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Effect of Several Solvents on the Chemical Compound, Antioxidant Activity, and Cytotoxicity Levels of *Acacia decurrens* Willd. Gum Extract

Pengaruh Perbedaan Jenis Pelarut terhadap Kandungan Senyawa Kimia, Aktivitas Antioksidan, dan Kadar Sitotoksisitas dari Ekstrak Gum Acacia Decurrens Willd

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ABSTRACT

This study aimed to determine the chemical compounds in A. decurrens gum and its bioactivity potential. Extraction was carried out in multilevel with the reflux technique using n-hexane, ethanol, and water as solvents. The chemical compounds were identified using Gas Chromatography-Mass Spectrometry (GC-MS). The amount of A. decurrens gum extract yield, total phenolic content (TPC), total flavonoid content (TFC), and total flavonols content (TVC) were then analyzed quantitatively. Furthermore, bioactivity in the form of antioxidant activity was analyzed with the 2,2-diphenyl-1-picrylhydrazil (DPPH) method, while the cytotoxicity level was assessed using the brine shrimp lethality assay (BSLT). The GC-MS analysis showed that the n-hexane-soluble fraction contained alkane, alkene, aldehyde, ester, and aromatics, while the ethanol-soluble fraction contained monosaccharide and fat group compounds. The water-soluble fraction contained only the monosaccharide group. The highest content value was obtained from the ethanol-soluble fraction, namely TPC, TFC, TVC, and antioxidant activity of 19.61±0.47 mgGA/g, 23.89±1.32 mgQE/g, 13.41±0.81 mgCE/g, and IC50: 3.842 g/mL, respectively. The cytotoxicity content obtained from each fraction sequentially was 0.47 ppm, 5.91 ppm, and 6.56 ppm for n-hexane, ethanol, and water, respectively.

KATA KUNCI
ekstraksi bertingkat,
TPC, TFC, TVC, bioaktivitas

INTISARI

Penelitian ini berfokus pada pencarian potensi senyawa kimia dan potensi bioaktivitas gum A. decurrens. Ekstraksi dilakukan secara bertingkat dengan teknik refluks. Pelarut yang digunakan yaitu n-heksana, etanol, dan air. Identifikasi kandungan senyawa kimia dianalisis dengan Kromatografi Gas - Spektrometri Massa (GC-MS). Besaran rendemen ekstrak gum A. decurrens, kadar fenolik total (TPC), kadar flavonoid total (TFC), kadar flavanol total (TVC) dianalisis secara kuantitatif. Bioaktivitas berupa aktivitas antioksidan dianalisis dengan metode 2,2-difenil-1-pikrilhydrazil (DPPH) dan kadar sitotoksisitas dianalisis dengan metode uji kematian larva udang (UKLU). Analisis GC-MS menunjukkan pada fraksi n-heksana terdapat senyawa dari kelompok alkana, alkena, aldehida, ester, dan aromatis. Pada fraksi etanol terdapat senyawa dari kelompok monosakarida dan lemak. Pada fraksi air terdapat senyawa dari kelompok monosakarida. Nilai tertinggi diperoleh dari fraksi larut etanol pada pengukuran TPC (19,61±0,47 mgGA/g), TFC (23,89±1,32 mgQE/g), TVC (13,41±0,81 mgCE/g), dan aktivitas antioksidan (IC50: 3,842 g/mL). Kadar sitotoksisitas yang diperoleh dari masing-masing fraksi secara berurutan yaitu n-heksana (LC50: 0,47 ppm), etanol (LC50: 5,91 ppm), dan air (LC50: 6,56 ppm).

Introduction

Gum is one of the products of secondary metabolism, which can be used for bioprospecting purposes in the health and pharmaceutical industry. There are at least 34 types of gum that belong to the Acacia genus (Nussinovitch 2010). Furthermore, as one of the countries included in the Indo-Malesian region, Indonesia is a good location for the distribution of the Acacia genus (Nielsen 1992). This situation provides an opportunity to explore the potential of gum from this genus. The first step is to streamline the process of finding bioprospecting targets by selecting species from the same genus of known species that produces valuable products (Brockelman 1997). The fact that *Acacia senegal* produces well-known and valuable gum Arabic becomes the basis of exploring the gum from the Acacia genus for bioprospection. Sudan has commercially produced Arabic gum from *A. senegal* and is globally distributed as a medicine for coughs, flu, pharyngitis, gastric irritation, inflammation, diarrhea, and dysentery (Nussinovitch 2010). Arabic gum is a rich source of non-viscous soluble fiber, has several health benefits, and has high antioxidant properties(Elnour et al. 2018).

In determining bioprospecting targets from natural materials, it is also crucial to observe the number of available genetic resources in nature and their uses by local communities (Brockelman 1997). The abundance of *A. decurrens in* Indonesia and the lack of study on its specific uses, including gum, become the reasons for selecting *A. decurrens* as a bioprospecting target in Indonesia. Furthermore, the species is dominant in the Mount Merapi area during the early stages of succession after its eruption in 2010 (Suryawan et al. 2015). This species is also found in the Gunung Merapi National Park (TNGM) area, on community lands of Kaliadem Hamlet, Kepuharjo Village, Cangkringan, Sleman (Suryanto et al. 2010), and Kemalang, Klaten, and Selo, Boyolali (Sunardi 2016).

In Indonesia, *A. decurrens* has become the source of animal feed plants, dyes, tannins, and timber (Susantyo 2011). So far, there is no study on its active ingredient and gum as medicinal raw material in Indonesia. However, several studies suggested that the bark has potential as an antioxidant because it contains flavonoids, phenols, and tannins (Bamidele et al. 2017). In Australia, *A. decurrens* produced a large amount of gum in spring, which has been used as a raw material for making jelly-like chewy candies (Maiden 1869). The *A. decurrens* gum is also a binding agent for gluing tanks or barrels (Nussinovitch 2010). For these reasons, it is crucial to explore the content of *A. decurrens* gum, especially its antioxidant content, using a similar method for the bark. 'Mirghani et al. (2018) suggested that species *A. senegal, Acacia polyacantha*, and *Acacia seyal* contained the phenolic compounds and antioxidant potential of the Arabic gum methanol fraction after being tested with 2,2-diphenyl-1-picrylhydrazil (DPPH) and other methods. Based on the total phenolic compounds (TPC) test using the Folin Ciocalteu method, Acacia, apricot, and karaya gums also contain phenolic compounds that indicate antioxidant potential (Hamdani et al. 2018). Arabic gum and gums from other species are safe for consumption and have no acute toxic properties (Nussinovitch 2010). However, Guar gum from *Cyamopsis tetragonolobus* species contains fluoro acetic acid, which is poisonous (Vartiainen & Gynther 1984). Therefore, a study on the content of poison compound in *A. decurrens* gum is crucial to understanding the gum's properties for future uses.

N-hexane, ethanol, and water become three types of solvents to extract *A. decurrens* gum. The N-hexane extracts non-polar compounds, such as hydrocarbon compounds (Harborne 1984). Ethanol extracts polar compounds because it is a suitable solvent for

polyphenol extraction and is safe for human consumption (Do et al. 2014). Water solvents extract water-soluble polar compounds because some gums are natural soluble compounds in heated water (Mantell 1949). The chemical compound identification of the *A. decurrens* gum extract uses Gas Chromatography-Mass Spectrometry (GC-MS). The phenolic compound analysis uses a quantitative test. The bioactivity analysis uses antioxidant bioactivity and cytotoxicity level tests. Based on this background, there is an urgency to study the potential of medicinal raw materials from *A. decurrens* gum extract in Indonesia. Therefore, this study will open opportunities to explore gums from Acacia species in Indonesia.

Materials and Methods

Preparation of *A. decurrens* Gum

Samples were collected at Palemsari Village, Cangkringan Sub-district, Sleman Regency, Yogyakarta, Indonesia. This research used 20 naturally regenerated *A. decurrens* trees after the eruption of Mount Merapi in 2010. The duration of gum collection was three weeks by tapping those at 1.5 m from the base. The gum harvesting occurred two weeks after the initial tapping and one week after the re-tapping. In each harvest, the gum yield from 20 trees was weighed as a total harvest.

Extraction of *A. decurrens* Gum

The gum (21.03 grams) was extracted sequentially with n-hexane, ethanol, and water as solvents. The extraction duration was six hours for each solvent using the reflux technique at the boiling point temperature of each solvent, namely 68°C, 78.3°C, and 100°C for n-hexane (Ramadhan et al. 2020), ethanol, and water, respectively (American Chemical Society 2019).

Yield Test of *A. decurrens* Gum Extract

The gum extract yield was extracted from six-hours extraction with four repetitions and calculated from the percentage of weight change of the initial gum extract sample weight. The yield calculation used the formula below:

$$\text{Yield (\%)} = \frac{(A-B)}{\text{Weight of Initial Sample}} \times 100$$

A = Flask weight and gum extract (g)

B = Empty flask weight (g) (Ketaren 1986).

Figure 1. Tapping form in 4th weeks (left) and the condition after a week (right)

Test of Chemical Compounds using Gas Chromatography-Mass Spectrometry (GC-MS)

The chemical compounds tests used the Gas Chromatography-Mass Spectrometry (GC-MS) Shimadzu QP 2010. The GCMS analysis employed the method proposed by Lukmandaru & Gazidy (2016). The comparison between the fragmentation of mass spectrum pattern with that of the reference compound indicated the chemical compounds (Hartono et al. 2017). The NIST11 *Database* became the source for the reference compounds. The identified chemical compounds were quantified using an internal standard (2-Naphthalenol), which was added before the isolation of the material. The deviation used the silylation method to substitute alkyl ethers with silyl for polar and non-volatile samples (Gandjar & Rohman 2012). The silylation used chlorotrimethylsilane (TMCS), bis(trimethylsilyl)-acetamide (BSA), and pyridine as the solvent (Moldeveanu & David 2018).

Phenolic Content Test

Total Phenolic Content (TPC) Test

Total phenolic content (TPC) was measured using the Folin-Ciocalteu method (Singleton et al. 1999). The gum extract from each solvent was diluted with the appropriate solvents to create one mg/mL solutions, namely n-hexane extract with DMSO, water extract with distilled water, and ethanol extract with ethanol. A total of 0.5 mL of the solution was mixed with 2.5 mL of Folin Ciocalteu reagent and then allowed to rest for two minutes at room temperature. Subsequently, two mL of 7.5% sodium carbonate were added and allowed to rest for 30 minutes at room temperature. The blank sample was a methanol solution with no mixture of extracts. The absorbance was then read at 765 nm using a UV-Vis spectrophotometer with three to four repetitions. A standard curve was created using 0.125, 0.063, 0.031, and 0.016 mg/mL gallic acid solution in methanol ($y = 0.0646x - 0.0022$; R2 = 0.9996). TPC results were prepared as mean ± standard deviation and expressed in milligram equivalents of gallic acid or mg GAE/g extract.

Total Flavonoid Content (TFC) Test

The total flavonoid content (TFC) calculation used the method suggested in a previous study (Brighente et al. 2007). The gum extract from each solvent was diluted with the appropriate solvents to create one mg/mL solutions, namely n-hexane extract with DMSO, water extract with distilled water, and ethanol extract with ethanol. A total of two mL aluminum chloride hexahydrate ($AlCl_3.6H_2O$) was reacted with two mL of the extract solution and incubated for one hour at 20°C. The blank sample was a methanol solution with no mixture of extracts. The absorbance was read at 415 nm using a UV-Vis spectrophotometer with three to four repetitions. A standard curve was created using 0.031; 0.016; 0.008; 0.004 mg/mL solution of quercetin in methanol ($y = 0.0318x + 0.0025$; R2 = 0.9996). TFC results were prepared as mean ± standard deviation and expressed as milligram equivalents of quercitin or mg QE/g extract.

Total Flavanols Content (TVC)Test

The total flavanols content (TVC) calculation used the method suggested in a previous study (Broadhurst & Jones 1978). The gum extract from each solvent was diluted with the appropriate solvents to create one mg/mL solutions, namely n-hexane extract with DMSO, water extract with distilled water, and ethanol extract with ethanol. Three ml of 4% vanillin solution and 1.5 mL of 37% hydrochloric acid were reacted with 0.5 mL of extract solution. This solution was then incubated for 15 minutes at room temperature. The blank sample was a methanol

solution with no mixture of extracts. The absorbance was read at 500 nm using a UV-Vis spectrophotometer with three to four repetitions. Subsequently, a standard curve was created using 0.125; 0.063; 0.031; 0.016 mg/mL solution of catechins in methanol ($y = 0.2422x - 0.0043$; $R^2 = 0.9977$). The results were calculated as mean ± standard deviation and expressed as milligram equivalents of catechins or mg CE/g extract.

Bioactivity Test of *A. decurrens* Gum Extract

Antioxidant Activity Test

The antioxidant activity test used the DPPH method (2,2-diphenyl-1-picrylhydrazyl). Samples were prepared of 0.1 mL of gum extract from each solvent fraction of n-hexane, ethanol, and water at different concentrations of 12,000 ppm, 6,000 ppm, and 3,000 ppm. The samples were added with five mL of DPPH free radical methanol solution, shaken, and then stored in a dark place for 30 minutes. This test used ethanol without extract as a blank and Gallic acid as a standard compound (Gao et al. 2006). Each test had three repetitions. The absorbance was measured using a UV-Vis spectrophotometer when the purple color disappeared at 517 nm. The antioxidant activity was expressed in the percentage of inhibition and was calculated as follows:

$$\text{Inhibition (\%)} \frac{(A0 - A1)}{A0} \times 100$$

A_0: Absorbance of the blank

A_1: Absorbance of the sample with extract (517 nm)

Cytotoxicity Test

Brine Shrimp Lethality Test (BSLT) became one of the methods to test Cytotoxicity levels (Wu 2014). The BSLT consisted of several steps. First, commercial *Artemia salina* Leach shrimp (Breeders brand) larvae hatching in a 3.8% sodium chloride solution equipped with an aerator at 26-28°C for 12 hours in light conditions and 12 hours in dark conditions. Second, 10mg of the gum extract was prepared and dissolved in one mL of DMSO (dimethylsulfoxide). The analysis used 1.25% of DMSO as a negative control (Geethaa et al. 2013). A 62.50 µL was taken from each solution of 10,000 ppm, 5,000 ppm, 2,500 ppm, and 1,250 ppm to create five mL samples with different concentrations of 125 ppm, 62.50 ppm, 31. 25 ppm, and 15.63 ppm with three repetitions. Third, ten hatched larvae were added to the solution samples and placed in incubation tubes for 24 hours at 26-28°C. Fourth, the mortality value was calculated using the number of dead shrimps. Lethal Concentration (LC50) with a 95% confidence interval was determined using the probability unit (probit) analysis (Lukmandaru & Gazidy 2016). The mortality value was calculated using the following formula:

$$\text{MT (\%)} = \frac{(Ma - Mk)}{(100 - Mk)}$$

Description:

MT: The observed mortality corrected by the control mortality (%)

Ma: Mortality observed (%)

Mk: Control mortality(%)

Data Analysis

Descriptive and statistical analyses were employed to identify the chemical compounds and bioactivity of the *A. decurrens* gum extract. Descriptive analysis was carried out in the compound content, total phenolic, flavonoid, and flavanols content analyses. Cytotoxicity analysis was carried out in each fraction using the probit test (probability unit) on SPSS 16.0 software. Furthermore, Pearson correlation using SPSS 16.0 software and conventional interpretation was used to analyze the relationship between the parameters (Schober et al. 2018).

Results and Discussion

Yield of *A. decurrens* Gum Extract

In this research, the tree diameter class used for gum tapping was >10 cm. Based on previous studies and field observations, this diameter class was dominant in the populations (Ramadhan et al. 2020), and therefore, it could meet the data collection requirements. The preliminary study suggested that the yields of gum tapping of *A. decurrens* trees varied ranged between 0.05 and 7.84 grams/tree/harvesting. These yields were obtained from gum tapping using the two-sided wounding per week with six repetitions and then extracted. The gum extract yields were presented in Table 1 below:

Table 1. Yield of *A. decurrens* gum extract

Solvent	Yield (%)
n-hexane	0.58
Ethanol	3.30
Water	19.00

From the 21.03 grams gum sample, the highest extract yield was obtained from the water fraction, followed by the ethanol and n-hexane fractions, namely 19%, 3.30%, and 0.58%, respectively. Furthermore, in filtering and separating the extract from the solvent, a freeze-dry device was used for the extract obtained from water. It was difficult to separate the water from the gum extract using a heater due to the large number of sugar compounds dissolved in the solvent, hence, the extract became very easy to burn.

The water fraction produced the highest yield, and this is consistent with previous reports that *A. decurrens* gum contains several water-soluble compounds, such as sugar groups. Tewari & Jindal (2010) reported that gum harvested in hilly parts of India contains monosaccharide compounds. However, some compounds in the sample were not dissolvable in organic solvents. This was because gums were often polysaccharides with long polymer bonds, which required a bonding simplification process, such as the hydrolysis method to identify the constituent monomers (Lyu & Untereker 2009).

Identification of Chemical Compounds in Gum Extract using Gas Chromatography-Mass Spectrometry (GC-MS)

The GC-MS analysis showed the chemical compounds of *A. decurrens* gum extract obtained from the n-hexane, ethanol, and water fractions, as shown in Tables 2, 3, and 4.

Based on Table 2, the chemical compounds in the n-hexane fraction of gum extract were dominated by hydrocarbons, including alkane, alkene, aldehyde, and ester groups. The result also showed the presence of an aromatic group. Some of these hydrocarbon compounds were known to have potential bioactivity. In the alkane group, there was 10-methyl-eicosane, generally known as an antifungal (Ahsan et al. 2017) and antioxidant (Rhetso et al. 2020). In the alkene group, there was (E)-2-tetradecene, which was reported to have several bioactivities, namely anticancer, antimicrobial, and antioxidant in plant extracts (Tonisi et al. 2020). In the aldehyde group, there was E-15-heptadecenal with antibacterial (Yogeswari et al. 2012) and antioxidant potential (Godara et al. 2019). There were also compounds in the aromatic group with bioactivity. Furthermore, 4-(1,1-dimethylpropyl)-phenol had anti-inflammatory and antioxidant potential (Zhao et al. 2020), while 4-(1,1,3,3-tetramethylbutyl)-phenol was commonly known as an antioxidant, antibacterial, and antifungal (OECD 1995). The extract from the n-hexane fraction had several bioactivity potentials, especially antioxidant activity.

A chemical analysis of gum extract from the ethanol and water fractions was also carried out using the GC-MS instrument. This process was performed simultaneously with the derivation of the isolated test samples. The results of chemical analysis on *A.*

Table 2. The result of chemical compounds analysis on *A. decurrens* gum extract from n-hexane fraction

Peak	Retention Time (minutes)	% Area	Compound	Similarity Index (SI)	Chemical Formula
1	8.99	1.88	Undecane	92	$C_{11}H_{24}$
2	16.76	3.14	(*E*)- 3-Tetradecene	94	$C_{14}H_{28}$
3	21.12	7.31	(*E*)- 2-Tetradecene	94	$C_{14}H_{28}$
4	23.61	1.92	4-(1.1-dimethylpropyl)-Phenol	85	$C_{11}H_{16}O$
7	23.98	2.18	2,6,6-trimethyl-, ethyl ester, 1,3-Cyclohexadiene-1-carboxylic acid	76	$C_{12}H_{18}O_2$
8	24.17	1.37	4-(1,1,3,3-tetramethylbutyl)-Phenol	85	$C_{14}H_{22}O$
10	24.33	2.54	1,3,5,5,6,6-hexamethyl- 1,3-Cyclohexadiene	76	$C_{12}H_{20}$
12	24.50	3.73	4-(1,1-dimethylpropyl)- Phenol	89	$C_{11}H_{16}O$
13	24.67	1.49	2-methyl-4-(1,1,3,3-tetramethylbutyl)- Phenol	81	$C_{15}H_{24}O$
14	24.97	10.49	Pentadecene	95	$C_{15}H_{30}$
15	28.45	10.06	E-15-Heptadecenal	95	$C_{17}H_{32}O$
16	30.15	1.21	10-methyl-Eicosane	90	$C_{21}H_{44}$
18	31.68	1.28	7-butyl- Docosane	91	$C_{26}H_{54}$
19	34.51	5.00	E-15-Heptadecenal	94	$C_{17}H_{32}O$
20	35.92	1.17	7-hexyl-Eicosane	87	$C_{26}H_{54}$
21	37.19	3.46	E-15-Heptadecenal	91	$C_{17}H_{32}O$
26	38.50	2.81	10-methyl-Eicosane	89	$C_{21}H_{44}$
27	39.68	7.23	Octadecyl trifluoroacetates	93	$C_{20}H_{37}F_3O_2$
28	40.89	2.41	11-decyl- Tetracosane	85	$C_{34}H_{70}$
29	42.12	4.97	Henekosil heptafluorobutyrate	87	$C_{25}H_{43}F_7O_2$

decurrens gum extract from the derivatized ethanol fraction were presented in Table 3.

From Table 3, the chemical compounds in the extract from the ethanol fraction were dominated by monosaccharides. Gum Benth from *Albizia procera* (Roxb.) was known to contain monosaccharides, such as arabinose and galactose (Pachuau et al. 2012). Furthermore, several studies revealed that gums with various types of sugar bonds increased viscosity, stabilized emulsions, and served as a suspension agent for other materials. Some of them were also used as sweet food products, cosmetics, textiles, paints, and polishes (Fraser-Reid et al. 2008).

Monosaccharides in gum became an antioxidant agent by reducing sugars, which also increased the antioxidant activity of chitosan (Sari et al. 2013). Bollenbach et al. (2016) reported that D-glucose reduced free radicals in water, while Kroemer et al. (2018) revealed the toxic properties of mannose-type monosaccharides in the digestive process in excessive

Table 3. The result of chemical analysis on *A. decurrens* gum extract from ethanol fraction

Peak	Retention Time (minutes)	% Area	Compound	Similarity Index (SI)	Chemical Formula
4	14.24	16.74	tris(trimethylsilyl) ether, Glycerol	86	$C_{12}H_{32}O_3Si_3$
6	19.86	32.31	2-Naphthaleno*	87	$C_{10}H_8O$
14	21.99	15.57	tetrakis(trimethylsilyl) ether (isomer 1). D-(-)-Ribofuranose	83	$C_{21}H_{52}O_6Si_5$
15	22.07	10.62	tetrakis(trimethylsilyl)-, D-Arabinose	84	$C_{17}H_{42}O_5Si_4$
16	22.31	13.93	6-deoxy-2,3,4,5-tetrakis-O-(trimethylsilyl)-, L-Manose	81	$C_{18}H_{44}O_5Si_4$
17	22.14	22.56	2,3,4,5-tetrakis-O-(trimethylsilyl)- D-Ribose	83	$C_{17}H_{42}O_5Si_4$
18	23.22	3.68	tetrakis(trimethylsilyl) ether (isomer 1), D-(-)-Ribofuranose	77	$C_{17}H_{42}O_5Si_4$
19	23.57	13.40	6-deoxy-2,3,4,5-tetrakis-O-(trimethylsilyl)-, L-Manose	87	$C_{18}H_{44}O_5Si_4$
23	26.85	9.07	tetrakis(trimethylsilyl) ether (isomer 1), D-(-)-Ribofuranose	81	$C_{17}H_{42}O_5Si_4$
25	27.44	11.76	2,3,4,5,6-pentakis-O-(trimethylsilyl)- D-Glucose	84	$C_{21}H_{52}O_6Si_5$
26	27.57	6.81	pentakis(trimethylsilyl) ether (isomer 2), D-Psychofuranose	80	$C_{21}H_{52}O_6Si_5$

*)2-Naphthalenol as an internal standard compound

Table 4. The result of chemical analysis on *A. decurrens* gum extract from the water fraction

Peak	Retention Time (minutes)	% Area	Compound	Similarity Index (SI)	Chemical Formula
6	19.85	60.76	2-Naphthalenol*	96	$C_{10}H_8O$
12	21.98	4.00	1,2,3,5-tetrakis-O-(trimethylsilyl)-Arabinofuranose	89	$C_{17}H_{42}O_5Si_4$
13	22.06	2.72	2,3,4,5-tetrakis-O-(trimethylsilyl)- Arabinose	86	$C_{17}H_{42}O_5Si_4$
14	22.31	2.88	6-deoxy-2,3,4,5-tetrakis-O-(trimethylsilyl)-, L-Manose	77	$C_{18}H_{44}O_5Si_4$
16	22.68	3.23	2,3,4,5-tetrakis-O-(trimethylsilyl)- Arabinose	90	$C_{17}H_{42}O_5Si_4$
17	23.21	1.86	tetrakis(trimethylsilyl) ether (isomer 2), D-(-)-Ribofuranose	81	$C_{17}H_{42}O_5Si_4$
18	23.56	1.37	2,3,4,5,6-pentakis-O-(trimethylsilyl)-D-Glucose	78	$C_{21}H_{52}O_6Si_5$

*2-Naphthalenol as internal standard compound

use. Glycerol, a poly-alcoholic compound, was also found in the extract.

As shown in Table 4, the *A. decurrens* gum extract contained an ethanol fraction dominated by sugar compounds while other compounds occupied only small areas. This situation led to two assumptions. First, the *A. decurrens* gum extract from the water fraction contained mostly sugar compounds, as suggested by Tewari & Jindal (2010). Second, there is a probability that the *A. decurrens* gum extract consisted of heavy molecules unidentified by the GC-MS. However, the sugar compounds (mono saccharides) from the water fraction indicated antioxidant activity. D-glucose (monosaccharide) had reducing properties (Bollenbach et al. 2016) that helped to increase the antioxidant activity in chitosan (Sari et al. 2013).

Phenolic Content of *A. decurrens* Gum Extract

Total Phenolic Content (TPC)

Based on Figure 2, the highest total phenolic content was obtained from the ethanol fraction, followed by the n-hexane and water fractions, namely 19.61, 8.47, and 5.38 mg GA/g samples, respectively. As an illustration, the total phenolic content of *A. senegal* gum was 46.94-251.45 mg GA/g (Elnour et al. 2018). This showed that the phenolic content of *A. decurrens* gum was lower than *A. senegal* gum, the producer of commercial gum (Arabic gum).

However, these quantitative test results opened an opportunity to find compounds with antioxidant activity. Pereira et al. (2009) stated that phenolics compounds had good antioxidant properties. Based on the chemical analysis using GC-MS, the *A. decurrens* gum extract from ethanol and water fractions contained no phenolic but monosaccharide compounds. The absence of the phenolic was related to the role of glucose in their synthesis. Plant phenolic compounds had two synthesis pathways namely shikimic acid and acetate malonate (polyketide). The shikimic acid synthesis required erythrose-4 phosphate from the pentose phosphate and phosphoenolpyruvate from glycolysis. Glucose became the first required molecule for synthesis (Khatri & Chhetri 2020). Furthermore, the analysis of phenolic derivatives content (flavonoids and flavanols) were carried out, and the results were presented in Figure 3 and 4.

Total Flavonoid Content (TFC) and Total Flavanols Content (TVC)

The results showed that the highest total flavonoids and flavonols were obtained from the ethanol fraction, namely 23.89 mg QE/g sample and 13.41 mg CE/g sample, respectively. Furthermore, the second highest value was recorded in the n-hexane fraction, namely TFC: 6.27 mg QE/g sample and TVC: 8.112 mg CE/g sample. The lowest total flavonoids and

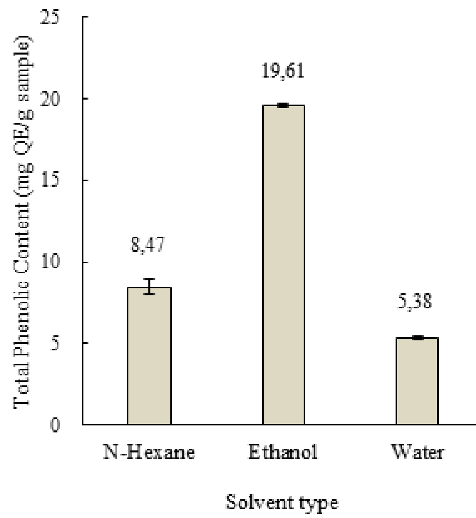

Figure 2. Total Phenolic Content of *A. decurrens* gum extract from multilevel extraction using 3 different solvents (means of 3 replications)

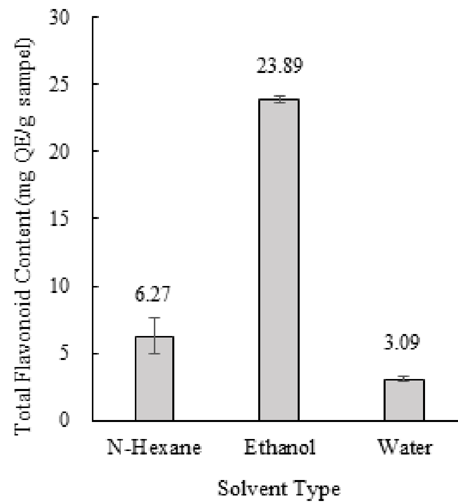

Figure 3. Total Flavonoid Content of *A. decurrens* gum extract from multilevel extraction using 3 different solvents (means of 3 replications)

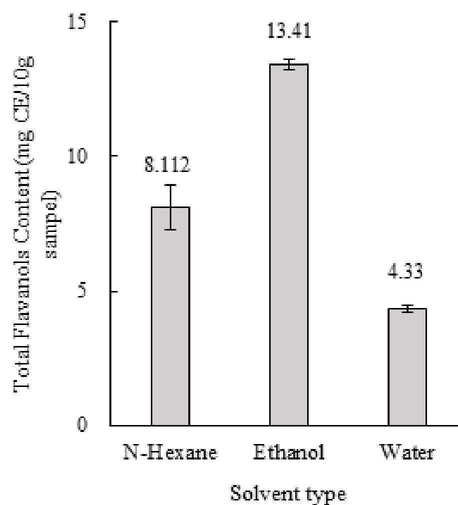

Figure 4. Total Flavanols Content of *A. decurrens* gum extract from multilevel extraction using 3 different solvents (means of 3 replication)

flavonols content was obtained from the water fraction, namely the 3.09 mg QE/g sample and 4.33 mg CE/g sample, respectively. Based on Figures 3 and 4, there were differences in the trend of values between the n-hexane and water fractions. This was caused by the difference in the polarity of the compounds in each solvent.

The results showed that the total flavonoid content of the n-hexane was higher than the water fraction because phenolic compounds had low polarity (non-polar to semi-polar). In the n-hexane fraction, the phenol compounds bounded to the benzene ring, and therefore, they were semi-polar. These phenolics contributed to the flavonoid content. However, the flavanols content analysis detected phenolic compounds bounded to the polar –OH group. These compounds were more soluble in polar solvents, such as ethanol and water. Consequently, the total flavanols content of the water fraction was higher compared to the n-hexane.

Bioactivity of *A. decurrens* Gum Extract

Antioxidant Activity of *A. decurrens* Gum Extract

The results showed that *A. decurrens* gum extract had antioxidant activity, as shown in Figure 5 the highest level of free radical inhibition of DPPH was recorded in the gum extract obtained from the ethanol fraction, followed by the n-hexane and water fractions. Based on Figure 5, a regression equation was calculated to determine the free radical inhibition ability of DPPH/50% free radical scavenging concentration (IC_{50}). The highest IC_{50} value of *A. decurrens* gum extract was obtained from the ethanol fraction, followed by n-hexane, and water fractions, namely 3.842 g/mL, 25.105 g/mL, and 287.472 g/mL, respectively. Compared with the standard compounds (gallic acid), ethanol was the most effective fraction to be used as an antioxidant. This finding was consistent with a previous report that the more extracted phenolic compounds gave the best DPPH free radical scavenging activity (Piljac-Žegarac et al. 2007).

Cytotoxicity Content of A. decurrens Gum Extract

Cytotoxicity analysis of *A. decurrens* gum extract in three different fractions was carried out using the Brine Shrimp Lethality Test (BSLT). The results are presented in Table 5.

Table 5 showed the results of the BSLT using different concentration levels of 125; 62.50; 31.25; and

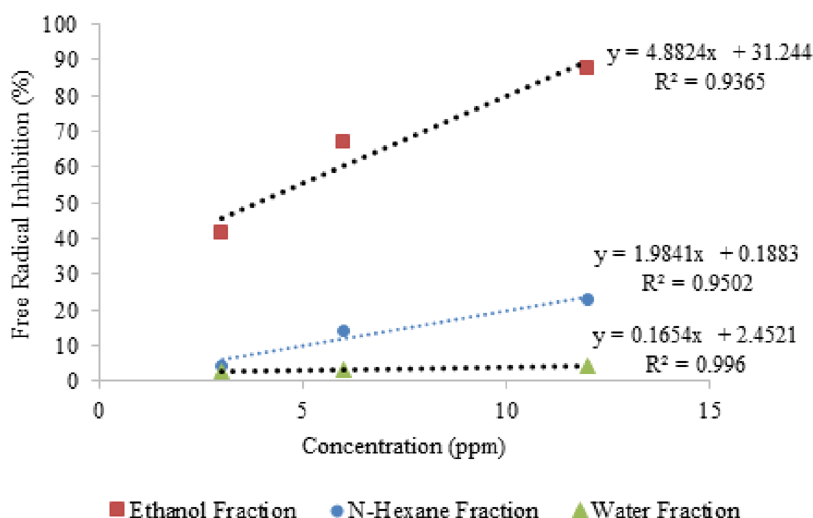

Figure 5. Inhibition value (%) of DPPH antioxidant activity assay treated with 3 different fraction on 3 different concentrations of 3,000 ppm, 6,000 ppm, and 12,000 ppm (means of 4 replications)

Table 5. Corrected mortality (%) of shrimp larvae treated with fractions of *A. decurrens* gum extract on 4 different concentrations (Means of 3 replications)

Fraction	Concentration(ppm)	Corrected Mortality(%)	LC50
N-hexane	125	96.67	0.473
	62.50	96.67	
	31.25	86.67	
	15.63	90.00	
Ethanol	125	93.33	5.906
	62.50	100.00	
	31.25	76.67	
	15.63	73.33	
Water	125	100.00	6.556
	62.50	93.33	
	31.25	83.33	
	15.63	76.67	
DMSO*	-	30	

*) 1.25% DMSO was tested on shrimp larvae as a negative control

15.63 ppm. The analysis showed that the highest cytotoxicity content was found in the n-hexane fraction with an LC50 of 0.47 ppm, followed by the ethanol and water fractions with values of 5.91 ppm and 6.56 ppm, respectively. The lower the LC50 value, the higher the bioactivity (Fikriah & Lestari 2011). Values of ≤ 30 ppm indicated that the fraction had the potential to be used as an anticancer (Lukmandaru & Gazidy 2016).

Although the majority of gums are nontoxic (Nussinovitch 2010), some of them contain toxic compounds, such as Guar gum (Vartiainen & Gynther 1984). The *A. decurrens* gum extract had cytotoxic potential. Based on GC-MS analysis, its n-hexane fraction contained several compounds, such as 10-methyl-eicosane, (E)-2-tetradecene, E-15-heptadecenal, 4-(1,1-dimethylpropyl)-phenol, and 4-(1,1,3,3-tetramethylbutyl)-phenol which had cytotoxic properties. The ethanol and water fractions contained mannose, a monosaccharide compound, which had been reported to have similar activities. Previous studies stated that it caused several human digestive disorders, but its allowable consumption limit had not been studied. These compounds might have a high cytotoxic value in the BSLT against *A. decurrens* gum extract.

Conclusion

The bioactivity test showed that *A. decurrens* gum potentially has good antioxidant activity and high cytotoxicity. The *A. decurrens* gum is a prospective biomaterial for the production of antioxidant products. However, its anticancer properties still require further clinical studies. The knowledge production related to *A. decurrens* gum still needs further development to determine the direction of its product development.

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