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Microwave-assisted extraction of *Eucalyptus robusta* leaf for the optimal yield of total phenolic compounds

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ABSTRACT

Eucalyptus robusta (*E. robusta*) has a significant value in traditional medicine and recently has been shown to possess many pharmacological properties *in vitro*. This study was designed to utilise microwave-assisted extraction (MAE) to yield optimal total phenolic content (TPC), total flavonoid content (TFC), proanthocyanidin levels and antioxidant capacity from *E. robusta* using water as the solvent, facilitated by the use of response surface methodology (RSM). A three-level-three-factor Box–Behnken design was implemented to elucidate the effect of irradiation time, power and sample-to-solvent ratio on the yields of these phytochemicals. The results highlighted the accuracy and reliability of RSM as a tool for predicting the yields of TPC, TFC, proanthocyanidins and total antioxidants using MAE. Sample-to-solvent ratio had the greatest impact on the TPC yield followed by power and irradiation time. The optimal MAE conditions for TPC and TFC were 3 min, 600 W power and 2 g/100 mL sample-to-solvent ratio. The experimental yield of TPC was 58.40 ± 1.03 mg GAE/g, and 19.15 ± 1.06 mg RE/g of TFC was obtained under these optimal conditions. These conditions, optimised for maximum TPC yield also liberated 62%, 64.6%, 66.3% and 67% of the maximum proanthocyanidins, ABTS, DPPH and CUPRAC values, respectively. This study revealed that MAE is a reliable and efficient method for extracting high yields of phytochemicals from *E. robusta*, with significant potential to be up-scaled for industrial, nutraceutical or pharmaceutical applications.

1. Introduction

Eucalyptus robusta is native to a narrow coastal area in south-eastern Australia (King and Skolmen, 1990). Due to the widely adaptable nature of the species, it has been introduced into many climatic regions around the world including tropical, subtropical and warm-temperate (King and Skolmen, 1990). Although, *E. robusta* is utilised in a range of ways including timber production, fuel, watershed protection, and windbreaks (King and Skolmen, 1990). *E. robusta* has been reported to possess many pharmacological properties that has seen it employed in traditional medicinal formulations. In Chinese traditional medicine, only the leaves are used to treat malaria (Konoshima and Takasaki, 2002), while in other parts of the world, both the leaves and bark have been used to treat an array of ailments including fever, skin diseases, dysentery, malaria and bacterial diseases (Nagpal et al., 2010).

The major non-volatile compounds found abundantly in *Eucalyptus* are phenolic compounds which contribute significantly to the antioxidant activities of extracts (Al-Sayed et al., 2012; Almeida et al., 2009). In general, several phenolic compounds such as gallic acid, protocatechuic acid, ellagic acid, quercetin, quercetin glycoside, naringenin, catechin, epicatechin, rutin, quercitrin, apigenin, and myricetin have been isolated from *Eucalyptus* extracts (Al-Sayed et al., 2012; Vázquez et al., 2012). Santos et al. (2012) identified epicatechin, catechin, quercetin-glucuronide, ellagic acid-rhamnoside, ellagic acid, galloyl-bis-hexahydroxydiphenoyl (HHDP)-glucose, gallic acid, chlorogenic acid and methyl-ellagic acid-pentose in *E. grandis, E. urograndis* and *E. maidenii* bark extracts. These results emphasized the high potential of *Eucalyptus* species as source of biologically active phenolic compounds (Santos et al., 2012). The anti-proliferative activity of the phenolic compounds present in *E. globulus* bark was illustrated by Mota et al. (2012). In addition, Santos et al. (2012) demonstrated that

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Table 1
Box–Behnken design and observed responses.

Run	Microwave conditions			Experimental values ($n=3$)					
	Irradiation Time (min)[a]	Power (%)[b]	Ratio (g:100 mL)	TPC (mg GAE/g)	TFC (mg RE/g)	Proanthocyanidins (mg CAE/g)	Antioxidant capacity (mg TE/g)		
							ABTS	DPPH	CUPRAC
1	1	40	5	37.97	13.16	8.42	104.85	99.76	96.52
2	1	50	8	42.05	17.32	12.68	97.81	49.94	92.04
3	1	50	2	43.76	15.05	7.96	65.45	66.06	169.28
4	1	60	5	45.42	14.05	15.07	104.72	85.45	106.17
5	2	50	5	30.05	11.11	10.03	91.15	53.51	100.26
6	2	60	8	38.32	9.47	16.67	98.01	77.23	164.57
7	2	50	5	29.08	12.52	13.75	104.53	57.87	132.48
8	2	40	2	39.45	15.64	8.57	64.19	68.11	148.85
9	2	50	5	30.21	11.86	10.53	104.79	56.36	100.33
10	2	40	8	33.18	15.83	12.72	97.82	55.32	116.35
11	2	60	2	48.59	16.38	11.76	65.49	67.56	153.35
12	3	60	5	45.91	18.11	9.52	104.75	100.96	224.56
13	3	40	5	40.53	18	15.78	104.64	108.66	225.93
14	3	50	2	55.26	22.22	8.5	65.56	69.52	148.66
15	3	50	8	45.33	21.09	15.77	70.88	65.33	236.92

[a] Extraction time is 2X of irradiation time as 10 s ON and 10 s OFF strategy was implemented.
[b] 40, 50 and 60% power were equivalent to 480 W, 600 W and 720 W, respectively.

there is a positive correlation between phenolic contents and the antioxidant activities of *E. grandis, E. urograndis* and *E. maidenii* bark extracts. Antioxidants such as flavonoids and other phenolics have gained more attention in recent years as potential agents for their therapeutic values and cardioprotective, anticarcinogenic and antimutagenic properties (Fu et al., 2010; Gharekhani et al., 2012; Luis et al., 2014).

A recent study by Fu et al. (2010) illustrated the significant antioxidant capacity and total phenolic contents of the fruits of the *E. robusta,* indicating a strong correlation between the two parameters. In addition, essential oil from *E. robusta* has been shown to possess anti-microbial properties against various bacterial and fungal pathogens *in vitro* (Cimanga et al., 2002; Sartorelli et al., 2007) as well as larvicidal and adulticidal activity (Lucia et al., 2012). These findings clearly suggest strong bioactivity in the phytochemical profile of *E. robusta* and as such, prospective application in other fields of medicine. Comprehensive characterization of the phytochemical profile and associated bioactivity of *E. robusta* is, therefore, crucial. Optimal extraction conditions for polyphenolics have not yet been established to allow for further study of *E. robusta*. This manuscript presents the first comprehensive study assessing parameters associated with optimal extraction conditions.

Although, a number of organic solvents are used for extraction of phenolic compounds from *Eucalyptus*, water is inarguably the safest, cheapest and most environmentally friendly and accessible polar extraction solvent. Moreover, it is traditionally employed for plant bioactive extractions in the form of decoction and infusions (Goldsmith et al., 2014; Vuong et al., 2011a). Water has also been employed in the extraction of phenolic compounds from *E. globulus* and *Eucalyptus* hybrids (Almeida et al., 2009; Hasegawa et al., 2008; Santos et al., 2011; Chapuis-Lardy et al., 2002).

Microwave assisted extraction (MAE) is a novel extraction method that has gained significance recently due to its shortened extraction time and reduced solvent consumption (Gharekhani et al., 2012; Ince et al., 2013). It is one of the dominant trends of the 'green chemistry' movement (Saifuddin et al., 2014). Conventional Soxhlet extraction usually requires long extraction times leading to thermal degradation of phyto-constituents (Gharekhani et al., 2012). In MAE, the internal pressure of solid media is increased by microwaves resulting in enhanced extraction efficiency (Bayramoglu et al., 2008). This also reduces the deterioration of phenolic compounds (Ince et al., 2013). The current literature does not provide any information regarding the optimal utilization of water and MAE for extraction of phenolic compounds from *E. robusta*. Therefore, the present study was undertaken to optimize the three MAE parameters of irradiation time, power and sample-to-solvent ratio for extracting maximal levels of phenolic compounds from *E. robusta* using response surface methodology (RSM) and water as the solvent system (Yemis and Mazza, 2012).

2. Materials and methods

2.1. Plant materials

Fresh leaves of *E. robusta* were collected on 2nd April, 2014 from cultivated plants located at Ourimbah, Central Coast, NSW, Australia (latitude of 33.4°S, longitude of 151.4°E). The plants were authenticated by one of the authors (A.C.C.) and a voucher specimen deposited at the Don McNair Herbarium (Accession number 10492), at the University of Newcastle, NSW, Australia. The leaves were immediately transferred to the laboratory and stored at −20 °C to limit the degradation of phenolic compounds. Using a dry air oven, the leaves were dried at 70°C for 5 h before commencing the experiments. The leaves then were ground to a fine powder using a commercial grade blender (Rio™ Commercial Bar Blender, Hamilton Beach) and stored at −20 °C until required.

2.2. Microwave-assisted extraction (MAE)

A household microwave equipped with inverter technology (1200 W, Frequency 2450 MHz, Sharp Carousel, Japan) was used for optimizing the MAE conditions. Water was used as the solvent system, and parameter permutations as designed by the response surface methodology software were implemented. The extraction was carried out in sealed vessels and no evaporation was observed.

2.3. Response surface methodology (RSM)

JMP software (version 11) was utilized for RSM experimental design and analysis. A three-level-three-factor, Box–Behnken design was applied with three central point replicates for designing experimental conditions based on the results of preliminary single-factor-test (Liu et al., 2013) to elucidate the influence of the three primary independent microwave parameters: irradiation time (1–3 min), power (40–60% or 480–720 W) and sample-to-solvent ratio 2–8 (g/100 mL). Prior to irradiation, pre-leaching was performed for each suspension for 5 min. The leaf samples were irradiated with different power settings (40–60% or 480–720 W)