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Extractive composition and bioactivity of *Uncaria acida* and *Uncaria glabrata* wood

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Masendra M., Purba B.A.V., Indrayanti L., Lukmandaru G., 2021. Extractive composition and bioactivity of *Uncaria acida* and *Uncaria glabrata* wood. Ann. For. Res. 64(2): 20-32.

Abstract *Uncaria acida* (red bajakah) and *Uncaria glabrata* (white bajakah) belong to the liana woody species. Both are naturally cultivated in Indonesia, particularly in Kalimantan (Borneo) island. This study, aims to investigate the extractive composition of *U. acida* and *U. glabrata* wood considering that extracts from different lianas usually are used as anticancer drugs (breast cancer). The phenolic, alkaloid, and saponin contents were measured by colorimetric and GC-MS methods, while the antioxidant, antifungal, and cytotoxicity were investigated using DPPH, *Phanerodontia chrysosporium* (white-rot), and brine shrimp lethality tests, respectively. The results showed that the total tannins, phenols, and saponins in *U. acida* were higher compared to *U. glabrata*, while the total flavonoids, alkaloids, polysaccharides, and antioxidant activity was lower. The GC-MS analysis indicated the presence of aromatic compounds, fatty acids, and triterpenoids in both species. High concentration of phenols, alkaloids, saponins, fatty acids, and steroids are known to provide support in terms of antioxidant, cytotoxicity, and antifungal activities.

Keywords: wood extractive, antifungi, bajakah, phytomedicine, polyphenols.

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Introduction

The huge biodiversity of tropical forests is partially attributed to the presence of lianas, a plant group that shares a similar growth strategy: to reach the forest canopy by climbing other plants as support (Schnitzer & Bongers 2002). Lianas have broad variety of species which consist of up to 45% of all lignocellulosic plant species in some areas (Uwalaka et al. 2021). *Uncaria* comprises a group of lianas from Rubiceae family, which is characterized by its "hook" utilized to climb other plants. Approximately, 34 species of *Uncaria* were discovered across the world, including Asia, Africa, and America (Ridsdale 1978, Quattrocchi 2000). Two species from this genus, namely *Uncaria acida* (Hunter) Roxb. and *U. glabrata* (Blume) DC., are growing naturally in Indonesia, and are also known locally, particularly in Kalimantan, as 'bajakah'.

Plants from *Uncaria* genus are deployed as traditional medicines in Indonesia, as well as in several other countries such as Japan and China (Imamura et al. 2011, Fujiwara et al. 2006). In Indonesia, *Uncaria*, as an ethnopharmacological plant, is used by ethnic group of Dayak in Borneo and Anak Rawa in Riau Provinces. *Uncaria gambir* is used as a traditional herbal drink by the Kerabat Dayaknese people in West Kalimantan against the stomachache and as typhus medicine by Wonokerto Community in Yogyakarta Special Province (Kuni et al. 2015, Utami et al. 2019, Nahdi & Kurniawan 2019).

Bajakah extracts were reported to contain alkaloids, triterpenoids and phenolics (Slade et al. 2005, Salim et al. 2011, Pavei et al. 2012, Zhang et al. 2020). Different alkaloids, including 19-epi-ajmallicine, rauniticine, 14α-hydroxyraunitine, uncarine A, glabratine, deoxycordifoline, and flavonoids termed (+)-catechin, were detected in the barks and leaves *U. glabrata* (Arbain et al. 1992, Arbain et al. 1993, Arbain et al. 1998). Antioxidant activities of other *Uncaria* species such as *U. tomentosa*, *U. guianensis*, and *U. gambir* have been reported (Sandoval et al. 2002, Navarro-Hoyos et al. 2018, Apea-Bah et al. 2009). The level of toxicity and the potential to be used as novel anticancer agents and anti-Alzheimer`s disease have been also investigated (Azad et al. 2018, Xu et al. 2021).

Other studies have revealed various other pharmacologically applications of *Uncaria* species, and that's why *U. acida* and *U. glabrata* might also be of interest for their medicinal value. However, existing studies showed limited information concerning the chemical composition for the wood part. This study is, therefore, recorded as the first investigation on extractive composition of *U. acida* (red bajakah) and *U. glabrata* (white bajakah) wood species and on their bioactivities.

Materials and Methods

Sample collection and extraction

The stems (diameter 3-5 cm) of the lianas were collected from Hampangen Educational Forest, Palangkaraya University, Central Kalimantan (Borneo) Province (113˚30'16" E and 1˚51'35" S, 35 m a.s.l.). The sites present a mean annual precipitation ranging from 2,776 to 3,393 mm and a mean annual temperature ranging from 23 to 32 ˚C. After collection, the specimens were deposited at the Indonesian Institute of Science. Subsequently, the samples were powdered and successively extracted using n-hexane, methanol, and hot water in a Soxhlet apparatus for 6 hours. The resulting solution was then dried through a rotary evaporator and the results were expressed in percentage of dry samples.

Total tannin content (TTC)

The total tannin content was investigated according to Padmaja (1989). 0.1 ml of the extract (1000 ppm) was diluted with 7.5 ml of distilled water followed by the addition of 0.5 ml Folin Denis' reagent and 1 ml

sodium carbonate (35%). Further dilutions were performed to obtain a 10 ml volume. Subsequently, the sample reaction was nurtured at room temperature for 30 min and the absorbance was recorded at 760 nm. The total tannin content was expressed as tannic acid equivalent (mg TAE/g sample).

Total phenolic content (TPC)

The TFC assessment was conducted according to Brighente et al. (2007), where 2 ml of the sample (1 mg/ml) were mixed with 2% $AlCl_3 6H_2O$ solution in methanol. The reaction was maintained at 20 ˚C and the sample absorbance was recorded at 415 nm. The results were expressed in quercetin equivalents (mg QE/g extract).

Total polysaccharides (TP)

The TP measurement protocol consists of 1 ml of the extract (1000 ppm) mixed with 1 ml phenol (5%) and 5 ml concentrated sulfuric acid (98%). Subsequently, the reaction was then paused for 20 mins at room temperature and the sample absorbance was read at 490 nm in line with glucose standard. The measurement unit was specified as mg glucose equivalent per g sample of extract (DuBois et al. 1956).

Total alkaloids content (TAC)

The 1000 ppm of the extract (1 ml) reacted with a mixture of 1 ml of 0.025 M $FeCl_3$, 0.5 M HCl, 1 ml of 0.05 M, and 1, 10-phenanthroline solution in ethanol. The mixture was nurtured for 30 min at 70 ˚C, and the absorbance was read at 510 nm. The quinine standard was utilized for the calibration of total alkaloid content (mg QnE/g sample) (Singh et al. 2004).

Total saponin content (TSP)

The total saponin content was documented from a previous reference (Makkar et al. 2007). 0.25 ml of extract (1000 ppm), 8% of vanillin reagent, and 72% of sulphuric acid were reacted for 10 min at 60 °C temperature. The sample was then cooled on ice for 4 min in order to stop the reaction and subsequently read at 544 nm. Diosgenin standard was applied for total saponin calibration (mg DE/g sample).

GC-MS Analysis

The methanol extract was sylilated according to Wijayanto et al. (2015). The process was performed by dissolving 1 mg of the sample in trimethylchlorosilane (15 µl) and N,O-bis (trimethylsilyl) acetamide (85 µl). Subsequently, the sample was evaporated after 1 h of incubation and the dry extract was dispersed in 1 ml MeOH. The *n*-hexane samples were prepared by direct injection. Meanwhile, the gas chromatography-mass spectrometry (GC-MS) data were collected using GCMS-QP 2010 (Shimadzu, Japan). The 1 µl of sylilated sample and non-sylilated *n*-hexane extracts were injected into the GC-MS machine. The GC conditions include Rtx-5MS capillary column (30 m x 0.25 mm I.D. and 0.25 µm; GL Sciences, Tokyo, Japan); column temperature was from 70 °C (1 min) to 290 ˚C at 5 ˚C /min at a injection temperature of 270 ˚C Detection temperature was of 290 ˚C and acquisition mass range of 50 - 800 amu using helium as the carrier gas. The sample mass spectrum was compared to the NIST library. The peak relative method was applied to calculate detected compounds.

DPPH scavenging activity

Antioxidant activity evaluation was performed according to Baba & Malik (2015). 0.1 ml extract (1000 ppm) of methanol reacted with 3 ml of 0.1 mM DPPH (1,1-diphenyl-2-picrylhydrazyl). The reaction was incubated for 30 mins and the absorbance was measured at 517 nm. The antioxidant activity was calculated by equation (1):

$$\text{DPPH scavenged}(\%) = 100 \times (Ao - A1)/Ao \quad (1)$$

where Ao is the blank absorbance and A1 represents the sample absorbance.

Antifungal activity

The antifungal activity was measured according to Lukmandaru (2013), employing *Phanerodontia chrysosporium* (white-rot). The wood samples were placed on the surface of 20 ml potato dextrose agar (PDA; Aldrich, Germany) medium in Petri dishes. 1 h after the sample spreading, the fungi were inoculated. The blank was conducted without extract and the samples were separately observed in three replications. Also, the positive control of commercial biocide was applied and the growth inhibition was calculated using equation 2:

Growth inhibition (%) = (A0-A1)/A0 x 100 (2)

Where $A = \pi \times (d/2)^2$, d = diameter of sample growth, A0 is the blank growth inhibition and A1 represents sample growth inhibition. The IC_{50} was calculated as 50% of fungi inhibition.

Brine shrimp lethality

To predict cytotoxicity, brine shrimp lethality test with some modifications was conducted (Sarah et al. 2017). The *Artemia salina* cyst (Breeders©) was hatched in artificial sea water (9 g of rock salt per 1 litre of water). After 24 hours of constant lightning and aeration, the nauplii were introduced in different culture media. The extracts were diluted to 10,000 µg/ml concentration using dimethyl sulfoxide (DMSO) and 50 µl, 5 µl, and 1 µl of the resulting solutions were added consecutively to 5 ml of artificial sea water containing 10 nauplii. After 24 hours, the percentage of dead nauplii and LC_{50} values were calculated. Negative controls were also applied using only DMSO, Positive controls were created with gallic acid in a similar procedure. The brine shrimp test was calculated from equation 3:

Lethality rate (%) = (A0-A1)/(A0) x 100 (3)

Where A0 is lethality of negative control and A1 is the sample lethality. The LC50 was calculated as the concentration of the extract or fractions assumed to kill 50% of nauplii.

Chemicals

Quercetin, gallic and tannic acids, quinine, diosgenin, DPPH, TMCS (trimethylchlorosilane), BSA (N, O-bis (trimethylsilyl) acetamide), Folin-Ciocalteu, and Folin-Denis' reagents were purchased from Sigma-Aldrich (Germany). The fungicide product was acquired from Bioindustries, Yogyakarta, Indonesia. The active biocides were methylene bis thiocyanate and 2-thyocyanomethyl thiobenzothiazole.

Statistical analysis

Two-way ANOVA was used to determine the significance of species and solvent factor by employing SPSS software (IBM, USA) with a 95% confidence level. The data showing significant variation were further analysed with Tukey HSD test. Correlations between parameters were evaluated using Pearson coefficient.

Results

Extractive content

Figure 1 shows the extraction content of the two studied liana woody species. The extractions were performed in n-hexane, methanol and hot water. The highest extractive content of *U. acida* was obtained in methanol (10.2%), while the highest extractive content for *U. glabrata* was obtained in hot water (5.76%). These conditions suggests that both samples contained mainly phenolics and sugars.

Extractive composition

Table 1 shows the extractive composition of *U. acida* and *U. glabrata* indicating the values of TTC, TPC, TFC, TP, TSP, and TAC. TTC, TPC, and TSC of MeOH of both species were larger compared to hot water extracts. For TAC and TP, only *U. glabrata* provided higher value in its MeOH extract. Meanwhile, the TP and TAC contents of *U. acida* MeOH extract were lower compared to hot water extract. By

applying ANOVA test a significant variation in the interaction between the species and fraction was observed.

GC-MS analysis

Table 2 shows the secondary metabolites identified in MeOH extracts. The constituents of both species were dominated by aromatic compounds. The major phenolic compounds involved benzoic acid, hydroquinone, and fatty acids in the form of palmitic and tetradecanoic, while the alkaloid was represented by 2-(5-[1,3] dioxolan-2-yl-pentyl)-3-methylaziridine. Previous reports also included the benzoic acid and hydroquinone derivatives in the species of *Cinamomiun verum* and *Terminalia arjuna* (Kankeaw & Masong 2015, Dutta et al. 2015). Moreover, the fatty acids (C16: 0) were detected in *Uncaria tomentosa* (Makoto et al. 2003). However, in the *n*-hexane extracts, the lianas were dominated by steroids and teriterpenoids i.e beta-sitosterol acetate and ursane-3,12-diol (Table 2).

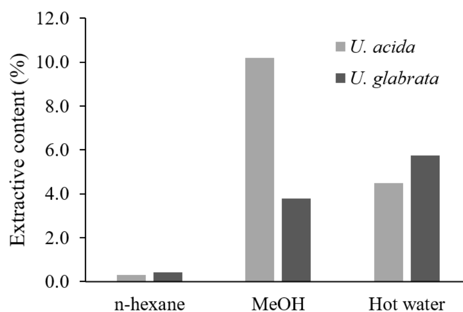

Figure 1 Extractive content of *U. acida* and *U. glabrata* woods

Table 1 Colorimetric measurement of *U. acida* and *U. glabrata* extracts.

Sample	Fraction	TTC (mg TAE/g)	TPC (mg GAE/g)	TFC (mg QE/g)	TP (mg GE/g)	TSC (mg DE/g)	TAC (mg QnE/g)
U. acida	MeOH	245.2 ±0.33[d]	166.5 ±1.83[d]	40.5 ±0.14[c]	31.2 ±0.09[b]	557.5 ±0.58[d]	59.0 ±0.10[a]
	Hot water	239.5 ±0.02[b]	144.6 ±0.79[b]	23.2 ±0.40[a]	55.9 ±0.26[c]	72.6 ±0.40[b]	120.3 ±0.70[c]
U. glabrata	MeOH	244.9 ±0.39[c]	160.2 ±1.61[c]	55.8 ±0.15[d]	119.8 ±1.00[d]	141.4 ±0.28[c]	117.6 ±0.20[b]
	Hot water	163.8 ±0.02[a]	50.6 ±1.16[a]	31.3 ±0.87[b]	14.3 ±0.04[a]	69.6 ±0.10[a]	119.0 ±0.25[c]

Note: TTC/total tannin content ($p<0.01$) HSD value=0.12; TPC/total phenolic content ($p<0.01$) HSD value=5.04; TFC/total flavonoid content ($p<0.01$) HSD value=1.76; TP/total polysaccharides ($p<0.01$) HSD value=1.89; TSC/total saponin content ($p<0.01$) HSD value=1.37; TAC/total alkaloid content ($p<0.01$) HSD value=1.36

Table 2 GC-MS results of MeOH (no. 1-16) and *n*-hexane (no. 17-23) extract from *U. acida* and *U. glabrata*.

No	Ret. time (min)	Constituents	Concentration (% of dried extract) *U. acida*	*U. glabrata*	Similarity index (%)
		Aromatics	**58.2**	**47.7**	
1	7.0	Isopropyl vinyl	2.3	tr	71
2	7.1	(1S)-Propanol, (2S)-[(tert.butyloxycarbonyl)amino]-1-phenyl	0.2	tr	60
3	11.4	Benzoic acid	15.9	15.7	85
4	12.5	4-Piperidinepropanoic acid,	9.7	8.0	89
5	12.9	Hydroquinone	10.2	6.0	81
6	13.5	5-Hydroxymethylfurfural	7.5	5.0	81
7	16.3	2,4-Dimethoxyphenol	5.9	1.0	81
8	20.3	Homovanillyl alcohol	3.8	1.0	80
9	22.3	2,2-Dimethyl-5-tert-butyl-1,3-oxathiane	1.0	2.0	60
10	22.5	2-(5-[1,3] Dioxolan-2-yl-pentyl)-3-methylaziridine	1.7	9.04	60

Table 2 GC-MS results of MeOH (no. 1-16) and *n*-hexane (no. 17-23) extract from *U. acida* and *U. glabrata*.

No	Ret. time (min)	Constituents	Concentration (% of dried extract)		Similarity index (%)
			U. acida	*U. glabrata*	
		Fatty acids	**41.5**	**46.8**	
11	27.4	Tetradecanoic acid	5.8	16.6	89
12	28.1	Palmitic acid	26.3	20.2	90
13	30.2	9,12-Octadecadienoic acid	1.9	2.0	83
14	30.9	Stearolic acid	4.1	3.0	84
15	31.3	Nonadecanoic acid	3.4	5.0	82
		Steroids and triterpenoids	**67.5**	**77.8**	
16	42.2	Stigmast-5-en-3-ol, oleate	8.0	tr	60
17	44.2	beta-Sitosterol acetate	59.5	38.9	72
18	47.8	Ergosta-4,6,22-trien-3.alpha.-ol	tr	10.2	64
19	48.7	Ursane-3,12-diol	-	28.6	55
		Other compounds	**31.5**	**21.8**	
20	14.2	Cyclohexasiloxane, dodecamethyl-	10	10	77
21	18.2	Nonane, 1-iodo-	6.9	-	71
22	18.9	Hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane	14.6	-	79
23	49.7	3,4-Dimethylbenzoic acid, tert-butyldimethylsilyl ester	-	11.8	50

Note:(tr): trace; (-): not detected

Bioactivities (antioxidant, antifungal, and cytotoxicity)

DPPH scavenging: The highest antioxidant activity was established in the MeOH extracts for *U. glabrata* and in hot water extracts for *U. acida* ($p<0.01$; HSD value = 31.14). The values were higher in *U. glabrata* compared to the quercetin positive control (Fig. 2).

Antifungal activity: Table 3 shows the inhibition of *P. chrysosporium* of *U. acida* and *U. glabrata* extracts. Both species confirmed a weak fungal activity compared to biocide positive control.

Cytotoxicity: The last bioactivity for *U. acida* and *U. glabrata* was conducted by brine shrimp lethality test. In Table 4 is shown that the maximum cytotoxicity level occurred in *U. glabrata n*-hexane soluble extract, while the lowest activity is reflected in MeOH extract. However, the *n*-hexane and hot water of *U. glabrata* extracts citotoxicity were higher compared to gallic acid positive control. Generally, all the recorded values were moderate.

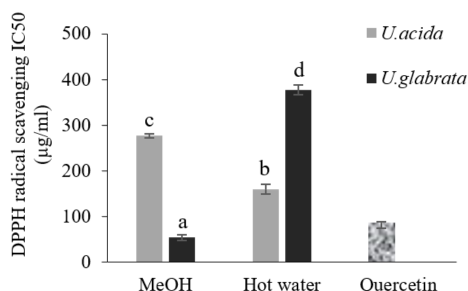

Figure 2 Antioxidant activity of *U. acida* and *U. glabrata* extracts.

Correlation between parameters

Table 5 shows the Pearson correlation between separate parameters. The highest negative value was obtained between TSC and TAC (r=-0.99*), while the optimum positive rate

Table 3 IC_{50} of inhibitory activity of *P. chrysosporium* (μg/ml).

Sample	Extract			Biocide
	***n*-hexane**	**MeOH**	**Hot water**	
U. acida	>2000	1901 ± 117	1561 ± 345	<500
U. glabrata	>2000	1569 ± 140	1758 ± 401	

Table 4 LC_{50} of brine shrimp test (μg/ml).

Sample	Extract			Gallic acid
	***n*-hexane**	**MeOH**	**Hot water**	
U. acida	138 ± 36[ab]	146 ± 63[ab]	159 ± 51[b]	108 ± 17
U. glabrata	31 ± 0.1[a]	248 ± 54[b]	90 ± 13[a]	

occurred between TTC and TPC (r=0.98*). Furthermore, strong negative correlations appeared between TP and TAC (r=-0.92**), DPPH and TPC (r=-0.71**), DPPH and TTC (r=-0.76**) as well as a strong positive correlation measured between TSC and TP (r=0.92**). Additional important correlations were also found between TP and TPC (r=0.65*), TTC and TP (r=0.59*), brine shrimp LC_{50}, and TTC (r=0.59*).

Discussions

The phenolic, alkaloids, and saponin contents of the samples were observed. Extractive composition was significantly influenced by solvent polarity and species variations. Generally, both species extracts were dominated by less polar compounds, apart from the TP and TPC of *U. acida*. Furthermore, the *U. acida* contained superior amounts of total saponins and phenolics, including tannins, whereas *U. glabrata* showed maximum concentration of total flavonoids, polysaccharides, and alkaloids. *U. acida* and *U. glabrata* have higher TPC and TFC levels than that of *U. gambir* (Kassim et al. 2011, Amir et al. 2012) and *U. tomentosa* (Navarro-Hoyos et al. 2015). However, both species have a lower content compared to the TPC and TFC levels of *U. rhynchophylla*.

The most noticeable dissimilarity between species occurred in the TSC value. Saponin is a glycoside compound containing aglycone (triterpenoid, steroid, or steroidal alkaloid) linked to glycone or oligosaccharide moieties (Mohan et al. 2016). Therefore, as a result of its high saponin content, *U. acida* is expected to be utilized in applications involving biopesticides, biosurfactants, and pharmaceuticals. However, the saponin compounds are harmful in general (Jiang et al. 2018). Due to the fact that previous research reported moderate toxicity of *U. acida* leaves extract (Azad et al. 2018) and due to the diversity of saponin structures in plants, the use of *U. acida* extracts requires further studies, before being deployed.

Table 5 Pearson correlation between DPPH and phytochemicals in *U. acida* and *U. glabrata.*

Parameter	TAC	TPC	TP	TSC	TTC	TFC	DPPH IC_{50}	Fungal IC_{50}
TPC	-0.45							
TP	-0.92**	0.65*						
TSC	-0.99**	0.52	0.92**					
TTC	-0.33	0.98**	0.59*	0.40				
TFC	-0.17	0.42	0.00	0.27	0.31			
DPPH IC50	-0.27	-0.71**	0.06	0.17	-0.76**	-0.51		
Fungal IC50	-0.43	-0.08	0.32	0.41	-0.13	-0.02	0.39	
Brine shrimp LC50	0.13	0.48	0.19	-0.11	0.59*	-0.26	-0.50	-0.12

* significant correlation (0.05 level)
** very significant correlation (0.01 level)

The MeOH extract of *U. glabrata* exhibited a higher level of inhibition compared to hot water extract in terms of antioxidant activity (Figure 2). This is probably due to the presence of high total tannins, phenolics, and flavonoids content. The polyphenol is one of the antioxidant agents derived from plants with the ability to reduce DPPH into DPPH-H as a result of the presence of -OH bond. Previous study shows the existence of cathechins in this species (Arbain et al. 1993). This compound is assumed to exhibit antioxidant activity of *U. glabrata* methanol extract. However, various patterns are known to occur in *U. acida* and the hot water extract generated higher antioxidant activity compared to MeOH. This indicates that the polyphenolic compound was bonded with sugars in the form of glycosides. The high concentration of total tannin and phenolic content in hot water extract also tend to cause the DPPH reduction. The antioxidant activity of *U. acida* and *U. glabrata* were in lower rate compared to *U. gambir*, the well-known Southeast Asia liana (Kassim et al. 2011) and the famous Chinese herbal of *U. rhynchophylla* (Kim et al. 2012).

Based on the GC-MS results, the two woody species contained high concentration of aromatic compounds known to promote antioxidant activity. In addition, the phenolic compounds i.e. benzoic acid and hydroquinone tend to act as DPPH inhibitors. Earlier studies reported that these derivatives were applied as efficient antioxidant agents (Velika & Kron 2012, Kankeaw & Masong 2015). The presence of these aromatic compounds also was detected in other lianas, i.e. *U. gambir* (Navarro-Hoyos et al. 2015) and *U. rhynchophylla* (Zhang et al. 2016).

The antifungal activity of the lianas on *P. chrysosporium* showed that the MeOH and hot water extracts exhibited stronger inhibition compared to n-hexane. This indicated the presence of the more polar compounds in the extracts. The phenolic compounds also tend to hinder the fungal activity. Previously, white-rot fungi inhibitors, i.e., lignan and latifolin, were reported as having antifungal activity (Sekine et al. 2009, Wijayanto et al. 2015). Therefore, the presence of phenol contents as well as aromatic compounds in *U. acida* and *U. glabrata* is assumed to be responsible for the *P. chrysosporium* inhibition. Furthermore, the fungal growth inhibitory potential of lianas was also studied using species such as *U. gambir* (Nandika et al. 2019) and *U. tomentosa* (Moraes et al. 2015).

Variation in recorded antioxidant and antifungal activity indicated that the cytotoxicity of *U. acida* and *U. glabrata n*-hexane extracts is the highest. In addition, the compounds were assumed to be responsible for the brine shrimp lethality due to the fact that n-hexane contained more steroids and triterpenoids. This represents a lower lethality rate of *U. acida* wood extracts compared to its leaves (Azad et al. 2018) and hooks of *U. tomentosa* (Sofiana et al. 2020) but brine shrimp lethality levels of both *U. acida* and *U. glabrata* were higher than of *U. cordata* leaves (Azad et al. 2018) and *U. rhynchophylla* hooks (Orlando et al. 2019).

The correlation of the compound type plays a significant role in bioactivity. In this study, two phenolic types, termed TPC and TTC, were compared with DPPH IC_{50}. A lower IC_{50} value reflected a stronger antioxidant activity. Meanwhile, a negative correlation indicates a positive interaction between the compound and the antioxidant activity. The role of phenolics as antioxidants has been previously reported (Zeka et al. 2019). Similarly, previous study related to *U. tomentosa*, reported a correlation between TPC and antioxidant activity (Navarro-Hoyos et al. 2018). This research identified the highest correlation with antioxidant activity using TTC. The result showed the antioxidant activity is attributed to the tannin compound in both species. Furthermore, correlation between antioxidant activity and tannin content also was recorded in an earlier report (Bizuayehu et al. 2015). No correlation of the TAC type agreed with previous research by Sandoval

et al. (2002), where the result of antioxidant activity from *U. tomentosa* and *U. guianensis* have not been correlated with their alkaloid content.

This experiment revealed a significant positive correlation between LC_{50} and TTC, indicating a negative correlation between toxicity and TTC. Tannin compounds are known to possess toxic effects due to the ability to reduce nutrient digestibility and protein availability (Mena et al. 2015). However, the tannin ingestion had also been reported to enhance the organism's survival rate such as of *Morone saxatilis* larvae (Ashraf & Bengtson 2007). The tannin toxicity was also described as dependent to its type and molecular sizes, where gallotannin was recorded to be more toxic than ellagitannin to *A. salina* nauplii (Yamasaki et al. 2002). This result indicates the toxicity of *U. acida* and *U. glabrata* were not attributed to its tannin compound.

Several correlations between compounds measured by colorimetric assay were also established, although this study measured TP and TTC. Hydrolysable tannins are polyesters of organic acids and polysaccharides (Plaza et al. 2018). This correlation tends to indicate the presence of polysaccharides in the tannin group. Furthermore, a correlation between TPC and TTC indicates tannin as a dominant compound in the phenolic group, while flavonoids being the less dominant. Some saponin compounds can be classified as steroidal alkaloid saponins containing steroidal alkaloid as its aglycone (Rahman & Choudhary 1999). The negative correlation between TAC and TSC is interpreted as the absence of steroidal alkaloids saponin in the extract.

Conclusion

The chemical extracts of *U. acida* and *U. glabrata* were investigated for the first time by colorimetric and GC-MS methods. For both species, the extracts contained phenols, alkaloids, and saponins. The GC-MS studies also confirmed that both species contained phenolic compounds, alkaloids, fatty acids, steroids and triterpenoids. Concerning the antioxidant activity, the highest antioxidant activity and lethality rate of brine shrimp occurred in *U. glabrata* when compared to *U. acida.* In summary, the enhanced phenol contents and aromatic compounds contained in bajakah suggested that these chemicals contribute to the antioxidant and antifungal activities whereas the high levels of steroids and triterpenoids are responsible for their cytotoxicity. Therefore, the *U. acida* and *U. glabrata* or bajakah wood may potentially act as phytomedicines.

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