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Full Length Article

Bioactive Composition, Antioxidant and Anticancer of *Apis dorsata* Nest Extract from Different Seasons

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Abstract

Honeybee hives are rich in bioactive compounds with broad-spectrum pharmacological potential. Exploration of potential antioxidant, antihyperlipidemic, bioactive antibacterial extract of *Apis dorsata* Binghami nest as endemic Sulawesi honeybee has started. However, previous work needs to adequately comprehend or explain nest extracts' bioactive content and bioactivity from different seasons. To answer this research gap, we present novel research on antioxidant bioactivity, anticancer and toxicity of *A. dorsata* nest extract from other seasons. *A. dorsata* nests obtained from January to July (SSM) and August to December (SSN) were extracted with 95% ethanol by maceration. The concentrated ethanol extract was then analyzed for bioactive content using high-performance liquid chromatography (HPLC), functional group detection with FTIR, DPPH free radical scavenging test, anticancer test on MCF-7 cells and the Brimp Shrimp Lethality Test (BSLT). The study found that extracts from the SSN group had more bioactive species than SSN. However, extracts from the SSM group had stronger antioxidant and anticancer activity on MCF-7 cells and toxicity to *Artemia salina* Leach larvae than the SSN group. Thus, the bioactive content with antioxidant and anticancer potential in *A. dorsata* nest extract is unaffected by the large variety of feed sources in a particular season.

Keywords: *Apis dorsata* Binghami; Nest extract; Cytotoxic; Radical scavenging

Introduction

As an endemic bee in the Wallace zone, *Apis dorsata* Binghami is the most productive honeybee in producing honey. Until now, scientists have not succeeded in cultivating *A. dorsata* in a closed form so that it still lives naturally in the forest. *A. dorsata* has an open nesting shape with one comb. Nests hang from tree branches, cliffs, and open ceilings. *A. dorsata* nests are 1–2 m wide and 1–2.5 m long. The diversity of feed plants for sources of nectar, pollen, and propolis *A. dorsata* is more than that *of A. mellifera*. Therefore, the characteristic of the bioactive content of the nest is also an accumulation of various plant species. The body size of *A. dorsata* is larger than that of *A. mellifera*, affecting the range of flying for food. *A. dorsata* can reach more than one kilometre from the nest point to find food plants, while *A. mellifera* reaches a maximum of 700 m (Mokosuli 2013; Mokosuli *et al.* 2019).

Honey beehives contain honey, propolis, pollen, wax and royal jelly. Honeycomb extraction will produce propolis and other bioactive components. Apart from honey, propolis is the most significant component in honeybee hives. Honeycomb extraction of more than 80% will attract propolis. Propolis is a balsamic and sticky resin with various colors and a pleasant smell collected by honeybees from various plants. Besides being used as a building material, propolis is a defense material that protects colonies from parasites and infections in honeybee hives. The constituents of propolis are different and vary according to climate, season, location, and year. The composition of the propolis content is highly dependent on the geographical area and the diversity of plant feed sources. Furthermore, there is a correlation between geographical area and plant diversity on the chemical content and antioxidant activity of propolis (Bobiș 2022; Kurek-Górecka *et al.* 2022; Semuel and Rombot 2023).

More than 500 chemicals have been identified in propolis, including flavonoids, phenolic compounds, polyphenols, terpenes, coumarins, steroids, amino acids, and aromatic acids. Raw propolis comprises around 50% resins, 30% waxes, 10% essential oils, 5% pollen and 5% other

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organic substances (Toreti *et al.* 2013; Wagh 2013). Furthermore, propolis contains phytochemicals such as essential oils, vitamins (A, B complexes, C and E), and minerals such as aluminum, salt, potassium, calcium, copper, magnesium, iron, and zinc, all of which play vital roles in biological activity. Propolis contains colors and substances produced by various plants, and its chemical makeup varies according to its geographical location, botanical sources, season, and bee species (Pasupuleti *et al.* 2017; Zullkiflee *et al.* 2022). In the last ten years, research on propolis has been directly based on a deeper understanding of its biological activity. It has been proven in numerous preclinical and clinical studies that a wide range of natural compounds in propolis, including flavonoids, phenolic compounds, polyphenols, terpenes, terpenoids, and aromatic acids, are potential anticancer, antiapoptotic, antidiabetic, anti-inflammatory, antioxidant, antibacterial, and antiviral agents (Huang *et al.* 2014; Chavda *et al.* 2023; Mountford *et al.* 2023).

In this present study, research focused on exploring the bioactive potential of *A. dorsata* nest extracts from different seasons for antioxidants and anticancer. Cancer is a degenerative disease with an increasing prevalence in the last five years. The bioactive content in beehives, especially propolis, has excellent potential as an anticancer and chemopreventive cancer. Many research reports on utilizing *A. mellifera* nest extract as an anticancer agent have been carried out. Isoflavonoids from Brazilian Fred propolis have the potential as anticancer therapeutic agents (Nani *et al.* 2018); *A. mellifera* propolis exhibits cell cycle inhibitory activity (Zabaiou *et al.* 2017). Propolis phenolic compounds have cytotoxic and larvicidal activities (Arruda *et al.* 2020). Phenolic compounds in propolis induce apoptosis in human Tongue Squamous Cell Carcinoma Cell (CAL-27) (Celinska-Janowicz *et al.* 2018). Even so, research on the anticancer activity of *A. dorsata* nest extract still needs to be reported or has yet to be reported.

Materials and Methods

Samples

Beehives of *A. dorsata* used in this screening were collected from natural habitats in North Sulawesi forests from August to December 2022 (SSN) and January to July 2023 (SSM). The beehive is about 3.5 months old. The beehives used for extraction are nests that have not been occupied or found with eggs or bee larvae but contain honey (Fig. 1). Nest characterization was conducted at the Zoology Laboratory, Faculty of Mathematics, Natural and Earth Sciences, Manado State University.

Extraction

A preparation of an ethanolic extract derived from the nesting of *A. dorsata* was conducted in order to isolate biologically active components. The nesting of *A. dorsata* was initially preserved at a temperature of four degrees Celsius and subsequently homogenized to get a tiny fragment. A solvent consisting of 95% ethanol was employed, with a nesting/solvent ratio of 1/4 (w/v). The extraction protocol was conducted via maceration as outlined in the methodology described by Semuel and Rombot (2023). A quantity of 250 g of nesting material was utilized. The combination was subjected to a period of 72 h of darkness at room temperature, using an orbital shaker set at a rotational speed of 40 revolutions per minute. The extraction techniques were conducted three times. The macerates were amalgamated, subjected to filtration, and afterwards concentrated to a state of dryness under decreased pressure at a temperature of 45°C and a rotational speed of 50 rpm, employing a HEIDOLP Rotavapor rotary evaporator. The outcome of the process of solvent evaporation is commonly known as honeycomb ethanol extract.

Flavonoid analysis HPLC method

The quantification of quercetin content in the samples was performed using high-performance liquid chromatography (HPLC). The HPLC analysis was conducted using isocratic elution at a flow rate of 1.0 mL/min. The mobile phase employed in the experiment consisted of methanol. The injected sample volume was 5 µL, and the detection wavelength utilized was 370 nm. A number of concentration gradients were utilized to manufacture standard quercetin. The study was conducted with a High-Performance Liquid Chromatography (HPLC) system (Shimadzu Corporation, Kyoto, Japan). The HPLC system consisted of many components, including a degassing unit (model DGU-20A5R), a pump (model LC-20AB), an autosampler (model SIL-20A HT), a column oven (model CTO-20AC), and a UV-Visible detector (model SPD-20A). The column used in this study was the Shim-Pack VP ODS C18 Shimadzu, with dimensions of 75 mm in length, 4.6 mm in internal diameter, and a pore size of 3 m. The elution mobile phase consisted of two components: 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). The utilization of the gradient elution system was implemented in the following manner: The elution gradient used in the experiment consisted of the following time intervals: 0–7 minutes for a concentration range of 2–20% B, 7–10 min for a concentration range of 20–30% B, 10–18 min for a concentration range of 30–50% B, 18–20.5 min for a concentration range of 50–98% B, 20.5–23 min for a concentration of 98% B, and 23–26 min for a concentration range of 98–2% B. Following this, the system was balanced until 35 min before the subsequent injection. The genotype samples were subjected to analysis under certain conditions. The injection volume for each sample was 10 µL. The mobile phase flow rate was set at 1 mL/min, and the detector wavelength was set at 320 nm. The analysis was

conducted at a temperature of 30°C for a duration of 35 minutes (Batubara *et al.* 2020).

Functional group analysis with FTIR

The objective of this study is to utilize an FTIR spectrophotometer to identify functional groups present in extracts obtained from SSN and SSM nests. The extract underwent analysis using KBr-FTIR spectroscopy. The spectra of KBr-FT-IR were obtained using a Nicolet 5700 instrument. A 2 mg portion of powdered material was combined with 200 mg of KBr in an agate mortar. The measurement range was from 4000 to 400 cm. The manufacture of potassium bromide (KBr) pellets was dependent on a standardized procedure. The researchers conducted background recording before each measurement and utilized software to automatically eliminate it (Najib *et al.* 2017).

BSLT test

Cysts of *Artemia salina* Leach were being incubated. Cysts of the species *A. salina* were subjected to a weighing process, with recorded weights reaching up to 50 mg. Subsequently, these cysts were placed into a container filled with filtered saltwater. Following this, aeration was provided, and the cysts were allowed to remain undisturbed for a duration of 48 h under the influence of lamplight, facilitating their entire hatching process. The hatched larvae are subsequently utilized for the purpose of conducting the toxicity test. The toxicity test was conducted in a segregated manner based on the specific honeycomb extract being evaluated. The procedural steps involved in conducting the toxicity test were as follows: A total of 10 *A. salina* larvae were introduced into a vial filled with seawater, followed by the addition of a honeycomb extract solution to achieve final concentrations of 1000, 100 and 10 parts per million (ppm). The researchers conducted observations at the 24-h mark by quantifying the mortality rate of larvae within the vials. Calculations were made by using a magnifying glass. The cumulative percent mortality data was analysed using LC50 probit analysis with a 95% confidence interval in the IBM SPSS 20 programme (Mc Laughlin *et al.* 1998; Mokosuli 2021).

Free radical scavenging test with the DPPH method

A solution of *A. dorsata* nest extract was generated in sterile test tubes at different concentrations (10, 50, 100, 200 and 250 ppm). Each test tube was supplemented with 500 µL of a 1 mM DPPH solution dissolved in methanol. The volume was adjusted to 5.0 mL and thereafter subjected to incubation at a temperature of 37ºC for a duration of 30 min. The measurement of absorbance was conducted at a specific wavelength of 515 nm using a Parkin Elmer UV Vis Spectrophotometer. The positive control in this study was Merck's Butyl Hydroxy Toluene (BHT), which was tested at doses of 2, 4, 6 and 8 ppm. The experiments were conducted in triplicate. The IC50 value is determined by applying the linear regression equation formula. The percentage of inhibition is determined by the following formula (Semuel and Rombot 2023):

$$\% \text{ inhibition} = \frac{[\text{Control absorbance} - \text{Sample absorbance}]}{[\text{Control absorbance}]} \times 100$$

Anticancer test

The anticancer test was carried out using MCF-7 cells according to the Central Laboratory of Padjadjaran University, Bandung method. The culture medium used was Roswell Park Memorial Institute Medium (RPMI) liquid culture medium containing 10% fetal bovine serum (FBS) and 50 µL/50 mL of antibiotic. The test concentration distribution used was Media + Cell, 2% DMSO, Ciplastin (positive control), nest extract successively: 1000 µg/mL, 500 µg/mL, 250 µg/mL, 125 µg/mL, 62.50 µg/mL, 31.25 µg/mL, 16.63 µg/mL, and 7.81 µg/mL. In summary, the anticancer test consists of the following stages: 1). The cell culture used in 96 well plates is then incubated (at 37°C and 5% CO_2 gas until the percentage of cell growth reaches 70%). 2). Cells were treated with *A. dorsata* nest extract, and the control was then incubated (for 48 h at 37°C and 5% CO_2 gas). 3). Adding presto blue working reagent to cells, and 5). Absorbance measurement using Multimode Reader. Absorbance was measured at 570 nm. The cells, after treatment, were observed with a microscope.

Results

Bioactive properties of bee nest

The results of the HPLC analysis showed that the standard quercetin retention time was 11,410 (Fig. 2a and Table 1). The extract of *A. dorsata* nest collection from January to July (SSM) obtained a retention time of 11,453, close to the standard quercetin retention time. Furthermore, 21 retention times were obtained with a distribution of 1.623 – 26.303. The results of HPLC analysis of *A. dorsata* nest extract in the SSM group showed 21 compounds, including quercetin (Table 2). The August to December (SSN) nest extracts obtained 26 peaks/retention times (Table 3). Compared to standard quercetin, four peak retention times were obtained, which were close to quercetin, namely 11,020, 11,233, 11,450 and 11,690. SSN retention time is in the distribution of 1.623 to 22.793 (Fig. 2).

FTIR data revealed that -OH, -CH, C=C, C=O and C=N at a wavelength of 411.22 cm^{-1} to 3310.41 cm^{-1} as functional groups in the nest extract. A very clear –OH group indicates that the SSN and SSM extracts contain phenolic compounds, especially flavonoids (Fig. 3).

Table 1: Retention time and quercetin standard area

UV-VIS results retention time	Area (µm^2)	Area (%)	Height (µm)	Height (%)
1.657	128804	4.77	10642	6.63
11.410	2573521	95.23	149824	93.37
Totals	2702325	100	160466	100

Table 2: Retention time and area of HPLC results of *A. dorsata* SSM nest extract

UV-VIS Results Retention Time	Area (µm^2)	Area (%)	Height (µm)	Height (%)
1.623	249485	4.77	20482	13.40
2.093	342310	18.12	39853	26.08
2.807	277948	14.71	20928	13.70
3.153	27998	1.48	3183	2.08
3.323	26771	1.42	2895	1.89
3.577	222946	11.80	26849	17.57
4.083	67718	3.58	3836	2.51
4.403	41674	2.21	3108	2.03
4.777	41240	2.18	2704	1.77
5.080	65719	3.48	5020	3.29
5.417	14559	0.77	1386	0.91
5.660	29209	1.55	2192	1.43
6.417	10481	0.55	1101	0.72
6.640	14124	0.75	1234	0.81
7.223	69356	3.67	4816	3.15
7.733	34692	1.84	2197	1.44
11.453	10180	0.54	739	0.48
16.567	33975	1.80	1413	0.92
21.427	83512	4.42	2572	1.68
22.247	15885	0.84	868	0.57
26.303	209597	11.09	5432	3.55
Totals	1889379	100.00	152808	100.00

Fig. 1: Samples of *A. dorsata* nests. (a) SSN and (b) SSM

Toxicity activity

A preliminary study used BSLT to test the cytotoxicity of *A. dorsata* nest extract against *A. salina* larvae. The average toxicity of *A. dorsata* nest extract from the SSM group was more toxic to *A. salina* compared to that from the SSN group. The highest toxicity of the SSM group extract was the MT extract (LC50: 205.41 mg/L), while the lowest was the BM extract (LC50: 219.54). The highest toxicity of the extract from the SSN group was the BM extract (LC50: 228.34 mg/L), while the lowest was the MI extract (LC50: 275.34 mg/L). However, the toxicity of the extracts from the SSN and SSM groups was below 1000 mg/L, so they were classified as toxic (Fig. 4).

Antioxidant activity

The DPPH, a free radical compound, was used to determine

Table 3: Retention time and area of HPLC results of *A. dorsata* SSN nest extract

UV-VIS results retention time	Area (µm^2)	Area (%)	Height (µm)	Height (%)
1.623	150476	10.30	8773	6.72
2.090	71741	4.91	15333	11.74
2.187	83204	5.69	8636	6.61
2.583	18735	1.28	2747	2.10
2.803	165342	11.32	19565	14.98
2.967	54611	3.74	3193	2.44
3.577	209174	14.32	26841	20.55
4.053	45470	3.11	2776	2.12
4.413	26796	1.83	2064	1.58
5.090	67838	4.64	5121	3.92
5.657	43652	2.99	2886	2.21
6.013	21160	1.45	1733	1.33
6.347	20206	1.38	1440	1.10
6.760	16687	1.14	1094	0.84
7.227	73974	5.06	5173	3.96
7.757	34820	2.38	2022	1.55
10.733	20306	1.39	1555	1.19
11.030	21544	1.47	1971	1.51
11.233	20743	1.42	2072	1.59
11.450	56925	3.90	2842	2.18
11.690	22418	1.53	1542	1.18
21.490	110791	7.58	3206	2.45
21.850	15195	1.04	1960	1.50
22.140	23774	1.63	2137	1.64
22.327	46766	3.20	2357	1.80
22.793	18720	1.28	1603	1.23
Totals	1461068	100.00	130642	100.00

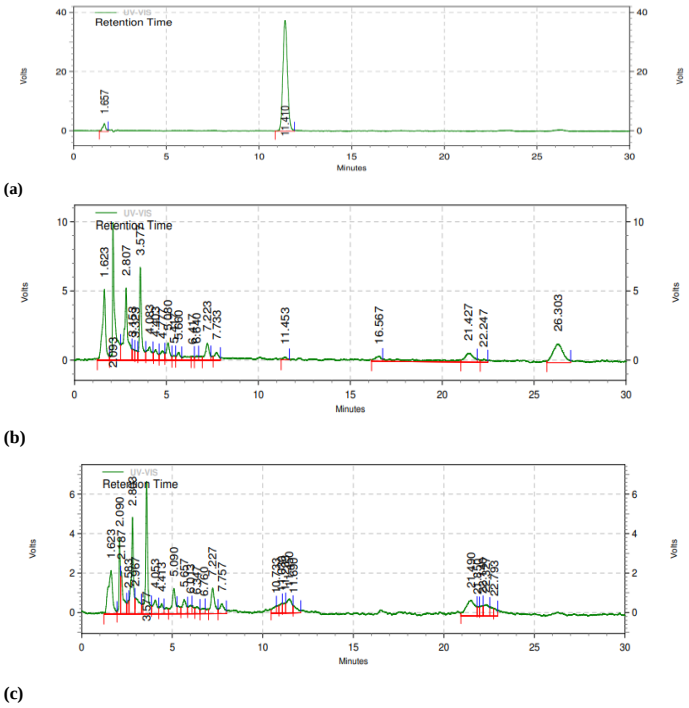

Fig. 2: Chromatogram of HPLC results. (a) Standard quercetin, (b) SSM sample and (c) SSN sample

the scavenging ability of free radicals in different *A. dorsata* nest extract samples by means of donating hydrogens through antioxidants. The antioxidant activity was evaluated through the scavenging effect of DPPH on the extracts by measuring the DPPH inhibition percentage of each of the extracts. The concentrations of extract used were also varied to study the relationship between the volume of extract used and its antioxidant effect. The free radical scavenging

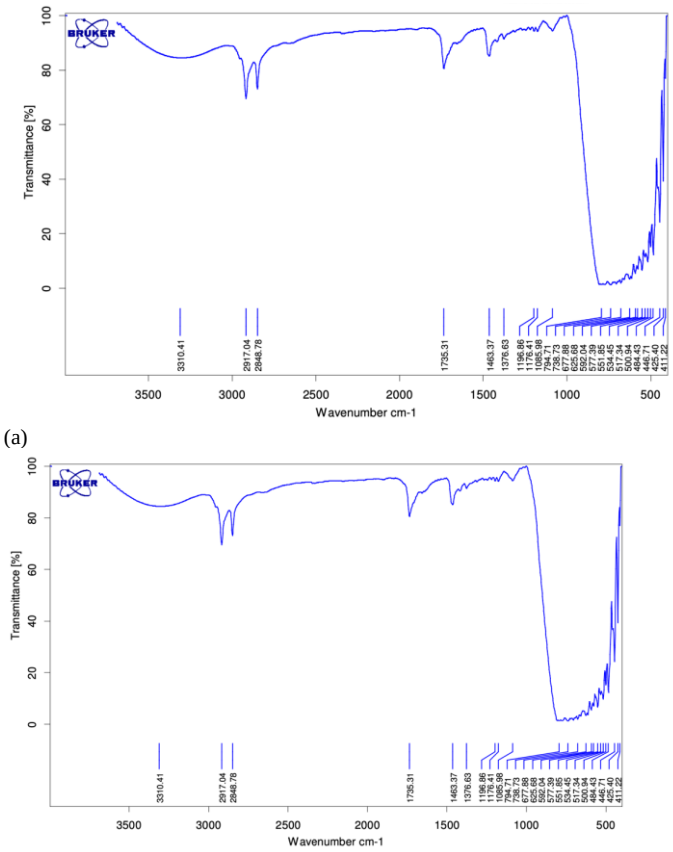

Fig. 3: FTIR spectra of *A. dorsata* nest extract. (a) SSN and (b) SSM

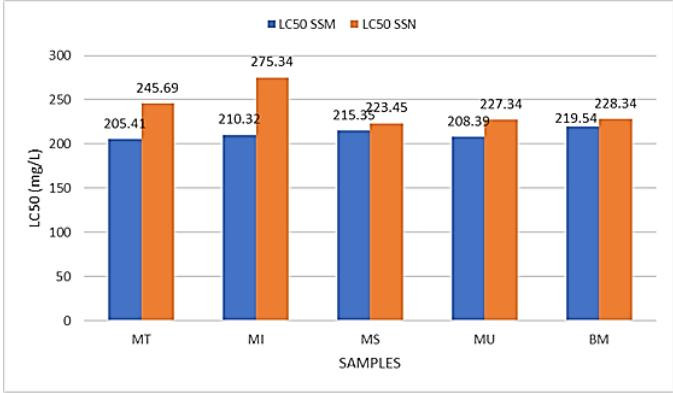

Fig. 4: Comparison of toxicity (LC_{50}) of *A. dorsata* extract in the SSN and SSM groups

activity of DPPH ethanol extract from the SSM group of the MT sample showed the best IC50, namely 85.43 mg/L, while the extract from the SSN group was the MU sample (119.03 mg/L). The SSM extract had a better IC50 than the BHT positive control. Even so, the IC50 of all SSN samples was still better than the BHT-positive control (Fig. 5).

Table 4 shows the percentage of DPPH scavenging activity and IC50 values of various *A. dorsata* nest extracts from the SSN and SSM groups. The results demonstrated that the percentage of DPPH scavenging activity of all extracts from the SSM group, particularly at the concentration of 250 mg/mL, was higher than BHT. However, the best DPPH percentage scavenging activity was shown by the MI extract (67.252%), followed by MS (66.049%), MT (64.299%), MU (64.199%) and BM (61.881%). MT value (85.426) was lighter than the control BHT (124.040). In extracts from the SSN group, the best percentages of DPPH scavenging activity were MU (65.38%), MI (62.05%), MT (61.05%), BM (59.75%) and MS (57.81 %). The highest DPPH radical scavenging

Table 4: Comparison of DPPH scavenging activity of SSN and SSM extracts

A. dorsata nest extract	Concentration	SSM		SSN	
		% inhibition (λ_{512} nm)	IC_{50}	% inhibition (λ_{512} nm)	IC_{50}
MT	10	11.285	*85.426*	14.801	*137.899*
	50	30.342		20.167	
	100	39.500		49.769	
	200	59.851		57.724	
	250	64.199		61.055	
MI	10	9.528	*142.190*	22.646	*134.210*
	50	19.796		26.152	
	100	39.501		49.769	
	200	57.816		53.904	
	250	67.252		62.051	
MS	10	11.470	*154.630*	11.841	*162.610*
	50	29.324		30.222	
	100	41.535		41.536	
	200	47.826		53.895	
	250	66.049		57.817	
MU	10	8.788	*119.030*	16.559	*142.140*
	50	31.267		29.417	
	100	46.716		40.518	
	200	59.759		53.469	
	250	64.199		65.384	
BM	10	7.678	*170.590*	8.973	*149.670*
	50	17.484		27.567	
	100	30.343		37.743	
	200	60.037		60.037	
	250	61.887		59.759	
BHT	10	7.678	*124.040*		
	50	17.484			
	100	38.668			
	200	42.646			
	250	50.340			

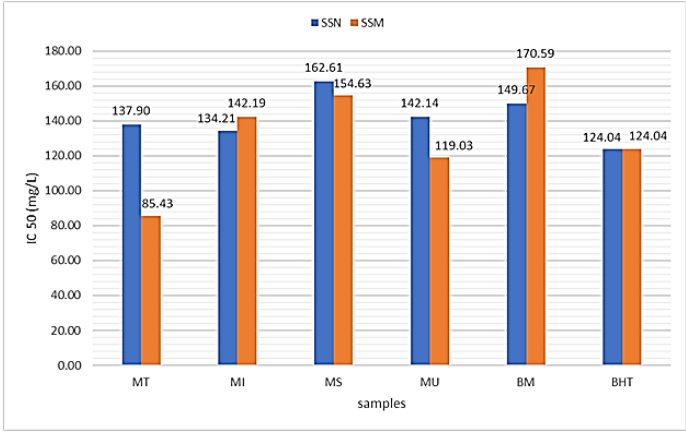

Fig. 5: Comparison of IC50 of *A. dorsata* hive extracts in the SSM and SSN groups

activity at a test concentration of 250 ppm was obtained in the SSM sample. This result is supported by the excellent correlation coefficient (*r*) in all samples > 0.90).

Anticancer *in vitro*

In vitro anticancer test on MCF-7 cells was carried out in eight concentration distributions where the lowest concentration was 7.81 µg/mL and the highest concentration was 1000 µg/mL (Fig. 3). Cisplatin was used as a positive control. *Cisplatin* is a chemotherapy drug used to treat several types of cancer, such as ovarian cancer, testicular cancer, or bladder cancer. This drug can be used as a single treatment or in combination with other anticancer drugs. Based on the results of the percentage of live cells in the distribution of test concentrations used for regression analysis. The regression analysis results using SPSS IBM 23 obtained the equation for SSN is Y = 102.167 + 0.001 x and SSM is Y = 100.020 + 0.000 x (Fig. 6).

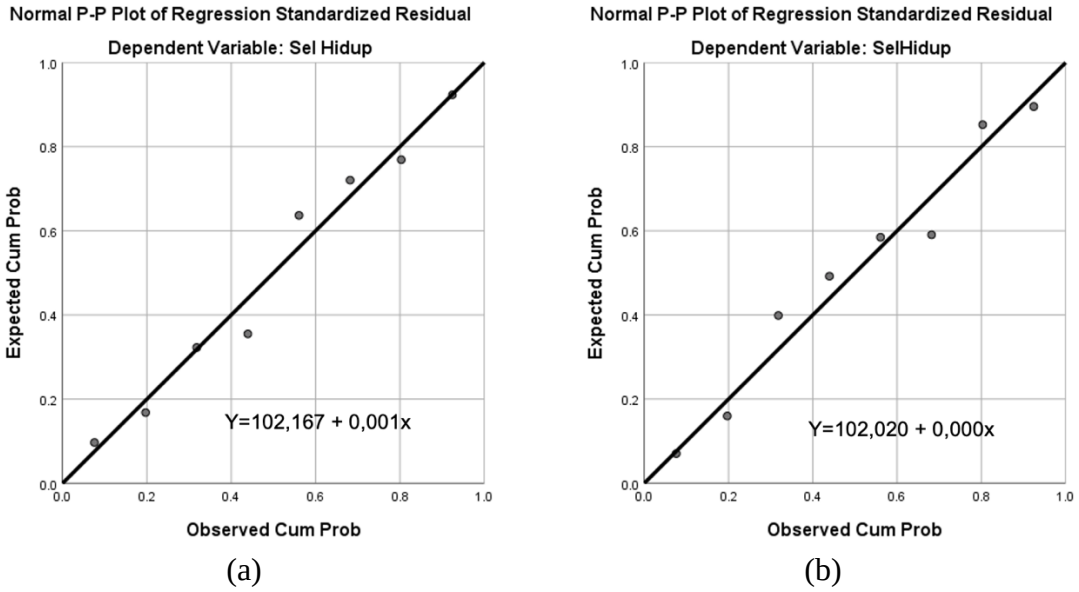

Fig. 6: Results of regression analysis of *A. dorsata* nest extract. (a) SSN and (b) SSM

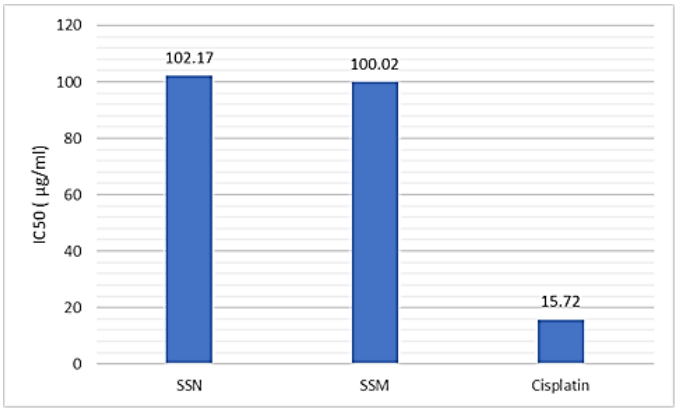

Fig. 7: Results of regression analysis of SSN and SSM nest extracts

The regression analysis showed that the IC50 of the SSN nest extract was 102.217 µg/mL, while that of the SSM nest extract was 100.02 µg/mL (Fig. 7). As for the control of the anticancer drug Cisplatin IC50: 15.72 µg/mL. Thus, the extract from the SSM group showed better cytotoxicity to MCF-7 cells than SSN.

The concentration distribution of the SSM and SSN nest extract tests on the microplate reader was measured for absorbance to determine the percentage of live cells (Fig. 5). Cells treated with extracts and positive control were analyzed microscopically for cell morphology. There were differences in cell morphology in the positive control (Cisplatin), media + cells, 2% DMSO and the distribution of test concentrations. Media + cells and DMSO showed more viable cells than the positive control and the concentration distribution of the SSM and SSN extract tests. The higher the concentration, the darker the colour of the media. The reaction of the formation of purple formazan crystals causes this. The colour change that occurs in the 96-well plate can be observed through the absorbance value using an ELISA (Enzyme-linked Immunosorbent Assay) reader at a wavelength of 600 nm for blue and 570 nm for pink (Fig. 8).

The morphology of MCF-7 cells after being treated with the extract is presented in Fig. 9. Live cells are marked with a light-coloured epithelium, while dead cells are marked with a round shape and dark colour (no glow). Live cells appear to shine brilliantly, and the membrane boundary with the media is visible, while dead cells appear round, dark, and opaque, and the cell membrane appears broken (Puspitasari and Ulfa (2009).

Discussion

Based on the results of the analysis of bioactive compound content detected using the HPLC method in extracts from the SSN group, more than those in SSM.

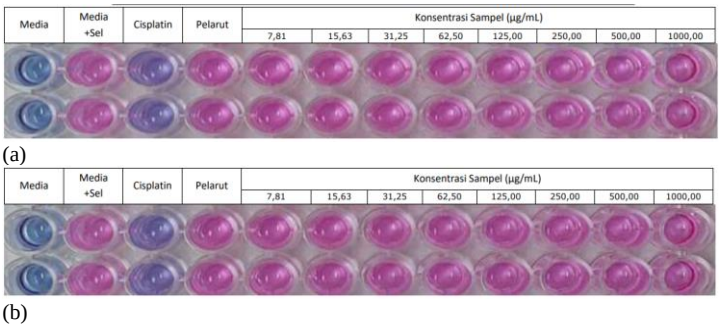

Fig. 8: Well plate extract results. (a) SSM and (b) SSN

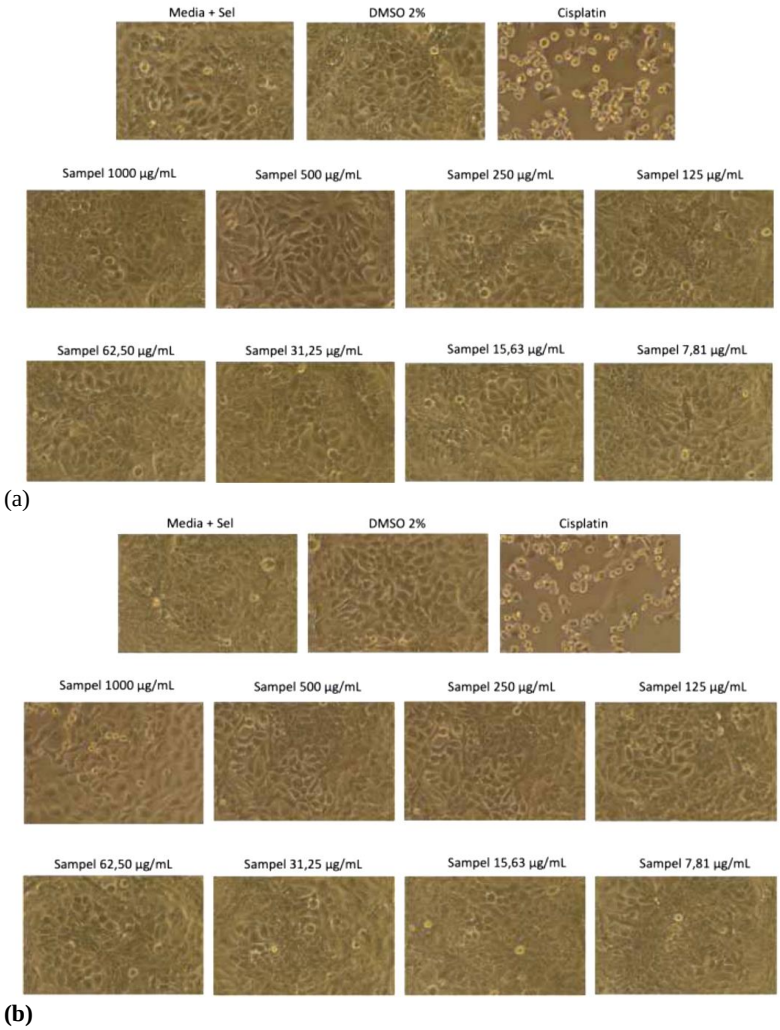

Fig. 9: Documentation of MCF-7 cell morphology after extract treatment. (a) SSM and (b) SSN

More flowering plants were found as a feed source in North Sulawesi, Indonesia, from July to December. January to July is not the season for fruit, so fewer flowering plants are available as a food source for *A. dorsata* bees that live naturally in the forest. The large variety of forage plants affects the bioactive composition of *A. dorsata* nests (Mokosuli and Rombot 2023). In SSN, HPLC analysis results found four retention times adjacent to quercetin. Thus, SSN has more quercetin and its derivatives than SSM. In SSM, only one retention time was found, close to or equal to standard quercetin. Based on the retention time distribution, the compounds detected in SSM and SSN are a

group of compounds belonging to the flavonoid class. FTIR analysis confirmed these results, which detected OH groups and other organic groups in the extract. *A. dorsata* nests are built with propolis and wax to form a hexagonal space structure where eggs are laid, and larvae develop. Extraction with 95% ethanol was proven to extract flavonoid compounds contained in *A. dorsata* nests.

The BSLT is a leading method for determining the presence of bioactive chemicals in natural goods, with results often connected to cytotoxic and anticancer activities (Ukwade *et al.* 2020). *A. salina* larvae are multicellular individuals with thin membrane characteristics, making them an ideal model for testing active compounds and their mechanisms of toxicity. The extract solution will diffuse into the cells to have a physiological effect on the larvae. The digestive tract of *A. salina* is a non-selective filter that makes it easier for toxic chemicals to enter the mouth (Aksono *et al.* 2022). Extract toxicity below 500 mg/L indicates *A. dorsata* nest extract is toxic to *A. salina* tissue. There is a positive correlation between BSLT and cytotoxic tests using cancer cell cultures, so BSLT is widely used to investigate the anticancer potential of extracts of natural ingredients (Carballo *et al.* 2002; Mokosuli 2008; Semuel and Rombot 2023). Extracts of natural ingredients have anticancer activity in the BSLT test if the LC50 value is less than 1000 mg/L. The results showed that both extracts from the SSN and SSM groups had LC50 at a distribution of 223.45 mg/L to 275.34. Thus, it is included in the moderate toxic category.

The DPPH free radical scavenging activity of *A. dorsata* nest extract was caused by the high flavonoid and phenolic compounds in both SSN and SSM extracts. However, the best IC50 was obtained from the MT extract from the SSM group. The abundant Flavonoid and phenolic compounds in *A. dorsata* nest extract have many hydroxy groups acting as electron donors with DPPH free radicals. Phenolics, including the flavonoids from propolis, are considered potent antioxidants acting as chain breakers, while the scavenging ability of polyphenols is due to the presence of hydroxyl groups in their structural composition (Alaerjani *et al.* 2022). This strong antioxidant activity is cancer chemopreventive. Bioactive in *A. dorsata* nest extract prevents free radicals from mutating DNA, resulting in cell growth changes.

The anticancer test of both SSN and SSM extracts on MCF-7 cells *in vitro* showed a potential anticancer response. Colour change is used to see the presence of cell proliferation. Cells that proliferate in mitochondria will absorb MTT, so these cells will turn purple due to the formation of tetrazolium (formazan) crystals. The intensity of the purple colour formed is proportional to the number of living cells, so the greater the intensity of the purple colour, the greater the number of living cells. Cell growth was observed under a microscope in each extracted sample from the results of the colour change in the SSN and SSM extracts. The results showed that the concentration of 1000 µg/mL had a higher number of dead cells than those at a lower concentration. IC50 SSM extract is more potent than SSN, but the difference is insignificant. IC50 > 200 µg/mL, indicating strong extract toxicity on MCF-7 cell culture.

Changes in cell morphology are a sign of cytotoxic activity resulting from a compound after treatment of cells compared to control cells. Nursid *et al.* (2010) stated that the more cells die, the less formazan crystals are formed and vice versa. The cells that died due to the extract treatment most likely no longer had the mitochondrial reductase enzyme, so when given MTT in the test microplates, MTT did not change into formazan crystals. Changes in cell morphology due to exposure to certain active compounds or chemotherapeutic agents reflect biochemical conditions that can lead to cell death either by apoptosis or necrosis. In cells that undergo apoptosis, the volume of the cytoplasm decreases, the cell nucleus shrinks, and the membranes and organelles remain together. In contrast, in cells that experience necrosis, the cell looks bulging or experiencing swelling, the cell nucleus undergoes lysis, and the plasma and nuclear membranes are damaged. and cell organelles disintegrate (Botteon *et al.* 2021; Forma and Brys 2021).

Several researchers reported the cytotoxic activity of extracts from honeycomb. Caffeic acid phenylethyl ester (CAPE) compounds belonging to flavonoids have proapoptotic and antimetastatic inhibition of proliferation, migration and invasion activities in many types of cancer cells, including MCF-7 (Zabaiou *et al.* 2017; Kabala *et al.* 2017; Ren and Kinghorn 2019; Gajek *et al.* 2020; Forma and Brys 2021). Variations in the anticancer activity of nine propolis ethanol extract variants were reported by Milek *et al.* where almost every sample inhibited migration of breast cancer cells at low propolis concentrations (0.04 µg/mL). Even at the lowest concentration (0.02 µg/mL), each propolis ethanol extract inhibited MCF-7 cell proliferation, but the level of inhibition varied between samples (Milek *et al.* 2022). Propolis has activity against several tumour cells *in vitro* and in vivo. In short, due to immunomodulatory action based on nonspecific antitumor immune response through activation of macrophages, producing soluble factors and acting directly on tumour cells or in other cell functions. It has been demonstrated that propolis can interfere with oncogenic signalling pathways, inhibit cell growth and proliferation, induce apoptosis, and reduce angiogenesis, among other effects (Gastaldello *et al.* 2021).

The cytotoxic activity of *A. dorsata* nest extract is strongly suspected due to the high content of variants of phenolic compounds and flavonoids in both types of extracts. Flavonoids and other phenolic compounds can induce apoptosis in cancer cells, causing cancer cell death. This study found SSM's anticancer activity was more potent than SSN's. This was also supported by the best antioxidant activity obtained from the SSM sample, namely the *A.*

dorsata MT extract. Toxicity to *A. salina* Larvae was also the strongest from SSM extract. The novelty of this research is that the many types of compounds in the extract are non-linear with antioxidant and anticancer bioactivity. Although the SSN samples detected more compounds by HPLC analysis than SSM, the antioxidant, anticancer and toxicity bioactivities were better found in SSM. The wide variety of bioactive compounds in the SSN group was because there were more flowering plants (known as fruit season) from August to December compared to the period from January to July. The large variety of forage plants affects the bioactive content of *A. dorsata* nests. Nevertheless, both extracts from the SSN and SSM groups have great potential as anticancer and antioxidant bioactive sources. *A. dorsata* nest contains antioxidant and anticancer bioactive components, which are not affected by season and availability of various feed sources.

Conclusion

A. dorsata nest extract obtained from January to June (SSM) had more potent antioxidant, anticancer and toxicity activities on *A. salina* larvae than *A. dorsata* nest extract obtained from August to December (SSN). Nevertheless, SSM and SSN are classified as potential sources of antioxidant and anticancer bioactives. Based on HPLC analysis, the variation of flavonoid compounds was found to be more numerous in SSN than in SSM.

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Author Contributions

MYS, AT and MM were involved in planning the research and DR performed the data acquisition/collection. AT, DR and MYS aided in interpreting the results. AT, DR performed data analysis. MYS, AT, MM revised the manuscript. All authors were involved in this research.

Conflicts of Interest

All authors have no conflict of interest about the results presented along this article.

Data Availability

The data will be made available on a fair request to the corresponding author.

Ethics Approval

Not applicable to this paper

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