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Chemistry Profile and Biological Activity of *Campnosperma auriculatum* Extracts

RINI MUHARINI[*1], IRA LESTARI[1], ERSANDO[1], MASRIANI[1], YANA AISYA PUTRI[1], FEBRILIA GERINA[1], ANTONIUS RB OLA[2]
[1]Study Program of Chemistry Education, Faculty of Teacher Training and Education, Tanjungpura University, Pontianak, Indonesia; [2]Chemistry Department, Faculty of Science and Engineering, University of Nusa Cendana, Kupang, Indonesia
*Corresponding author: rini.muharini@fkip.untan.ac.id
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ABSTRACT

As our ongoing investigation for bioactive natural products from tropical plant, we performed preliminary study on one of important tropical plants in West Kalimantan, Terentang putih, *Campnosperma auriculatum*. The aims were to determine effective solvent used for extraction, chemistry profile, total phenolic content, free-radical scavenging, cytotoxicity, and anti-termite activities of leaves, stems, and roots extracts of *C. auriculatum*. Variation of solvent for extraction was selected based on its polarisation, namely, ethanol, ethyl acetate, and *n*-hexane. The effectiveness of solvent was determined by observing the rendemen of each extract, where amount of sample and solvent volume, duration of extraction, temperature, and maceration technique were controled. Determination of total phenolic content was performed using Folin-Ciocalteu method. IC_{50} value for free-radical scavenging activity was calculated by plotting standard concentration and absorption data observed through DPPH method. Cytotoxicity evaluation was performed to each ethanolic extract against 4T1 cancer cell line using MTT assay. Anti-termite activity was conducted against *Coptotermes curvignathus* by calculating percentage of termite mortality and paper weight loss. This research showed that ethanol solvent was the most effective extraction solvent giving the highest yield in each part of plant. Phytochemically, all extracts showed that they contain phenolics and alkaloids. Ethanolic extract of stems showed the highest total phenolic content with 737.6 ± 0.56 ppm (GAE) and the most active as free-radical scavenger with IC_{50} value of 135.51 ± 0.91 ppm. Meanwhile, the roots extract exhibited pronounce cytotoxicity toward 4T1 cancer cell line with IC_{50} value of 1.55 ± 3.29 µg/ml and high selectivity index. Furthermore, the roots extract displayed most active as anti-termite as well as antifeedant. Hitherto, this study is the first report on phytochemistry and biological activity from leaves, stems, and roots of *C. auriculatum*. Moreover, this plant can be explored further for its potential on medicinal and agricultural industries.

Keywords: Anti-proliferative, anti-termite, *Campnosperma auriculatum*, free-radical scavenging, phytochemical investigation

INTRODUCTION

Campnosperma auriculatum, known as Terentang putih by local, is one of tropical plants found abundant on peat swamp forest of primary forest in West Kalimantan. Beside for its wood, local people traditionally used young leaves of this plant for treating burn skin. In Johor, shoots and roots were prepared for treating coughing with blood (Sabran *et al*., 2016). Meanwhile, leaves and roots were used as herbal remedies forstomach ache and headache (Ismail *et al*., 2015). The oil produced from the wood was reported having irritation effect on skin (Sanusi *et al*., 2018)

To the best of our knowledge, there is little information related with secondary metabolites and its bioactivity from *C. auriculatum*. The only information about chemical compounds from *C. auriculatum* was from Lamberton's research dated back more than 60 years ago. It mentioned the isolation of a 5-hydroxy-cyclohex-2-en-one from *C. auriculatum* oil (Lamberton, 1958). This type of alkyl-hydroxycyclohexenones was reported also to be found in *Campnosperma zeylanica* Thwaites leaves (Edireweera *et al*., 2018). The compounds were campnospermenone A, B, and C, in which exhibited promising antiproliferative towards several breast cancer cell lines (Edireweera *et al*., 2018).

As our ongoing exploration and investigation on bioactive compound from tropical plant, we performed preliminary study on *C. auriculatum* collected at Sungai Pinang village, Melawi district, West Kalimantan. This paper described the extraction results on leaves, stems, and roots of *C. auriculatum* by using different polarity solvents, determination of total phenolic contents, free-radical scavenging activity, and anti-termite properties of each ethanolic extract.

MATERIALS AND METHODS

Plant Material Preparation and Extraction

The leaves, stems, and roots of *C. auriculatum* were collected in October 2020 at Sungai Pinang village, Melawi district, West Kalimantan. Its specimens were determined and deposited at Herbarium Bogor, Bogor, with code number B-1559/IPH.3/KS/XII/2020. The maceration technique was performed to each part of plant by using ethanol, ethyl acetate, and *n*-hexane as solvent. Each resulted extract was concentrated using rotary vacuum evaporator (Buchii, Germany) and calculated for its rendement.

Chemistry Profile Evaluation

Aliquote of each extract was screened phytochemically using several specific reagents for phenolics, alkaloids, triterpenoids, and steroids, as well as thin layer chromatography (TLC) techniques with $CeSO_4$ 1.5% as a universal TLC visualization reagent. Reverse phase HPLC (Agilent EZChrom Elite 1220, USA) was used to identify constituents profile contained in each extract.

Determination of Total Phenolic Content

Total phenolic content of each extract were determined using Folin-Ciocalteu assay described previously (Armania *et al*., 2013) with some modification. Gallic acid was used as comparison. The absorption data of the mixture of each extract (10 mg/ml) with Folin-Ciocalteu reagent was observed with UV Spectrophotometer at λ 765 nm. Measurement to each extract was done in two replicates. Total phenolic content was represented as milligram gallic acid equivalent per gram extract (mgGAE/g extract).

Free-radical Scavenging Assay

Free-radical scavenging evaluation for each extract were done by conducting TLC-DPPH method described by Ciesla (2015). IC_{50} values were determined by using DPPH method (Gulcin & Alwasel, 2023). Ascorbic acid was used as a positive control. Correlation between total phenolic content and antioxidant was carried out using Pearson's correlation with p= 0.05.

Cytotoxicity Assay

Cytotoxicity evaluation of each ethanolic extract was conducted on 4T1 cancer cell line, a murine mammary carcinoma cell line from BALB/cfC3H mouse, using MTT assay with modification (Damasuri *et al*., 2020). Each ethanolic extract was prepared as a mixture of one hundred µl of culture medium in various concentration (31.25 – 500 µg/ml) on 96-well microplate. Cisplatin was used as positive control. The medium culture without extract was played as negative control. The assay was done in triplicate. Absorbance for each well was measured using ELISA microplate reader at λ 570 nm. The absorbance values were directly proportional to the number of live cells. The viability was calculated using formula as in Eq. (1):

$$\%\ viability = 100\% - \left(\frac{As-Ae}{As-Ab}\right) \times 100\% \quad \text{Eq. (1)}$$

where A_s is absorbance of cell control, A_e is absorbance of extract, and A_b is absorbance of media control. IC_{50} values were determined by probit analysis on log concentrations and percentage of viability. The selectivity index (SI) was determined by comparing IC_{50} ratio in Vero cell versus 4T1 cells (Rollando *et al*., 2022). Selectivity index will be considered high when its value is higher than 3.

Anti-termite Assay

Anti-termite evaluation was performed by feeding termites *Coptotermes curvignathus* with filter paper Whatman No.1 (d = 3 cm) impregnated with extract using previously described procedure (Adfa *et al*., 2017) with slight modification. About 30 workers and 3 soldiers of termites were employed. Negative

control was Whatman filter paper without extract. Each day, termite condition was checked. After 21 days, the number of dead termites were calculated for its percentage mortality with formula as in Eq. (2):

$$\%\ termite\ mortality = \frac{number\ of\ \ dead\ termites\ \ after\ treatment}{number\ of\ termites\ before\ treatment} \times 100\% \quad \text{Eq. (2)}$$

In the same time, percentage of paper weight loss was determined to observe how many termites consumed their food. It can be calculated using Eq. (3):

$$\%\ paper\ weight\ loss = \frac{paper\ weight\ before\ treatment - paper\ weight\ after\ treatment}{berapaper\ weight\ before\ treatment} \times 100\% \quad \text{Eq. (3)}$$

Finally, calculation of absolute antifeedant coefficient for each extract experiment was performed in order to determine antifeedant activity with formula as in Eq. (4):

$$A = \frac{KK - EE}{KK + EE} \times 100\% \qquad \text{Eq. (4)}$$

where A is absolute antifeedant coefficient, KK is paper weight loss of control paper, EE is paper weight loss of extract impregnated paper.

Statistics Analysis

All measurements were done in replication. Data represented as a mean and a standard deviation. The mean difference between obtained data were determined using t-test with p value < 0.05.

RESULTS AND DISCUSSION

Extraction's process began with preparation of leaves, stems, and roots of *C. auriculatum* as dried powder 165.0, 579.9, and 135.0, g respectively. Each sample was divided equally to be macerated with ethanol, ethyl acetate, and *n*-hexane to give nine extracts all together (Figure 1). The volume of solvent, maceration duration, and number of shaking the mixture were controlled and made to be exactly the same with each other. Therefore, the effect of given solvent can be observed.

Ethanol, ethyl acetate, and *n*-hexane extract of each sample were analysed by comparing its rendements and phytochemical profiles. Based on rendement, ethanol extract gave the highest yield compared to ethyl acetate and *n*-hexane extract (Table 1). Theorytically, organic solvent will take secondary metabolites with similar polarity out from plant cell tissue (Sticher, 2008), in which ethanol, as universal polar organic solvent, is able to extract almost all secondary metabolites contained in plant cell. This is related with the size of ethanol that small enough to break the membrane of plant cell (Tzanova *et al*., 2020). Further analysis on rendement of each extract obtained that ethanolic extract from *C. auricalatum* roots gave the highest rendement with 8.31%, indicating roots part contained the highest content of secondary metabolites. This data suggested that *C.auriculatum* likely deposited its secondary metabolites more in roots part other than leaves or stems. Moreover, rendement analysis showed that leaves possessed more semi polar to non polar compounds than polar compounds. The yield of ethyl acetate extract from leaves was quite similar to ethanol extract, whereas its *n*-hexane extract gave more than 2%. Thus, *C. auriculatum* stems and roots were suggested to have more polar compounds rather than non polar compounds (Table 1). This suggestion was supported by TLC and HPLC chromatogram analyses on each extract.

Phytochemical screening test showed that the leaves possessed phenolic, alkaloid, terpenoids, and steroids (Table 2). The existence of terpenoids and steroids in leaves was particularly detected in ethyl acetate and *n*-hexane extracts. The roots extract showed that phenolics and alkaloids were dominant secondary metabolites in this part of plant. Previous study reported that secondary metabolites isolated from genus Campnospermae were phenolics and terpenoids. They were lanaroflavon (**1**), a flavonoid isolated from *C. panamese* leaves (Weniger *et al.*, 2004); campnospermenone A (**2**), campnospermenone B (**3**), campnospermenone C (**4**), isolated from *C. zeylanica* Thwaites leaves (Ediriweera *et al*., 2018); and 5-hydroxy-cyclohex-2-en-one (**5**), isolated from *C. auriculatum* oil (Lamberton, 1958) (Figure 2). Thus, the finding of the existence of alkaloids, where it is prevailing in root part of *C. auriculatum*, becomes new phytochemical information for this genus.

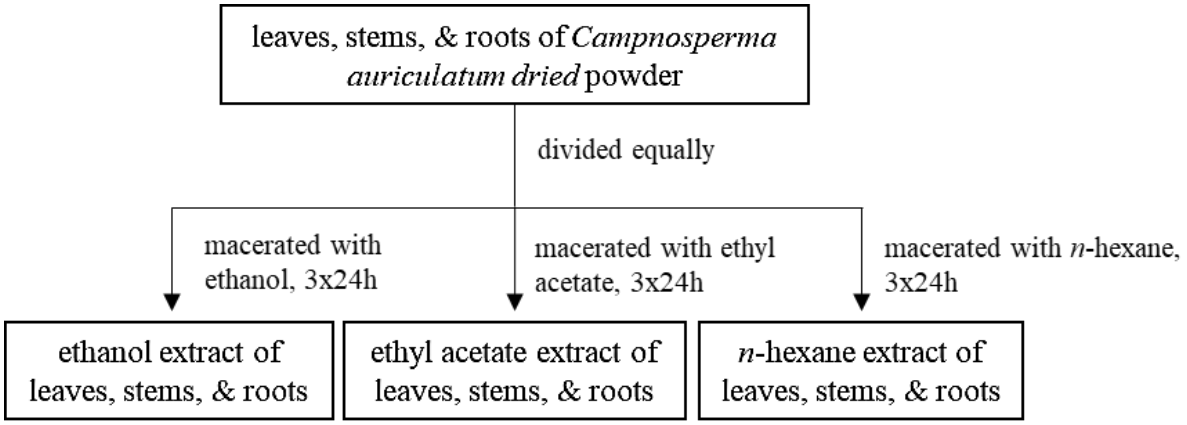

Figure 1. Procedure scheme of leaves, stems, and roots of *C. auriculatum* extraction

Table 1. Rendement yield of each extract

Part of plant	Extract	Amount (g)	Rendement (%)
leaves	Ethanol	3.68	6.69
	Ethyl acetate	3.35	6.08
	n-Hexane	1.64	2.98
stems	Ethanol	3.36	1.73
	Ethyl acetate	0.63	0.32
	n-Hexane	0.58	0.30
roots	Ethanol	3.74	8.31
	Ethyl acetate	0.98	2.19
	n-Hexane	0.19	0.43

Table 2. Phytochemical screening result using several specific reagents towards each extract

Extracts*	Specific Reagent**			
	a	b	c	d
CDE	+	+	++	-
CDA	++	+	+	+
CDH	-	++	+	+
CBE	++	+	-	-
CBA	+	+	-	-
CBH	-	-	+	-
CAE	++	+++	-	-
CAA	++	+++	-	-
CAH	-	+	-	-

*CDE = ethanol leaves extract, CDA = ethyl acetate leaves extract, CDH = n-hexane leaves extract, CBE = ethanol stems extract, CBA = ethyl acetate stems extract, CBH = *n*-hexane stems extract, CAE = ethanol roots extract, CAA = ethyl acetate roots extract, CABH = *n*-hexane roots extract

**a = $FeCl_3$ 5% reagent; b = Mayer reagent; c = salkowsky reagent; d = liebermann-burchad reagent; +, ++, +++ = positive result with increasing concentration; - = negative result

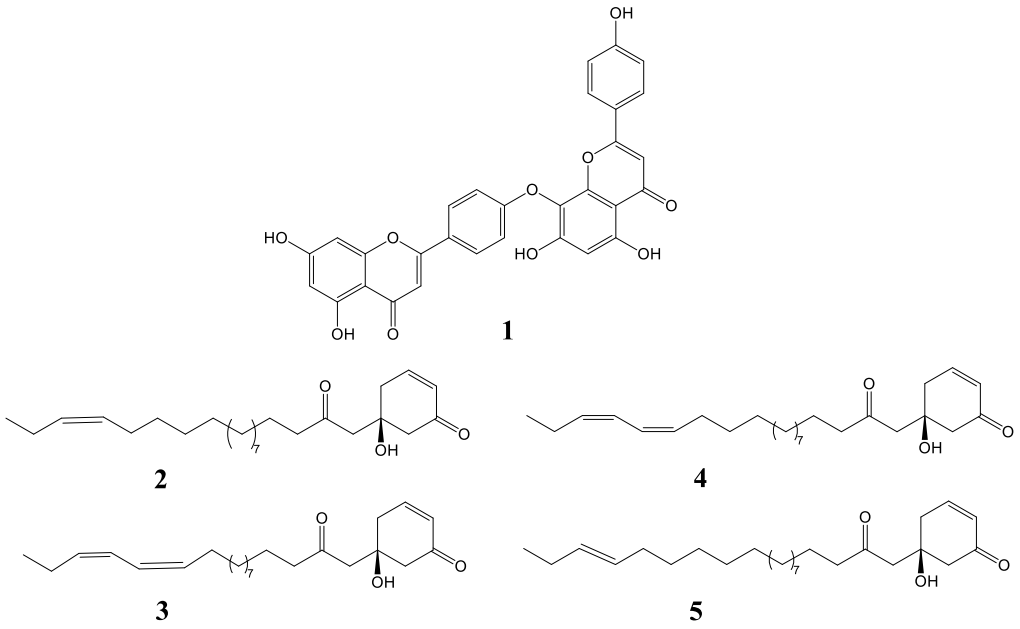

Figure 2. Reported compounds isolated from genus *Campnospermae*

Thin layer chromatography (TLC) technique was performed to analyse chromatogram profile from each extract. Two eluent systems with different polarity (*n*-hexane – ethyl acetate, 8 : 2; chloroform 100%) were used to give better understanding on TLC chromatogram. The chromatogram showed that ethanolic leaves extract differed than that ethanolic stems and roots extracts, by giving spots on non polar region with retention factor above 0.7 (Figure 3). Further, the HPLC chromatogram profile of each ethanolic extract showed similar pattern (Figure 4), particularly for roots and stems extracts. Meanwhile, ethanolic leaves extract chromatogram slightly differed by showing overlapping peaks at retention time 12.5 – 15 minutes (more non polar region). Combining this data with phytochemical screening's result gave facts that phenolics and alkaloids detected in each extract possessed similar polarity and variation. Meanwhile, peaks at retention time 12.5 – 15 minutes in ethanolic leaves extract's chromatogram might belong to terpenoids or steroids.

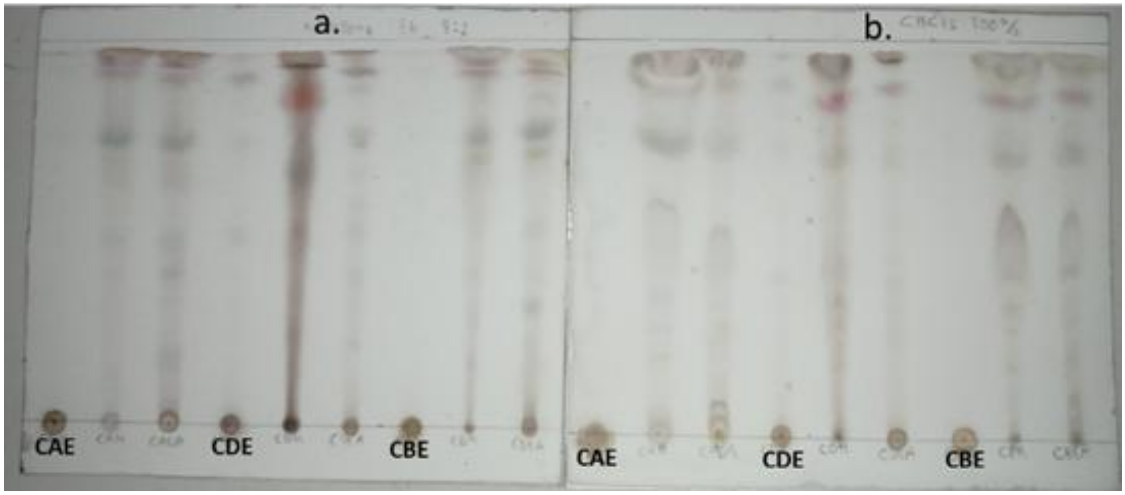

Figure 3. TLC chromatogram for ethanol extract of roots (CAE), leaves (CDE), dan stems (CBE) after spraying with $CeSO_4$ 1.5% with eluent sistem *n*-hexane – ethyl acetate, 8 : 2 (a), and chloroform 100% (b)

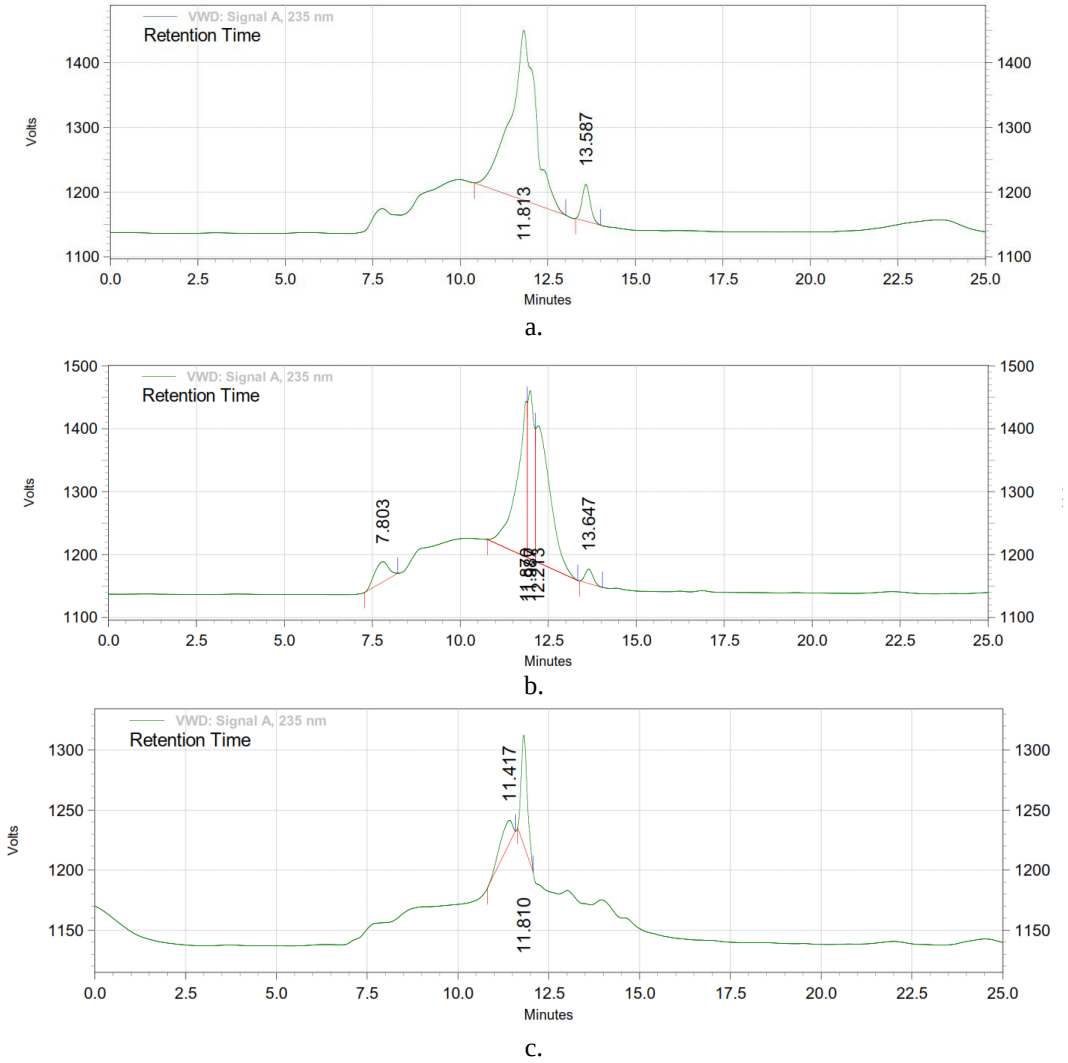

Figure 4. HPLC profile of ethanol roots (a), stems (b), and leaves (c) extracts

Total phenolic content (TPC) from ethanol stems extract was higher than from the ethanol roots and leaves extract. This finding was interesting because $FeCl_3$ 5% test for phenolics of the stems extract showed less intense color than from the roots. In this case, this proved that TPC determination is important to describe quantitatively the amount of phenolics in the plant extract.

Antioxidant evaluation was performed using DPPH assay and showed that ethanol extract of stems and roots exhibited potential antioxidant activity with IC_{50} values of 135.66 and 194.54 ppm (Table 3). Free-radical scavenging activity of an extract or a compound was reported to have strong correlation with other biological activities, such as cytotoxicity, antiinflammation, antidiabetes, anticancer, and antiviral (Lesjak *et al*., 2018; Grigalius *et al*., 2017). In this case, both extracts were predicted to show good results in other bioactivity assays. Therefore, further bioactivity evaluation toward these extracts was necessary to be performed. Correlation analysis between free-radical scavenging activity and total phenolic content of these extracts indicated that there was strong correlation between both variable (r = -0.93). This data indicated that phenolics contained in leaves, stems, and roots of *C. auriculatum* were responsible for radical scavenging activity showed by these extracts. Thus, this study showed that phenolics contribute on free-radical scaveging activity in the plant extract (Muharini, 2021).

4T1 breast cancer cell line is one type of breast cancer cell that commonly used in preclinical research, particularly for studying breast cancer metastasis (Nakayama *et al*., 2021). Cytotoxic activity could be categorized into four groups. They are high cytotoxic (IC_{50} < 20 µg/ml), moderate cytotoxic (21 µg/ml ≤ IC_{50} ≤ 200 µg/ml), weak cytotoxic (201 µg/ml ≤ IC_{50} ≤ 500 µg/ml), and no cytotoxic activity (IC_{50} > 500 µg/ml) (Damasuri *et al*., 2020). Based on this classification, the evaluation result showed that ethanol roots extract exhibited high cytotoxicity, stems extract possessed moderate cytotoxicity, and leaves extract is inactive toward 4T1 cancer cell lines (Table 4). Nevertheless, both roots and stems extracts possessed high selectivity with SI index more than three. Thus, the result indicated that latter extracts has pronounce potential for biopharmaceutical purposes.

Campnosperma auriculatum plant is woody plant used by local for furniture and traditional house floor because it sustains toward termites. The activity of anti-termite was classified into seven categories as very strong (mortality (m) ≥ 95%), fairly strong (75% ≤m ≤ 95%), sufficiently strong (60% ≤ m ≤ 75%), moderate (40% ≤ m ≤ 60%), weak (25% ≤ m ≤ 40%), quite weak (5% ≤ m ≤ 25%), and inactive (m < 5%) (Prijono, 1998). Anti-termite evaluation toward leaves, stems, and roots extracts showed that all of extracts are extremely active against *C. curvignathus*, where roots extract exhibited the most active as antitermite (Table 4). Meanwhile termite's resistance towards extract-impregnated paper was observed through paper weight loss and calculated for antifeedant absolute coefficient (Ohmura *et al*., 2000). There are four criteria of antifeedant activity, i.e very strong (75% ≤ antifeedant (A) ≤ 100), strong (50% ≤ A < 75%), moderate (25% ≤ A < 50%), and inactive (0 ≤ A< 25%) (Dungani *et al*., 2012). Calculation of antifeedant absolute coefficient towards paper weight loss of each extract showed that roots extract exhibited pronounce antifeedant activity. On the other side, the anti-termite activity of leaves and stems extracts was due to the toxicity of its constituents towards *C. carvignathus*. By comparing the anti-termite activity of leaves, stems, and roots extracts, it can be suggested that the constituents responsible for anti-termite contained in roots extract differed from those in leaves and stems extracts. Furthermore, it revealed that the ethanol leaves extract is toxic toward termites *C. carvignathus*, but non-toxic toward 4T1 cancer cell lines. Nevertheless, these findings confirm well the utility of this plant as furniture or house flooring material.

Table 3. Total phenolic contents (TPC) and free-radical scavenging activity data of each ethanolic extract of *C. auriculatum*

Extracts[a]	Free-radical scavenging activity[b] (IC_{50}, ppm)	TPC[b] (GAE, ppm)
CDE	> 1000	151.6 ± 0.19
CBE	135.51 ± 0.91	737.6 ± 0.56
CAE	194.54 ± 1.63	420.3 ± 69.07

[a]CDE = ethanol leaves extract, CBE = ethanol stems extract, CAE = ethanol roots extract
[b] a mean and standard deviation in two replicates

Table 4. Cytotoxicity Data of *C. auriculatum* extracts

Extracts	IC_{50} (µg/ml)		Selectivity Index
	4T1	Vero	
CAE	1.55 ± 3.29	100.66 ± 4.42	64.94
CBE	51.15 ± 1.82	547.32 ± 3.48	10.70
CDE	> 500	> 500	1

CONCLUSION

This study on *C. auriculatum* showed that ethanolic roots extract contained the highest content of secondary metabolites. In general, major secondary metabolites in *C. auriculatum* were phenolics and alkaloids. Meanwhile, the ethanol stems extract showed the highest amount of phenolics based on its TPC value, which also exhibited the highest radical

scavenging activity. It can be concluded that ethanolic stems and roots extracts of *C.auriculatum* were promising to be natural sources for searching antioxidants. All ethanolic extracts exhibited pronounce anti-termite activity, with the ethanolic roots extract gave antifeedant property. These results are able to give a hint for further investigation toward *C. auriculatum* plant. Hitherto, there was no antioxidant and anti-termite of phenolic or alkaloids reported from *C. auriculatum* previously. Thus, further investigation on secondary metabolites from leaves, stems, and roots of *C. auriculatum* was necessary to be done thoroughly.

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