x10^{-5}$	$3.0±0.0x10^{-5}$
RRG-2	66.6±3.9	24.1±1.7	0.74±0.06	$4.0±0.0x10^{-4}$	$2.0±0.0x10^{-4}$	$1.0±0.0x10^{-4}$	$3.0±0.0x10^{-5}$
RRG-3	67.0±0.7	18.5±0.2	0.66±0.09	$5.0±0.6x10^{-4}$	$2.0±0.3x10^{-4}$	$9.5±0.4x10^{-5}$	$3.0±0.0x10^{-5}$
RRG-4	70.6±0.8	19.0±0.7	0.60±0.05	$5.0±0.0x10^{-4}$	$3.0±0.0x10^{-4}$	$1.0±0.0x10^{-4}$	$3.7±0.3x10^{-5}$
YB-1	54.1±0.7	30.2±3.3	0.29±0.04	$2.7±0.7x10^{-4}$	$9.7±0.3x10^{-5}$	$5.7±0.9x10^{-5}$	$1.7±0.3x10^{-5}$
YB-2	56.1±0.3	23.2±1.5	0.42±0.00	$5.3±1.4x10^{-4}$	$2.3±0.3x10^{-4}$	$8.7±0.7x10^{-5}$	$3.0±0.6x10^{-5}$
YB-3	46.0±0.5	32.8±0.6	0.50±0.00	$1.7±0.3x10^{-4}$	$9.3±0.7x10^{-5}$	$5.0±0.6x10^{-5}$	$1.3±0.3x10^{-5}$
YB-4	64.2±0.7	23.4±1.9	0.48±0.01	$5.3±1.3x10^{-4}$	$2.3±0.3x10^{-4}$	$1.0±0.0x10^{-4}$	$3.7±0.3x10^{-5}$
WB-1	59.1±2.0	30.4±2.1	0.38±0.06	$3.7±0.7x10^{-4}$	$1.7±0.3x10^{-4}$	$7.7±0.7x10^{-5}$	$2.3±0.3x10^{-5}$
WB-2	55.4±2.8	31.6±1.8	0.37±0.05	$5.0±0.6x10^{-4}$	$1.7±0.3x10^{-4}$	$7.0±0.0x10^{-5}$	$2.0±0.0x10^{-5}$
WB-3	58.2±2.0	30.4±3.0	0.36±0.11	$3.0±0.0x10^{-4}$	$1.3±0.3x10^{-4}$	$7.0±1.5x10^{-5}$	$1.7±0.3x10^{-5}$
WB-4	64.3±2.0	27.9±2.3	0.56±0.07	$5.0±0.6x10^{-4}$	$2.0±0.6x10^{-4}$	$8.7±0.7x10^{-5}$	$2.3±0.3x10^{-5}$
M-1	53.2±1.5	21.1±0.8	0.40±0.01	$4.7±0.3x10^{-4}$	$2.7±0.3x10^{-4}$	$1.0±0.0x10^{-4}$	$4.0±0.0x10^{-5}$
M-2	47.9±2.2	25.0±3.8	0.38±0.03	$3.0±0.6x10^{-4}$	$2.0±0.0x10^{-4}$	$7.7±0.3x10^{-5}$	$2.7±0.3x10^{-5}$
M-3	64.4±5.4	17.1±1.7	0.58±0.15	$1.1±0.1x10^{-3}$	$5.7±0.3x10^{-4}$	$2.0±0.0x10^{-4}$	$3.3±0.3x10^{-5}$
M-4	26.9±2.4	20.8±1.9	0.24±0.03	$2.0±0.0x10^{-4}$	$1.2±0.4x10^{-4}$	$6.0±1.5\ x10^{-5}$	$2.3±0.3x10^{-5}$
GIB-1	70.9±0.2	18.0±0.2	0.71±0.10	$3.0±0.0x10^{-4}$	$2.0±0.0x10^{-4}$	$9.3±0.3x10^{-5}$	$3.3±0.3x10^{-5}$
GIB-2	70.7±4.1	18.4±3.0	0.52±0.03	$7.7±0.9x10^{-4}$	$3.3±0.3x10^{-4}$	$1.3±0.3x10^{-4}$	$3.7±0.3x10^{-5}$
GIB-3	70.4±0.5	15.8±1.1	0.56±0.03	$8.7±0.3x10^{-4}$	$3.7±0.3x10^{-4}$	$1.5±0.4x10^{-4}$	$4.0±0.0x10^{-5}$
GIB-4	70.8±0.6	20.8±1.4	0.74±0.02	$5.7±1.4x10^{-4}$	$2.7±0.3x10^{-4}$	$9.0±0.6x10^{-5}$	$3.3±0.3x10^{-5}$
GIB-5	69.8±0.8	31.0±1.3	0.72±0.02	$3.7±0.3x10^{-4}$	$2.0±0.0x10^{-4}$	$8.7±0.7x10^{-5}$	$2.7±0.3x10^{-5}$

CIB-1	54.8±2.0	35.3±0.8	0.42±0.02	$1.7\pm0.3\times10^{-4}$	$8.7\pm0.9\times10^{-5}$	$3.7\pm0.3\times10^{-5}$	$1.0\pm0.0\times10^{-5}$
CIB-2	61.3±1.9	20.1±2.2	0.44±0.03	$4.0\pm1.0\times10^{-4}$	$1.0\pm0.0\times10^{-4}$	$7.0\pm0.0\times10^{-5}$	$3.7\pm1.7\times10^{-5}$
CIB-3	49.6±2.0	35.1±1.1	0.31±0.02	$1.3\pm0.3\times10^{-4}$	$6.3\pm0.3\times10^{-5}$	$2.7\pm0.3\times10^{-5}$	$9.0\pm0.6\times10^{-6}$
BTIB-1	67.7±1.4	30.7±0.8	0.75±0.02	$2.2\pm0.8\times10^{-4}$	$1.2\pm0.4\times10^{-4}$	$5.3\pm0.7\times10^{-5}$	$1.7\pm0.3\times10^{-5}$
BTIB-2	70.8±0.6	22.5±3.4	0.53±0.11	$8.0\pm1.5\times10^{-5}$	$4.0\pm1.5\times10^{-5}$	$2.3\pm0.9\times10^{-5}$	$8.3\pm0.9\times10^{-6}$
BTIB-3	67.8±0.1	29.4±1.5	0.53±0.05	$3.3\pm0.3\times10^{-4}$	$1.7\pm0.3\times10^{-4}$	$6.3\pm0.7\times10^{-5}$	$1.7\pm0.3\times10^{-5}$
BTIB-4	61.5±0.7	30.7±2.4	0.33±0.05	$2.0\pm0.0\times10^{-4}$	$9.7\pm0.3\times10^{-5}$	$5.7\pm0.3\times10^{-5}$	$2.0\pm0.0\times10^{-5}$
MIB-1	36.8±0.8	35.7±2.3	0.37±0.00	$1.3\pm0.3\times10^{-4}$	$7.7\pm1.5\times10^{-5}$	$4.0\pm0.6\times10^{-5}$	$1.0\pm0.0\times10^{-5}$
MIB-2	67.9±0.3	22.3±1.5	0.43±0.09	$4.3\pm0.7\times10^{-4}$	$2.3\pm0.3\times10^{-4}$	$8.7\pm0.9\times10^{-5}$	$2.7\pm0.3\times10^{-5}$
MIB-3	64.7±1.5	28.4±0.8	0.52±0.06	$1.3\pm0.3\times10^{-4}$	$6.7\pm0.9\times10^{-5}$	$3.7\pm0.3\times10^{-5}$	$1.0\pm0.0\times10^{-5}$
MIB-4	72.2±0.8	26.4±0.4	0.82±0.01	$4.3\pm0.7\times10^{-4}$	$2.0\pm0.0\times10^{-4}$	$8.3\pm0.3\times10^{-5}$	$2.3\pm0.3\times10^{-5}$
MIB-5	49.4±3.1	30.9±4.4	0.43±0.04	$9.3\pm0.7\times10^{-5}$	$6.7\pm0.9\times10^{-5}$	$3.7\pm0.3\times10^{-5}$	$1.0\pm0.0\times10^{-5}$
NLIB-1	71.4±0.7	19.3±0.5	0.70±0.10	$4.7\pm0.7\times10^{-4}$	$2.3\pm0.3\times10^{-4}$	$9.3\pm0.3\times10^{-5}$	$2.7\pm0.3\times10^{-5}$
NLIB-2	73.9±4.5	21.6±3.5	0.64±0.10	$2.7\pm1.2\times10^{-4}$	$1.0\pm0.0\times10^{-4}$	$4.7\pm1.3\times10^{-5}$	$2.0\pm0.0\times10^{-5}$
NLIB-3	70.0±1.4	19.5±0.4	0.51±0.03	$4.7\pm0.3\times10^{-4}$	$2.3\pm0.3\times10^{-4}$	$8.3\pm0.9\times10^{-5}$	$2.3\pm0.3\times10^{-5}$
NLIB-4	67.4±2.5	22.2±2.3	0.56±0.04	$3.7\pm0.3\times10^{-4}$	$1.7\pm0.3\times10^{-4}$	$9.0\pm1.0\times10^{-5}$	$2.7\pm0.3\times10^{-5}$
P-1	53.4±3.2	30.5±2.7	0.28±0.03	$1.1\pm0.5\times10^{-4}$	$8.7\pm0.3\times10^{-5}$	$5.0\pm0.0\times10^{-5}$	$2.0\pm0.0\times10^{-5}$
P-2	33.1±0.6	20.5±1.8	0.22±0.03	$3.3\pm0.7\times10^{-4}$	$2.7\pm0.3\times10^{-4}$	$1.0\pm0.0\times10^{-4}$	$3.3\pm0.7\times10^{-5}$
P-3	66.0±0.6	23.9±4.4	0.76±0.18	$3.7\pm0.3\times10^{-4}$	$2.0\pm0.0\times10^{-4}$	$9.3\pm0.7\times10^{-5}$	$3.0\pm0.0\times10^{-5}$
P-4	48.7±4.0	30.5±3.2	0.39±0.01	$4.0\pm1.0\times10^{-4}$	$1.7\pm0.3\times10^{-4}$	$9.3\pm0.3\times10^{-5}$	$3.0\pm0.0\times10^{-5}$
C-1	70.4±0.9	18.8±1.9	0.56±0.04	$6.3\pm0.7\times10^{-4}$	$2.7\pm0.3\times10^{-4}$	$9.3\pm0.7\times10^{-5}$	$2.7\pm0.3\times10^{-5}$
C-2	69.3±2.0	17.8±1.7	0.50±0.01	$8.3\pm0.7\times10^{-4}$	$3.7\pm0.3\times10^{-4}$	$1.3\pm0.3\times10^{-4}$	$4.0\pm0.0\times10^{-5}$
HG-1	73.0±0.9	22.1±0.5	0.83±0.02	$5.3\pm1.2\times10^{-4}$	$3.3\pm0.3\times10^{-4}$	$1.0\pm0.0\times10^{-4}$	$3.3\pm0.3\times10^{-5}$
HG-2	49.6±1.1	22.0±0.6	0.47±0.06	$2.7\pm0.3\times10^{-4}$	$1.0\pm0.0\times10^{-4}$	$5.3\pm0.3\times10^{-5}$	$2.0\pm0.0\times10^{-5}$
HG-3	58.3±2.4	19.4±1.9	0.33±0.03	$3.7\pm0.3\times10^{-4}$	$2.0\pm0.0\times10^{-4}$	$7.7\pm0.3\times10^{-5}$	$2.7\pm0.3\times10^{-5}$
HG-4	71.7±0.3	19.5±2.8	0.44±0.09	$5.7\pm0.3\times10^{-4}$	$3.0\pm0.0\times10^{-4}$	$1.0\pm0.0\times10^{-4}$	$4.0\pm0.0\times10^{-5}$
HG-5	71.1±1.3	21.8±1.9	0.60±0.05	$3.0\pm0.6\times10^{-4}$	$1.7\pm0.3\times10^{-4}$	$7.0\pm1.0\times10^{-5}$	$2.3\pm0.3\times10^{-5}$
HG-6	68.8±2.4	28.0±3.9	0.58±0.04	$4.3\pm0.9\times10^{-4}$	$1.7\pm0.3\times10^{-4}$	$7.3\pm0.3\times10^{-5}$	$2.0\pm0.0\times10^{-5}$
GB-1	65.4±3.9	27.8±1.4	0.74±0.06	$3.7\pm0.3\times10^{-4}$	$2.3\pm0.3\times10^{-4}$	7.0 ± 2.010^{-5}	$3.0\pm0.0\times10^{-5}$
WSB-1	74.0±0.9	16.0±0.1	0.80±0.01	$1.1\pm0.1\times10^{-3}$	$4.0\pm0.0\times10^{-4}$	$2.0\pm0.0\times10^{-4}$	$4.0\pm0.0\times10^{-5}$
Mall-1	63.7±1.3	23.0±0.6	0.72±0.03	$2.3\pm0.3\times10^{-4}$	$1.3\pm0.3\times10^{-4}$	$7.7\pm0.3\times10^{-5}$	$3.0\pm0.0\times10^{-5}$
Mah-1	65.9±2.7	28.8±1.9	0.55±0.03	$4.3\pm0.9\times10^{-4}$	$1.7\pm0.3\times10^{-4}$	$9.0\pm0.6\times10^{-5}$	$3.3\pm0.3\times10^{-5}$

Gallic acid equivalent (GAE). Mean±SEM

Table 3 DPPH EC_{50} and free radical scavenging capacity for honey varieties of the same primary botanical source, where honeys were obtained from ≥3 geographical regions

Sample Identifier	Maximum effect (%)	EC_{50} mg/mL honey	GAE µg/g honey	Radical Scavenging Capacity $mg.mL^{-1}.min^{-1}$			
				1 min	10 min	30 min	150 min
NZ Manuka (n=4)	73.2±1.89 [a]	19.8±1.64	0.81±0.03 [c]	$7.7±1.0x10^{-4}$	$3.6±0.5x10^{-4}$	$1.7±0.1x10^{-4}$ [h]	$4.6±0.3x10^{-5}$ [j]
River red gum (n=4)	69.1±1.34	19.7±1.50	0.66±0.03	$4.6±0.3 x10^{-4}$	$2.3±0.2x10^{-4}$ [e]	$9.7±0.2x10^{-5}$	$3.2±0.2x10^{-5}$ [k]
Yellow box (n=4)	55.1±3.74	27.4±2.43	0.42±0.05 [d]	$3.8±0.9x10^{-4}$	$1.6±0.4x10^{-4}$	$7.4±1.2x10^{-5}$ [i]	$2.4±0.6x10^{-5}$
White Box (n=4)	59.2±1.86 [b]	30.1±0.78	0.42±0.05 [d]	$4.2±0.5x10^{-4}$	$1.7±0.1x10^{-4}$	$7.6±0.4x10^{-5}$	$2.1±0.1x10^{-5}$ [l]
Messmate (n=4)	48.1±7.86	21.0±1.61	0.40±0.07 [d]	$5.1±1.9x10^{-4}$	$2.9±1.0x10^{-4}$	$1.1±0.3x10^{-4}$	$3.1±0.4x10^{-5}$
Grey I.B. (n=5)	70.5±0.20	20.8±2.67	0.65±0.05	$5.7±1.1x10^{-4}$	$2.7±0.3x10^{-4}$ [f]	$1.1±0.1x10^{-4}$	$3.4±0.2x10^{-5}$ [k]
Caley's I.B. (n=3)	55.2±3.38	30.2±5.03	0.39±0.04 [d]	$2.3±0.8x10^{-4}$	$8.3±1.0x10^{-5}$ [g]	$4.5±1.3x10^{-5}$ [i]	$1.9±0.9x10^{-5}$
Blue Top I.B. (n=4)	67.0±1.95	28.3±1.97	0.54±0.09	$2.1±0.5x10^{-4}$	$1.1±0.3x10^{-4}$	$4.9±0.9x10^{-5}$ [i]	$1.6±0.3x10^{-5}$ [m]
Mugga I.B. (n=5)	58.2±6.59	28.7±2.24	0.51±0.08	$2.5±0.8x10^{-4}$	$1.3±0.4x10^{-4}$	$5.7±1.2x10^{-5}$ [i]	$1.6±0.4x10^{-5}$ [l]
Narrow Leaf I.B (n=4)	70.7±1.36	20.7±0.73	0.60±0.04	$3.9±0.5x10^{-4}$	$1.8±0.3x10^{-4}$	$7.8±1.1x10^{-5}$	$2.4±0.2x10^{-5}$ [l]
Peppermint (n=4)	50.3±6.80	26.4±2.49	0.41±0.12 [d]	$3.6±0.2x10^{-4}$	$1.8±0.4x10^{-4}$	$8.4±1.1x10^{-5}$	$2.8±0.3x10^{-5}$
Hillgum (n=6)	65.4±3.84	22.1±1.28	0.54±0.07	$4.1±0.5x10^{-4}$	$2.1±0.4x10^{-4}$	$7.9±0.8x10^{-5}$	$2.7±0.3x10^{-5}$ [l]

Maximum effect: a>b. Gallic acid equivalent (GAE): c>d. Radical scavenging capacity at 10 min: e,f>g; at 30 min: h>l; at 150 min: j,k>m; j>l. Mean±SEM, P<0.05.

CRediT authorship contribution statement

Soheila Beiranvand: Investigation, Validation, Methodology, Writing – original draft. **Ashley Williams:** Investigation, Validation, Methodology, Writing – original draft, Review and editing. **Symsia Long:** Investigation, Methodology, Review and editing. **Peter Brooks:** Investigation,

Validation, Supervision, Review and editing, Funding acquisition. **Fraser Russell:** Conceptualization, Investigation, Validation, Supervision, Review and editing, Funding acquisition, Project administration.

Use of kinetic data to model potential antioxidant activity: Radical Scavenging Capacity of Australian *Eucalyptus* Honeys by Soheila Beiranvand, Ashley Williams, Symsia Long, Peter Brooks and Fraser Russell.

Highlights

- Antioxidant activity determined for 53 Australian honeys from 16 *Eucalyptus* species
- Kinetic DPPH$^{\bullet}$ data modelling of the radical scavenging capacity of honeys
- Honey activity was differentiated by radical scavenging capacity but not EC_{50}
- Most active honeys were from *E. paniculate* and *E. camaldulensis* species