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Dracorhodin Content and Selected Bioactivities of *Calamus ruber*' Dragon Blood Resin from Different Drying Conditions

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ABSTRACT Dragon blood resin from the fruit barks of *Calamus ruber* contains a bioactive compound, dracorhodin, that has many medicinal benefits. The study aimed to investigate the effects of different drying methods on dracorhodin content, cytotoxicity and antioxidant activities of the resin. Hot-air and hot-steam-air drying at 55°C and 60°C were employed in this study. Resin dried with the hot-steam-air drying at 55° was most toxic to *Artemia salina* (LC_{50} of 430.61 ppm). Resin dried with hot-steam-air drying at 60°C contained the highest dracorhodin content (4.34%) and was the best at scavenging DPPH• (1,1-diphenyl-2-picrylhydrazyl) (IC_{50} of 32.73 ppm), cupric ion (TEAC of 63.15 ppm) and ferric ion (TEAC of 8.73 ppm). Resin from the hot-air-drying at 55°C was the best at scavenging $ABTS^{•+}$ (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) (TEAC of 469.72 ppm). Drying method and temperature affected the dracorhodin content and IC_{50} of the resin, respectively.

INTRODUCTION

Dragon blood resin is a red-coloured resin exuded by four plant genera, namely, *Daemonorops* spp, *Dracaena* spp., *Croton* spp, and *Pterocarpus* spp (Purwanti et al. 2019; Andini et al. 2021; Sari et al. 2022). Pterocarpus and Croton are plants endemic to South America. Conversely, the Dracaena and Daemonorops grow primarily in Africa and Southeast Asia (Indonesia, India and Malaysia) (Purwanti et al. 2019; Andini et al. 2020b; Andini et al. 2021). The Daemonorops genus comprises approximately 115 species. However, only 10 to 15 species (or approximately 10%) produce dragon blood resin (Andini et al. 2020a). One such species is *Daemonorops rubra*, which is used for this research. It has been renamed *Calamus ruber* (*C. ruber*) according to the comprehensive phylogenetic-based plant classification report (Baker 2015).

The dragon blood resin obtained from the *Calamus* species is known as *jernang*. Dragon blood resin has been used as a traditional chest-pain-relieving medicine and hemostatic agent to alleviate postpartum bleeding, internal trauma and menstrual irregularities by the local communities (Baja-Lapis 2009; Andini et al. 2020a). Recent studies have demonstrated its additional medicinal benefits, including antioxidant, anti-tumour, anti-cancer, anti-diarrhoeal, anti-ulcer, anti-microbial and anti-inflammatory activities (Krishnaraja et al. 2019; Purwanti et al. 2019; Andini et al. 2020a; Park et al. 2022; Sari et al. 2022). This resin is a highly valuable medicinal commodity with a selling price of up to USD 120 per kilogram in its dry powder form (Andini et al. 2020a).

The various medicinal benefits of this resin originate from the presence of several active components. Among them, dracorhodin is a flavylium chromophore of the anthocyanin family and is used as a resin identifier and quality determinant. This bio-

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compound exhibits antioxidant, ultraviolet-blocking, anti-tumour and wound-healing activities (Andini et al. 2020a; Sari et al. 2022; Xiong et al. 2022).

Dracorhodin is a main component in the Shengji Huayu formula, which is a traditional Chinese medicine that has been widely used for decades to treat diabetic ulcers (Zhang et al. 2023). Furthermore, dracorhodin perchlorate (DP), which is a chemically synthesised analogue of dracorhodin, exhibits potential cytotoxic effects and can induce apoptosis in cancer and tumour cells (Xia et al. 2004; Lu et al. 2019). Additionally, DP is recommended for the adjuvant treatment of diabetic-wound healing (Liu et al. 2019; Deng et al. 2022; Xiong et al. 2022) or therapeutic treatment of the osteoclast-related bone diseases (Liu et al. 2020).

The preparation of dragon blood resin as a medicinal ingredient includes resin extraction from the barks of rattan fruits through a soaking process in methanol/hot water/other solvents, followed by a drying process. Drying reduces the moisture content (MC) of a biomaterial and inhibits the growth of microorganisms in medicinal plants (Kaur et al. 2019). The air drying of resin in an open field and under a shade is a common practice adopted by resin farmers. Air drying is a simple technique commonly used to prepare any medicinal or ornamental plant parts (Sayeed and Thakur 2020; Momin et al. 2022). However, the air drying of resin is generally time consuming. Additionally, long exposure in an open field potentially increases the vulnerability of resin to microbial attacks or causes contamination, which may further affect the quality and cleanness of resin.

The use of an artificial dryer to dry resin can potentially address the issues associated with the air drying of resin, such as long periods and microbial contamination. In this study, hot-air and hot-steam-air drying are proposed as potential artificial methods for drying dragon blood resin. The hot-air-drying process uses the heat originating from the electrically heated resistive elements inside a dryer or oven. It is a commonly used method for drying herbs and is widely adopted in various industries due to its rapid heat transfer and stable temperatures (Thamkaew et al. 2021; Bai et al. 2023). In contrast, a general hot-steam-air dryer uses hot steam as its heat source. Hot steam has been previously employed as a drying medium in the superheated-steam drying of various food products. This particular drying technology produces decent quality dried products and saves operational-energy costs owing to short exposures at temperature below 100^0C (with vacuum addition) or above 250^0C (Patel and Bade 2020; Roslan et al. 2020; Adewumi et al. 2021; Sobulska et al. 2022).

Thus far, the hot-air or hot-steam-air drying of dragon blood resin has not been extensively explored. The impact of these drying methods on the dracorhodin content or bio-activities, such as cytotoxicity and antioxidant activity/capacity, of the resin has also not been reported. A drying process should be carefully conducted because it could affect the quality and essential biological activities of medicinal plants, such as antioxidant properties (Kaur et al. 2019; Zhang et al. 2019). Additionally, heat application can potentially deteriorate dracorhodin, which is a flavonoid compound. Therefore, the determination of an appropriate drying condition for dragon blood resin is essential to minimise the risk of its quality deterioration.

Objectives

This study aimed to investigate the effects of hot-air and hot-steam-air drying on the dracorhodin content, cytotoxicity and antioxidant activity/capacity of dragon blood resin.

METHODOLOGY

Material

Primary materials used in this study were *C. ruber* rattan fruits (locally known as *javanese jernang*) purchased from a resin entrepreneur in Sukaharja subdistrict, Ciamis regency, West Java province, Indonesia. Other materials used were *Artemia salina* (*A. salina*) shrimp larvae, methanol, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2¹-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 6-hydroxy-2,5,7,8-tetrametylchroman-2-carboxylic acid (Trolox), CuCl solution, NH 4 Ac buffer pH 7, neokuproin and ferric reducing antioxidant power (FRAP) reagent.

Resin Extraction and Drying Experiments

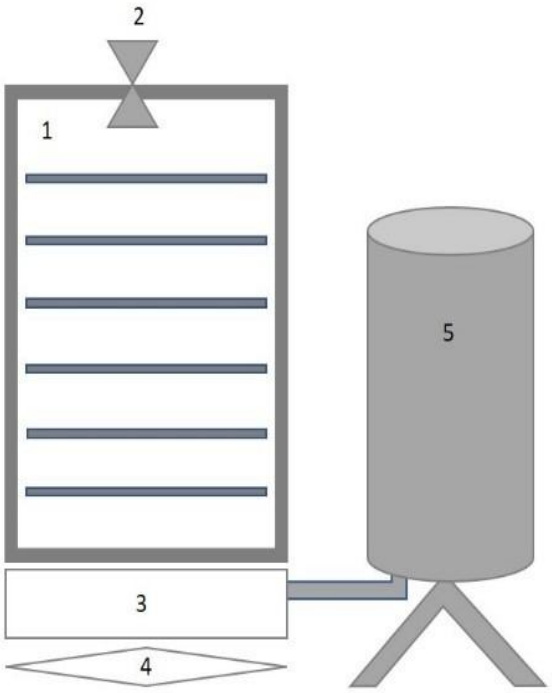
Resin was immediately extracted from the bark of the rattan fruit to avoid fruit spoilage. First, 1 kg of the rattan fruit was soaked in 1000 mL of metha-

nol for 2 hours. The dissolved resin and water were transferred into small containers and left for 1 week to obtain resin deposits at the container base. Approximately 0.01 kg of wet resin was obtained from 1 kg of the rattan fruit. All resin deposits were packed in vacuum-sealed plastic bags and stored in a refrigerator to maintain their freshness.

The hot-air-drying experiment was performed in a laboratory oven (Memmert). The hot-steam-air dryer consisted of a drying chamber equipped with an exhaust fan on the chamber top, water bath under the chamber connected by pipes to the chamber, storage tank beside the chamber to distribute water into the water bath and gas burner under the water bath to aid the water-boiling process (Fig. 1). The hot-steam air produced via the water-boiling process occurring in the water bath was distributed into the chamber through the connecting pipes.

Two drying temperatures, 55°C and 60°C, were employed in each drying method. Generally, the maximum temperature applied for drying herbs is 60°C (Thamkaew et al. 2021). Under each drying condition, three samples with an initial weight of 0.1 kg were tested. Approximately 0.02 kg from the initial sample of 0.1 kg wet resin was used for the initial MC estimation using the gravimetric method. The initial MC of all the resin samples was in the range of 66.55 percent to 82.26 percent.

The remaining amount, that was, 0.98 kg, of each resin sample was spread evenly in a 200 mm × 200 mm square pan, forming an average thickness of 1 mm. Each resin sample was dried until it had a dry surface, non-sticky characteristic and frangible texture with an average MC of 16 percent. Based on preliminary research conducted at the laboratory, this MC range was generally achieved when the weight of the resin samples was reduced by approximately 36 to 40 percent of their initial weight. The final MC in the resin samples was estimated via the gravimetric method at the end of the drying process as 10.80 percent to 17.36 percent. Additionally, the MC of the dry resins was confirmed through an Aufhauser procedure, yielding values of 8.40 percent to 10.15 percent. The recorded drying-time ranges were 250 to 370 and 235 to 375 minutes for hot-air and hot-steam-air drying, respectively. All the dried resins were packed in airlock pouches and stored in a closed wooden cabinet inside a room.



1. Drying chamber
2. Exhaust fan
3. Waterbath ("connected by hidden pipes to drying chamber")
4. Gas burner
5. Water tank
6. Connecting pipe from tank to waterbath
7. Drying shekves

Fig. 1. The structure diagram of the hot-steam air dryer used for the research

Dracorhodin Content Test

The dracorhodin content was determined according to the standardised HPLC protocol for this biocompound (Waluyo et al. 2018). Dracorhodin samples were prepared by dissolving 0.4 g of dragon blood resin with 5 mL of 3 percent phosphoric acid solution in methanol. The mixture was weighed (A gram), refluxed for 30 minutes, cooled, and reweighed. Subsequently, 3 percent more phosphoric acid was added until the weight of the mixture reached A g. The mixture was homogenised and centrifuged at 10,000 rpm for 10 minutes. A 0.45 m filter membrane, with a 13 mm diameter, was used to remove liquid from the mixture. The mobile phase, acetonitrile (sodium dihydrogen phosphate 0.05 mol/L (40:60)), was prepared by dissolving 0.4167 g of sodium dihydrogen phosphate with distilled water until reaching 1000 mL solution. The solution was stirred until homogeneous. The primary standard solution was prepared by dissolving 5 mg of dracorhodin standard with methanol up to the boundary mark of the 50-mL flask. Subsequently, the mixture was homogenised. The working standard solutions were prepared by dissolving 2, 5, 10 and 15 mL of the primary standard solution with methanol. The HPLC parameters employed included a Zorbax SB C18 column, a mobile phase comprising acetonitrile: sodium dihydrogen phosphate 0.05 percent, a flow rate of 1.0 mL/min, a wavelength of 440 nm, a temperature of 35°C, and an injection volume of 20 L. The dracorhodin content was determined as follows:

$$dracorodin\ (\%) = \frac{(sample\ area\text{-}intercept)}{slope} x\ A\ x\ 100/mg\ sample$$

Cytotoxicity Assay

The cytotoxicity of the obtained resins was determined by the brine shrimp lethal test (BSLT) protocol with modifications in the sample preparation (Lestari et al. 2015; Rahmadi et al. 2021). The protocol comprised of the hatching of 0.01 g of *A. salina* shrimp larvae in a hatching vessel filled with 250 mL of marine water, preparation of the resin sample by diluting 0.02 g of the resin in 10 mL marine water, followed by solution homogenisation to obtain 2000 ppm sample stocks, and the conduction of the bioassay test using several resin and marine-water compositions in 1 mL solution (1:0, 0.5:0.5, 0.1:0.9 and 0.01:0.99) and by mixing each composition with another 1 mL of marine water containing approximately 10 shrimp larvae. Each mixture was exposed to light irradiation for 24 hours and subsequently left for 24 hours. Three (3) repetitions were used for each mixture. Afterwards, the numbers of alive and dead larvae were counted and used to determine the level of toxicity, LC_{50}, which is defined as the substance concentration that causes the death of half of the initial number of shrimp larvae (or approximately 50%).

Antioxidant Activity/Capacity Assays

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Assay

During this assay, the anti-oxidant donates hydrogen to reduce DPPH radicals and convert them to DPPH-H. The assay was conducted following the method proposed by Sobottka et al. (2021). For this assay, a 2×10^{-4} M DPPH solution was first prepared as the reference absorbent. The resin was dissolved in methanol to obtain different series of resin solutions with the addition of the DPPH solution. Each solution was stirred until all ingredients were mixed and then further conditioned for 30 minutes. Afterwards, the absorption of each solution was determined using a spectrophotometer at 517 nm. The results were expressed with IC_{50} (in ppm), which is defined as the substance concentration required to scavenge half of initial DPPH radicals (or approximately 50%).

The ABTS/TEAC ((2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)/ Trolox Equivalent Antioxidant Capacity) Radical Scavenging Assay

The ABTS reagent was prepared according to the method proposed by Nurcholis et al. (2022) with modifications such that 20 µL of the resin was mixed with 180 µL of the ABTS reagent on a 96-well clear polystyrene microplate. The mixture was incubated for 6 minutes. The absorbance of the solution was determined using a microplate nano spectrophotometer at 734 nm. The results were expressed with productivity in µg Trolox equivalent per g sample (ppm TE/g sample).

The Cupric Ion-reducing Antioxidant Capacity (CUPRAC) Assay

This assay was conducted following the method proposed by Nurcholis et al. (2022). Briefly, 50 µL of the resin was added to a mixture of 50 µL of 10–2 M $CuCl_2$ solution, 50 µL of NH4 Ac buffer pH 7 and 50 µL of 7.5×10^{-3} M neokuproin on a microplate. The mixture was incubated at room temperature in a dark room for 30 minutes. Afterwards, the absorbance was measured at 450 nm using a microplate spectrophotometer. The results were expressed with productivity in ppm for TE/g sample.

The Ferric Ion-reducing Antioxidant Power (FRAP) Assay

The FRAP assay was conducted according to the procedure proposed by Benzie and Devaki (2018) with modifications such that samples (10 µL) were placed on a 96-well clear polystyrene microplate, mixed with 145 µL FRAP and exposed to subsequent incubation for 4 minutes in the dark room. The sample absorbance was determined using a spectrophotometer at 593 nm. The results were expressed with productivity in ppm for the TE/g sample.

Supporting Data: Gas Chromatography Mass Spectrometry (GCMS): Chemical Composition Analysis

The GCMS test was performed under standard operating conditions to obtain additional information on other constituents present in the obtained dragon blood resin.

Data Collection and Analysis

The main parameters analysed were the dracorhodin content (in percentage), cytotoxicity (LC_{50} in ppm), antioxidant activity against the DPPH$^{\bullet}$ radical (IC_{50} in ppm) and Trolox-equivalent antioxidant capacity against the ABTS$^{\bullet+}$ radical, cupric ion and ferric ion (TEAC in ppm). All data were tabulated, and the average values and standard deviation were calculated. A two-way analysis of variance, using the Minitab 16 version, was employed to investigate the effects of the drying methods and temperature on the dracorhodin content, cytotoxicity and antioxidant activity/capacity. The significantly different groups were determined using the Tukey test. Furthermore, the correlation between the dracorhodin content and cytotoxicity or antioxidant activity/capacity was also investigated. The GCMS-chemical composition results were used as supporting data.

RESULTS

Table 1 lists the dracorhodin content, cytotoxicity and antioxidant activity/capacity of the *C. ruber* resin previously exposed to hot-air and hot-steam-air drying. The dracorhodin content in the hot-steam-air-dried dragon blood resin (4.10%-4.34%) was higher than that in the hot-air-dried resin (3.84%-3.99%). Among the analysed resin samples, the resin from hot-steam-air drying at 60°C possessed the highest dracorhodin content (4.34%).

Furthermore, the dried dragon blood resin exhibited toxicity towards *A. salina*. The cytotoxicity (LC_{50}) ranges of the hot-steam-air-dried and hot-air-dried resins were 430.61-449.47 ppm and 432.48-469.72 ppm, respectively. The resin from the hot-steam-air drying at 55^{0}C exhibited the highest toxicity due to its lowest LC_{50} value (430.61 ppm).

All the dried resins showed good scavenging activity against DPPH$^{\bullet}$, with the IC_{50} value ranges of 32.73-34.19 and 33.18-36.15 ppm for the hot-steam-air-dried and hot-air-dried resins, respectively. Among the prepared resins, the resin from the hot-steam-air drying at 60^{0}C exhibited the highest scavenging activity against DPPH$^{\bullet}$ due to its lowest IC_{50} value (32.73 ppm). For the hot-steam-air-dried resins, the TEAC ranges against the ABTS$^{\bullet+}$ radical, cupric ion and ferric ion were 80.40-84.78, 62.46-63.15 and 7.95-8.73 ppm, respectively. The TEAC value ranges determined for the hot-air-dried resin against the ABTS$^{\bullet+}$ radical, cupric ion and ferric ion were 83.95-86.60, 60.26-62.61 and 7.70-7.92 ppm, respectively. The resin obtained from the hot-steam-air drying at 60^{0}C exhibited the highest scavenging activity against cupric and ferric ions due to their highest TEAC values (63.15 and 8.73 ppm for cupric and ferric ions, respectively). In contrast, the resin from the hot-air drying at 55^{0}C exhibited the highest scavenging activity against the ABTS$^{\bullet+}$radical due to its highest TEAC value (86.60 ppm).

The hot-steam-air-dried resin tended to preserve more amounts of dracorhodin and showed higher scavenging activities against DPPH$^{\bullet}$ and

Table 1: Dracorhodin content, cytotoxicity and antioxidant activity/capacity of dragon blood resin from the hot-air and the hot-steam-air drying

Drying method	*Temperature*	*Dracorhodin content*	*Cytotoxicity (LC_{50})*	*Antioxidant activity/capacity*			
				IC_{50} DPPH	*TEAC/ABTS*	*TEAC/CUPRAC*	*TEAC/FRAP*
	^0C	*%*	*ppm*	*ppm*	*ppm*	*ppm*	*ppm*
Hot-air	55	3.99 ± 0.09^{ab}	432.48±211.65^{a}	36.15 ± 0.88^{acd}	86.60 ± 3.99	62.61 ± 2.47	7.92 ± 1.29
	60	3.84±0.04^{a}	469.72±145.03^{a}	33.18 ± 0.74^{bcd}	83.95 ± 2.38	60.26 ± 3.60	7.70 ± 0.40
Hot-steam-air	55	4.10±0.05^{ab}	430.61±43.40^{a}	34.19± 1.35^{c}	84.78 ± 2.13	62.46 ± 1.32	7.95 ±0.41
	60	4.34±0.27^{b}	449.47± 121.25^{a}	32.73±0.80^{d}	80.40 ± 1.67	63.15 ±1.72	8.73 ± 1.08

Remarks: Results were in mean ± SD. ANOVA was carried out individually for each parameter. A subsequent Tukey test was carried out following a significant ANOVA result for each parameter. Figures with different letters indicated significant differences between the means being compared in each parameter. Figures with same letters indicated no significant differences between the means being compared in each parameter

ferric ions than the hot-air-dried resin. As the drying temperature increased, the resin dried using hot-steam air at 60°C showed an improved dracorhodin content (4.34%), cytotoxicity (LC_{50} = 449.47 ± 121.25 ppm) and scavenging activity against the DPPH$^{\bullet}$ radical (IC_{50} value of 32.73 ± 0.80 ppm), cupric ion (TEAC value of 63.15 ± 1.72 ppm) and ferric ion (TEAC value of 8.73 ± 1.08 ppm). In contrast, the resin from the hot-air drying only showed an increase in cytotoxicity (LC_{50} = 469.72 ± 145.03 ppm) and scavenging activity against the DPPH$^{\bullet}$ radical (IC_{50} value of 33.18 ± 0.74 ppm) when the drying temperature was increased to 60°C.

Further statistical analysis results listed in Table 1 confirmed that the drying method had a significant effect on the dracorhodin content, whereas the drying temperature significantly affected the scavenging activity of the resin against the DPPH$^{\bullet}$ radical. Both factors or their interactions did not significantly affect the cytotoxicity or antioxidant activity of the resin against the $ABTS^{\bullet+}$ radical, cupric ion and ferric ion. Correlation tests between the dracorhodin content and LC_{50} or IC_{50}/TEAC values of the resins yielded low to moderate Pearson coefficient scores (less than 0.5–1). Furthermore, the correlation test results with p-values greater than 0.05 indicated small to medium degrees of correlation between the dracorhodin content and cytotoxicity or antioxidant activity/capacity of the resin (Table 2).

DISCUSSION

Dracorhodin Content

The dracorhodin content in dragon blood resin is a key property that determines its commercial utilisation. The required minimum value of the dracorhodin content is 1 percent (Lu et al. 2021). All the dried resins obtained from *C. ruber* contained greater than 1 percent of dracorhodin and therefore met the minimum standard requirement for commercialisation. This result suggests that all the drying conditions reported in this paper can be applied to prepare dragon blood resin as a medicinal material.

Notably, the hot-steam-air-dried resin possessed a higher dracorhodin content than the hot-air-dried resin. Among the prepared resins, the resin from hot-steam-air drying at 60°C possessed the highest dracorhodin content (4.34%). As the tem-

Table 2: Correlation between the dracorhodin content and cytotoxicity or antioxidant activity/capacity of *Calamus ruber* dragon blood resin

		Cytotoxicity (LC_{50})	*DPPH* (IC_{50})	*TEAC/ ABTS*	*TEAC/ CUPRAC*	*TEAC/ FRAP*
Dracorhodin	Pearson	-0.040	0.002	0.403	-0.110	-0.222
	p value	0.910	0.995	0.193	0.733	0.488

perature increased, the dracorhodin content in the hot-steam-air-dried resin tended to increase but not necessarily in the hot-air-dried resin. Furthermore, the dracorhodin content in the hot-steam-air-dried resin was higher than those obtained in previous studies where the resins were dried via solar drying or intermittent drying processes (Pasaribu et al. 2021; Yuniarti et al. 2022). Moreover, statistical analysis results showed that the dracorhodin content was significantly affected only by the drying method, particularly at the drying temperature of 60°C (Table 1).

The sensitivity of dracorhodin to the drying process is due to the heat-sensitive characteristics of flavonoid, which is its main constituent. Flavonoid generally has a low thermal stability and therefore, is vulnerable to changes under the conditions of various thermal processing factors (heating methods, temperature and duration) (Gao et al. 2022). During hot-air drying, a loss of bio-active compounds may occur (Thamkaew et al. 2021). However, a higher flavonoid content may be retained after drying using hot steam as the heat source than that after conventional convection heating (oven drying) as reported for the cases of tea leaves, black cumin seeds and cocoa beans (Zzaman et al. 2013; Liang et al. 2018; Roslan et al. 2020).

Cytotoxicity

The BSLT is a rapid, reliable, affordable and effective bio-assay tool to assess the cytotoxicity effects of plant extracts. Due to a significant correlation between the BSLT results with the inhibition of tumour cell growth in human assay is often used as a preliminary screening method for substances/ plant extracts with anti-tumour and anti-cancer activity (Tanvir et al. 2018). According to Meyer's toxicity index, a plant extract is categorised as toxic when its LC_{50} value is less than 1000 µg/mL (ppm) . Additionally, Clarkson's toxicity index classifies plant extracts into four categories, that is, non-toxic when their LC_{50} is >1000 ppm, low toxic when their LC_{50} is 500-1000 ppm, moderate toxic when their LC_{50} is 100-500 ppm, and very toxic when their LC_{50} is 0-100 ppm (Rahmadi et al. 2021).

The LC_{50} values of the hot-steam-air-dried and hot-air-dried resins prepared in this study were less than 500 ppm. Therefore, based on Meyer's and Clarkson's indexation, the obtained resins are classified as toxic/moderate toxic. Accordingly, the dried resins have considerable potential for further development as anti-cancer or anti-tumour agents.

As previously reported in this study, small significant differences are observed in the LC_{50} values of the hot-steam-air-dried and hot-air-dried resins. Therefore, all the drying conditions reported in this paper can be employed for the preparation of resins as cytotoxic agents. Furthermore, due to a low degree of correlation between the cytotoxicity and dracorhodin content of the resin, the cytotoxicity effects of the dragon blood resin derived from *C. ruber* is assumed to be due to contributions from other bio-compounds. A previous report suggested that the high lethality of zebrafish embryos after being fed with dragon blood resin was due to other constituent compounds, apart from dracorhodin, in the resin (Krishnaraja et al. 2019).

GCMS screening results (Table 3) revealed several other bio-compounds with potential capacity as antitumour/anticancer agents, namely, coumarin and 3,5,7-trihydroxy and (Z,Z)-9,12-octadecadienoic acid (CAS) linoleic acid. Several studies have demonstrated the potential of coumarin or its derivatives and linoleic acid as antitumour/anticancer agents (Bhattarai et al. 2021; Wu et al. 2020). The concentration of each compound was different under different drying conditions, indicating the influence of drying factors on each bio-compound.

Antioxidant Activity/Capacity

Four different assays, namely, DPPH•, FRAP, CUPRAC and ABTS assays, were selected to represent two general antioxidant activity mechanisms of a substance, that is, hydrogen atom transfer

Table 3: GCMS results for *Calamus ruber* dragon blood resin previously dried with hot-steam-air (HS) and hot-air (HA) drying method

S.No.	Retention time	Area	Chemical compounds	Concentration (%)			
				HS-dried resin		HA-dried resin	
				55^0C	60^0C	55^0C	60^0C
1	3.493	31088246	Methanamine, N-Methyl- (CAS) Dimethylamine			0.18	
2	3.490	208416022	Trideuteroacetonitrile		0.72		
3	3.811	207008502	Carbamic Acid, Monoammonium Salt (CAS) Ammonium Carbamate	1.05			
4	7.944	996949002	Benzene, Methyl- (CAS) Toluene		3.46		
5	9.826	364793964	Benzene, Ethyl- (CAS) EB	1.23	1.43	2.06	
6	10.347	1869847603	1,3,5,7-Cyclooctatetraene (Cas) Cyclooctatetraene	7.94	6.96	10.57	11.14
7	11.592	97906490	Benzene, Propyl- (CAS) N-Propylbenzene		0.34		
8	12.088	118911766	Benzene, 1-Propenyl- (CAS) Propenylbenzene	0.49	0.48	0.67	0.39
9	12.493	201224271	Phenol (Cas) Izal	1.16	0.76	1.14	2.88
10	12.642	491150332	Benzene, 1-Ethenyl-2-Methyl- (CAS) O-Methylstyrene		1.70		
11	12.649	425275932	Benzene, 1-Ethenyl-3-Methyl- (CAS) M-Methylstyrene			2.40	
12	12.675	210029660	Benzene, 1-Propenyl- (CAS) Propenylbenzene	1.06			
13	13.397	435874492	Ethanone, 1-Phenyl- (CAS) Acetophenone	4.76	1.55	2.46	5.38
14	13.646	712567043	Benzoic Acid, Methyl Ester (CAS) Methyl Benzoate		2.98	4.03	
15	14.175	31742554	2-Propanone, 1-Phenyl- (CAS) Phenylacetone	0.15		0.18	
16	14.322	46716045	Benzene, 1-Ethenyl-3-Methoxy- (CAS) ANISOLE, M-VINYL-			0.26	
17	14.339	45969343	Benzene, 1-Ethenyl-4-Methoxy- (CAS) P-Vinylanisole	0.23			
18	14.579	141970038	Benzene, (Methoxymethyl)- (CAS) Benzyl Methyl Ether		0.56	0.80	
19	14.584	114819250	3-Hydroxy-1-Phenyl-Propan-1-One	0.58			
20	14.599	109381526	Benzene, (Methoxymethyl)- (CAS) Benzyl Methyl Ether				0.57
21	14.808	128613601	Phenol, 3-Ethyl- (CAS) M-Ethylphenol	0.47	0.53	0.73	
22	14.840	97506801	Phenol, 4-Ethyl- (CAS) P-Ethylphenol				0.51
23	15.439	855422339	Benzoic Acid (CAS) Retardex	3.88	3.18	4.84	3.36
24	15.625	70373461	Benzenepropanoic Acid, Methyl Ester (CAS) Methyl Hydrocinnamate				0.37
25	15.817	6725169	(1 alpha,2 alpha,3 alpha)-Dimethyl 1,3-Dimethyl-4-Cyclohexene-1				0.03
26	15.892	15218880	Benzocycloheptatriene				0.08
27	15.967	13244471	3-Methoxy-2-Methylphenol				0.07
28	16.130	97681653	Phenol, 2-Methyl-5-(1-Methylethyl)- (CAS) Carvacrol	0.55		0.55	0.30
29	16.275	65889626	Cycloheptane, 1,3,6-Trimethylene-	0.33			
30	16.292	38179206	Bicyclo[3.1.0]Hex-2-Ene, 4-Methylene-1-(1-Methylethyl)- (CAS) 2,4(10)-Thujadiene				0.20
31	16.492	131403549	Phenol, 2,6-Dimethoxy- (CAS) 2,6-Dimethoxyphenol	0.65	0.70	0.74	0.67
32	16.709	42183487	Benzoic Acid, 2-Methoxy-, Methyl Ester (CAS) Methyl Anisate			0.24	
33	16.728	78685814	2-Phenyl-1,1-Difluorocyclopropane		0.27		
34	16.755	55412161	2-Phenyl-1,1-Difluorocyclopropane	0.28			
35	16.760	50770563	2-Phenyl-1,1-Difluorocyclopropane				0.26
36	16.917	46389893	Beta-D-Lyxofuranosid, Heptyl-2,3-O-Ethylborandiyl-5-O-Acetyl				0.24
37	17.167	23718743	Ether, Methyl Diphenylmethyl				0.12
38	17.308	71145283	1,2,4-Trimethoxybenzene			0.40	

Table 3: Contd...

S.No.	Retention time	Area	Chemical compounds	Concentration (%)			
				HS-dried resin		HA-dried resin	
				55^0C	60^0C	55^0C	60^0C
39	17.320	205617048	4-Methoxy-3-(Methoxymethyl)Phenol		0.71		
40	17.328	103882199	Benzene, 1,2,3-Trimethoxy- (CAS) 1,2,3-Trimethoxybenzene (CAS) Methylsyringol				0.54
41	17.567	47804017	Triacontanedioic Acid, Dimethyl Ester				0.25
42	17.594	90416188	1,3-Diphenylbutan-1-One		0.31		
43	17.780	133282682	Phenol, 3,5-Dimethoxy- (CAS) 3,5-Dimethoxyphenol	0.94	2.22	0.75	
44	17.820	161386155	Benzenemethanol, 3-Hydroxy-5-Methoxy-				0.84
45	17.945	113197868	Benzene, 1,1'-(1,2-Ethanediyl)Bis- (CAS) Dibenzyl	1.84		0.64	
46	17.958	265243575	2-(1-Benzyloxy-2-Bromo-Ethyl)-Oxirane				1.38
47	18.057	208482334	4-Methoxy-3-(Methoxymethyl)Phenol	0.52	1.16	1.18	1.32
48	18.380	175683375	4-Methoxy-3-(Methoxymethyl)Phenol	1.40		0.99	
49	18.592	30039668	2-Phenyl-2,3-Dihydroindene			0.17	
50	18.708	37690609	7-Benzofuranol, 2,3-Dihydro-2,2-Dimethyl- (CAS) Nia 10272			0.21	
51	18.708	72967151	2-Cyclohexen-1-One, 4-Hydroxy-3,5,5-Trimethyl-4-(3-Methyl-1,3-	0.37			
52	18.985	2351787911	2,4-Dimethoxy-Phenol	11.99	10.94	13.29	10.23
53	19.467	244355849	1-Penten-4-Yn-3-Ol, 1-Phenyl-				1.27
54	19.541	249717924	Benzene, 1,1'-Ethenylidenebis- (CAS) 1,1-Diphenylethene	1.26			
55	19.618	138185975	2-Allyl-1,4-Dimethoxy-3-Methyl-Benzene				0.72
56	19.792	570165610	Ethanone, 1,2-Diphenyl- (CAS) Deoxybenzoin				2.96
57	19.797	478196566	Ethanone, 1,2-Diphenyl- (CAS) Deoxybenzoin	2.42			
58	19.845	882945545	1-(4-Methoxymethyl-2,6-Dimethyl-Phenyl)-Ethanone		3.07		
59	19.849	315571751	Beta.-Ionone				1.78
60	19.992	211793756	Phenol, 4-(Phenylmethyl)- (CAS) P-Benzylphenol			1.20	
61	20.042	207877418	Ethanone, 1-(4-Hydroxy-3,5-Dimethoxyphenyl)- (CAS) Acetosyringone	1.05			
62	20.317	231891783	Methyl 2-(3,5-Dimethoxyphenyl)-Ethanoate	1.74	1.11	1.31	0.93
63	20.542	126117373	Hexadecanoic Acid, Methyl Ester (CAS) Methyl Palmitate	0.66	1.60	0.71	
64	20.625	204051816	Phenol, 4-(2-Phenylethyl)- (CAS) P-HYDROXY-1,2-DIPHENYLETHANE	1.03			
65	20.982	192479497	Hexadecanoic Acid (CAS) Palmitic Acid	1.55	1.67	1.09	4.43
66	21.154	321637376	Coumarin, 3,5,7-Trihydroxy- (CAS) 3,5,7-TRIHYDROXYCOUMARIN	2.65	2.32	1.82	
67	21.542	314532839	1,2-Diethoxy-4-Ethylbenzene		1.09		
68	21.545	177022603	3-Cyclobutene-1,2-Dicarboxylic Acid, 3-Methyl-4-Propyl-, Dimethyl Ester, Trans- (				0.92
69	21.605	80518722	2-Phenylnaphthalene	0.97		0.46	
70	21.758	242435661	10,13-Octadecadienoic Acid, Methyl Ester (CAS)	1.92	1.70	1.37	2.13
71	22.303	485632860	9,12-Octadecadienoic Acid (Z,Z)- (CAS) Linoleic Acid	4.17	3.48	2.75	3.62
72	22.531	54687063	1-Benzoyloxy-2-Formyloxybenzene			0.31	
73	22.667	535443600	9,12,15-Octadecatrienoic Acid, Methyl Ester, (Z,Z,Z)- (CAS) Methyl Linolenate				2.78

Table 3: Contd...

S.No.	Retention time	Area	Chemical compounds	Concentration (%)			
				HS-dried resin		HA-dried resin	
				55 ^{0}C	60^{0}C	55^{0}C	60^{0}C
74	22.678	183470821	Bicyclo[2.2.1]Heptane, 2-Methyl-3-(1-Methylethenyl)- (CAS) BICYCLO[2.2.1]HEPTANE	1.77	1.86	1.04	2.2
75	22.777	276347515	D(15)-Norpregnan-20-One, (5.Alpha.)- (CAS) D-Norpregnan-20-One, (5.Alpha.)-	1.40			
76	22.785	127507387	2-Cyano-9,10-(Dideuteroetheno)-Anthracene		0.44		
77	22.892	288238362	Sulfon, Methyl 4-Benzyliden-2-Phenylcyclopentyl		1.00		
78	22.992	101415953	Tetracontane				0.53
79	23.217	54323893	1,1':3',1''-Terphenyl (CAS) M-Terphenyl	0.85	1.13	0.31	1.09
80	23.613	153047030	Bis(2-Furyl)Methylphenylsilane				0.80
81	23.680	97939379	2-Cyclohexen-1-One, 2-Hydroxy-3-Methyl-6-(1-Methylethyl)- (CAS) Diosphenol	0.77		0.55	
82	23.796	351192993	4H-1-Benzopyran-4-One, 5,7-Dihydroxy-2-Phenyl- (CAS) Chrysin		1.22		
83	23.810	120397868	5-Hydroxy-6-Methoxy-3-Methyl-2-Phenylbenzofuran (Parvifuran)				0.63
84	24.292	138344786	2H-1-Benzopyran-7-Ol, 3,4-Dihydro-5-Methoxy-6-Methyl-2-Phenyl- (CAS) 5-METHOXY	1.22	1.41	0.78	1.61
85	24.442	22255340	6-Methoxy-4-Methylthio-2-Phenyl-Quinazoline	0.23		0.13	
86	24.592	137297998	Tetrabenzo[A,E,I,M]Cyclohexadecene				0.71
87	24.994	129177586	7a,9c-(Iminoethano)Phenanthro[4,5-Bcd]Furan, 4a.Alpha., 5-Dihydro-3-Methoxy		0.45		
88	25.167	89994332	Docosanoic Acid, Methyl Ester (CAS) Methyl Behenate		0.31		
89	25.192	36625440	Alpha-D-Galactopyranose, 6-O-(2,3,5-Tri-O-Acetyl-Beta-D-Lyxofuranosyl				0.19
90	26.225	2540790728	4H-1-Benzopyran-4-One, 2,3-Dihydro-5,7-Dihydroxy-2-Phenyl-, (S)- (CAS) 5,7-	13.03		14.36	11.83
91	26.570	2009249782	2H-1-Benzopyran-7-Ol, 3,4-Dihydro-5-Methoxy-6-Methyl-2-Phenyl- (CAS) 5-METHOXY	9.83	21.14	11.36	11.21
92	27.125	152072226	9,10-Anthracenedione, 1,4-Dihydroxy-2,3-Dimethyl- (CAS) Anthraquinone, 1,4			0.86	
93	27.208	213371591	1-[1-(4-Methylpiperazino)Naphthalen-3-Yl]Propan-1-One	1.08			
94	27.240	236409894	2-Tert-Butyl-4-Methyl-6-(1-Methyl-1-Phenylethyl)Phenol				1.23
95	27.242	544465418	1-Methyl-4-Iodo-3,5-Dimethoxy-1H-Pyrazole		1.89		
96	27.325	122870535	Lily Aldehyde			0.69	
97	27.592	135741767	2H-1-Benzopyran-7-Ol, 3,4-Dihydro-5-Methoxy-6-Methyl-2-Phenyl- (CAS) 5-METHOXY			0.77	
98	27.969	565939625	4H-1-Benzopyran-4-One, 3-Hydroxy-7-Methoxy-2-Phenyl- (CAS) 7-METHOXYFLAVANOL	3.86	5.07	3.20	4.48
99	28.892	273195172	3-(1-Phosphorylethyl)-5-Phenyl-2-Isoxazoline		0.95		
100	28.895	163809640	[1,2]Dithiolo[1,5-B][1,2]Oxathiole-7-SIV, 2,4-Diphenyl- (CAS)	0.83			
101	28.925	126429002	Ethanone, 1-Phenyl-2-(4-Phenyl-3H-1,2-Dithiol-3-Ylidene)- (CAS) 1-PHENYL-2			0.71	
102	28.867	123357930	1,3,5-Triphenylpyrazole				0.64

Table 3: Contd...

S.No.	Retention time	Area	Chemical compounds	Concentration (%)			
				HS-dried resin		HA-dried resin	
				55^0C	60^0C	55^0C	60^0C
103	29.392	127345260	Ethanone, 2-Methoxy-1-[(2-Methoxy-1-Phenylethylidene) Hydrazono]-1-Phenyl- (				0.66
104	29.408	163746951	3 4-Benzyl-5,6-Dihydro-2-Phenylamino-4H-1,3-Thiazin-5-One	0.83			
105	29.453	107372802	5,6-Dimethoxy-1-Indanone			0.61	
106	29.545	426228012	4-Benzyl-5,6-Dihydro-2-Phenylamino-4H-1,3-Thiazin-5-One		1.48		
107	29.617	36394623	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-Hexamethyl- (CAS) Squalene	0.24			0.19
108	29.975	47125922	5,7-Dimethoxy-2-Methylindan-1-One				
109	29.992	33528799	Ethyl 3-Phenylpropionate				0.17
110	30.142	183673618	Cyclopentane, 1-Phenyl-3-(Diphenylmethylene)-		0.64		
111	30.592	100778606	Pentatriacontane (CAS) N-Pentatriacontane		0.35		
112	30.942	90202717	1H-Inden-1-One, 2,3-Diphenyl- (CAS) 2,3-Diphenyl-Inden-1-One		0.31		
113	31.292	92433056	[1,2]Dithiolo[1,5-B][1,2]Oxathiole-7-SIV, 2,4-Diphenyl- (CAS)		0.32		
114	31.826	188420813	3-Acetoxy-9,10-Secoergosta-5,7,10(19),22-Tetraene		0.65		
115	46.075	195757744	1-Benzoyl-2,2-Dimethyl-2,3,3a,4,5,6-Hexahydro-1H-Indol-3a-Yl Benzoate	0.98	0.75	1.11	0.74

(HAT) and single electron transfer (SET) reactions. The ABTS assay represents the HAT or mixed SET/HAT mechanism. DPPH$^{\bullet}$, FRAP and CUPRAC assays represent the SET mechanism (Sun et al. 2018; Avanti et al. 2021).

The antioxidant assay involving the DPPH method is a standard method. The DPPH$^{\bullet}$ free radical shows a purple colour that turns into a yellow colour when the radical is reduced into a non-radical (diphenyl picrylhydrazyl) compound by an antioxidant agent. The antioxidant capacity of an agent against the DPPH$^{\bullet}$ free radical is expressed using the parameter IC_{50}. A small IC_{50} value indicates a strong antioxidant agent (Avanti et al. 2021).

The IC_{50} values of all the dried resins of *C. ruber* determined via the DPPH assay were less than 50 ppm, indicating the high antioxidant capacity of the resins. The IC_{50} values of the resins were lower than those of the same type of resin obtained from *Daemonorops longipes* Mart., *Daemonorops draco* BL. and *Daemonorops melanochaetes* BL. The IC_{50} values of the resins obtained from the three aforementioned *Daemonorops* species were in the range of 50-100 ppm (Purwanti et al. 2019; Waluyo and Pasaribu 2013). These results indicate that the *C. ruber* dried resin has potential for application as an antioxidant agent and scavenges DPPH$^{\bullet}$ free radicals better than the same type of resin obtained from the three aforementioned *Daemonorops* species.

The antioxidant activity of the resin against ABTS, CUPRAC and FRAP is expressed in terms of TEAC. The TEAC assay measures the relative ability of a substance to scavenge and reduce $ABTS^{\bullet+}$ free radicals and metal oxidants, cupric and ferric ions, compared with that of Trolox, which is the synthetic form of water-soluble vitamin E (Dorta et al. 2018; Sun et al. 2018; Frangu et al. 2020). The TEAC assay results (Table 1) indicate that the scavenging ability of the dried dragon blood resin obtained from *C. ruber* against $ABTS^{\bullet+}$ free radicals is higher than that against ferric and cupric ions.

The hot-steam-air-dried resin tended to have better scavenging activity against DPPH$^{\bullet}$ radicals and ferric ions than the hot-air-dried resin. The superiority of hot-steam air as a drying medium over hot air in increasing the antioxidant activity against DPPH• and ferric ions have also been reported in the cases of tea leaves and cocoa beans. For both cases, the drying processes were performed in a superheated-steam dryer for a short period at tem-

peratures above 100°C (Roslan et al. 2020; Zzaman and Sifat 2023).

Increasing the drying temperature by 5°C from 55°C improved the scavenging activity towards DPPH$^{\bullet}$ radicals but decreased the antioxidant activity against ABTS$^{\bullet+}$ radicals for both the hot-air-dried and hot-steam-air-dried resins. An increase in scavenging activity against cupric and ferric ions with increasing temperature was only observed for the hot-steam-air-dried resin. The significant effect of the drying temperature on the antioxidant activity of the resin was confirmed only against DPPH$^{\bullet}$ radicals. This result suggests that drying at 60°C can potentially be employed for the preparation of resins as antioxidant agents, particularly for reducing the DPPH$^{\bullet}$ free radicals.

The impact of the drying temperature on antioxidant activity will vary depending on the material being dried and the antioxidant assay being performed. Several studies have demonstrated a reduction in the antioxidant activity of plum (*Prunus divaricata* L.), mahaleb (*Prunus mahaleb* L.), mortiño fruit (*Vaccinium meridionale* Swartz), kinnow mandarin, eureka lemon, maltodextrin, white quinoa, wild guava leaves and *Vernonia amygdalina* leaves with increasing drying temperature (Hartiati and Mulyani 2015; López-vidaña et al. 2017; Juhaimi et al. 2018; Alara et al. 2019; Ghafoor et al. 2019; Z^{3}otek et al. 2019). However, other studies have also demonstrated an inevitable increase in the antioxidant activity of oven-dried tomato powder, xuan-mugua fruit and red quinoa with an increase in the drying temperature (Kim and Chin 2016; Zlotek et al. 2019; Chen et al. 2022).

The differences observed in the antioxidant activity as a response to variations in the drying temperatures are assumed to be due to the differences in the chemical composition of these biomaterials. Furthermore, the correlation tests conducted in this study revealed a relatively low degree of correlation between the dracorhodin content and antioxidant activity/capacity of the dragon blood resin derived from *C. ruber*. This result indicates that the antioxidant activity/capacity of the *C. ruber* dragon blood resin is governed not only by dracorhodin but also possibly by other constituents.

Furthermore, GCMS screening results (Table 3) revealed several other constituents with potential capacity to act as antioxidant agents, namely, hexadecanoic acid (CAS, palmitic acid), hexadecanoic acid, methyl ester (CAS, methyl palmitate) and (Z,Z)-9,12-octadecadienoic acid (CAS, linoleic acid). The variations in the concentration of these constituents under each drying condition indicated the effects of drying factors on these compounds. The potential antioxidant activities of the aforementioned constituents have been reported in several previous papers (Pinto et al. 2017; Abdel-Hady et al. 2018; Hidajati et al. 2018; Fecker et al. 2022).

CONCLUSION

The hot-steam-air dried resin from 60°C contained the highest dracorhodin content (4.34%) and exhibited the best scavenging activity against DPPH$^{\bullet}$ radical (IC_{50} value of 32.73 ppm), cupric ion (TEAC value of 63.15 ppm), and ferric ion (TEAC value of 8.73). The hot-steam-air-dried resin from 55°C exhibited the best cytotoxicity properties due to its lowest LC_{50} (430.61 ppm). The hot-air-dried resin from 55°C demonstrated the highest scavenging activity against ABTS radical. Notably, the drying methods significantly affected the resin's dracorhodin content, whereas the drying temperatures affected the resin' IC_{50} values. The as-obtained dried resins exhibit the potential for further development as an antioxidant, antitumor and anticancer agents.

RECOMMENDATIONS

Based on the obtained results, resin farmers are encouraged to adopt artificial drying techniques, such as oven and/or hot-steam-air drying, for processing wet resin. The use of a hot-steam-air dryer is recommended because it provides more advantages than an oven/hot-air dryer, such as the maintenance of the dracorhodin content in resin and improvement of the cytotoxicity and antioxidant properties of resin.

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viding technical laboratory assistance during the cytotoxicity and antioxidant assays.

DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no impending clashes of interest to the research, authorship and/or publication of this paper.

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