Isolation of Ethyl *p*-Methoxycinnamate from *Azadirachta indica* Root Bark as Hong Kong Caterpillar (*Tenebrio molitor*) Antifeedant

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Received: March 4, 2021
Accepted: July 7, 2021

DOI: 10.22146/ijc.64487

Abstract: *This study aims to identify the antifeedant activity of* Azadirachta indica *A. Juss root bark against Hong Kong caterpillar (*Tenebrio molitor*).* A. indica *A. root was macerated in* n*-hexane, ethyl acetate, and methanol. The investigation of antifeedant activity was conducted by using the no choice leaf disk method. The antifeedant activity (%AI) tests showed that the extracts of* n*-hexane, ethyl acetate, and methanol with each concentration of 0.5% were 6.71, 71.78, and 40.14%, respectively. The ethyl acetate extract demonstrated the most active antifeedant activity. Ethyl acetate extract was fractionated to obtain five subfractions (A-E). Each subfraction with each concentration of 0.5% showed the %AI of 70.55, 85.29; 67.40, 82.70 and 82.22%, respectively. Furthermore, GC-MS results demonstrated that ethyl acetate extract contained 19 compounds. The main compounds are methyl hexadecanoate and methyl 8-octadecenoate. Further purification of fraction A, which has the highest amount, was then carried out. The obtained isolate, i.e., A3*$_2$*, was determined as ethyl* p*-methoxycinnamate confirmed by* ^{13}*C- and* ^{1}*H-NMR, FTIR, and MS spectra. The presence of ethyl* p*-methoxycinnamate makes* A. indica *A. root is potential as* T. molitor *antifeedant. The implication of these findings is to reference herbal antifeedants and reduce the use of synthetic pesticides.*

Keywords: Azadirachta indica *A. Juss root bark; antifeedant; ethyl* p*-methoxycinnamate;* Tenebrio molitor

■ INTRODUCTION

Aceh is a tropical region in Indonesia overgrown by various medicinal plants, like *Artocarpus camansi* [1-3], *Ficus racemosa*, *Morus alba* [4-5], and *Azadirachta indica* A. Juss. *A. indica* A. Juss or Meliaceae, known as Neem trees, originally came from India, Bangladesh, Thailand, Nepal, and Pakistan. However, this plant is commonly found in Aceh [6]. *A. indica* A. Juss is a tree with 10–15 m of height. It has roots, stems, leaves, flowers, fruit, and seeds. The stems structures are straight and woody [7]. This plant is one of the essential medicinal plants, which the UN declared as a 21st Century Tree. In India, the neem is called the divine tree, which means the tree of life, the natural pharmacy, and the elixir for all diseases [8-11]. Various chemical constituents are found in neem leaves, such as nimbin, nimbanene, 6-desacetylnimbinene, nimbandiol, nimbolide, ascorbic acid, *n*-hexacosanol, amino acids, 7-desacethyl-7-benzoylazadiradione,7-deacethyl-7-benzoylgedunin and nimbiol [11-13]. The secondary metabolites of *A. indica* A. Juss plants belong to the terpenoid, steroid, and flavonoid compounds [14]. These have been isolated from various parts of the *A. indica* A. Juss plant, and some of them showed antifungal, antitumor, and antimalarial activity [15]. The steroid saponin compounds, such as 2α,4α-dihydroxy-pregn-5-en-16-one-3α-*O*-*D*-glucopyranose, 6-deacetylnimbin, 6-deacetylnimbinal, nimbandiol, nimbolide, 2',3'-dehydrosalannol, 3β,4β,20α-trihydroxy-5-pregnen, 2α,3β-dihydroxy-5-pregnen-16-one, (+)-dehydro-vomifoliol, 3β-hydroxy-5α,6α-epoxy-7-megastigmen-9-one, and quercetin-3-gluco-pyranoside have been isolated from methanol extracts of

A. indica leaves, which known to have antibacterial activity [16].

Generally, antifeedant compounds are defined as compounds that temporarily or permanently stop the insect's appetite. The antifeedant identification of *A. indica* A. Juss seed water extract within a concentration of 80 g/L using the "No Choice Leaf Disk Method" resulted in 98.19% of antifeedant activity and potential repellent to the *Plutellaxylostella larvae* [17]. However, further study of the antifeedant activity from other parts of the *A. indica* A. Juss plant, including the root bark, is still in progress.

The Hong Kong caterpillar *Tenebrio molitor* is one of the pest organisms found in agricultural products [18]. The synthetic pesticides applied to the plant are unable to decompose, but plant roots can absorb them. Meanwhile, it will be accumulated in roots and leaves, and fruits, which animals eat. Moreover, the accumulated compounds will enter the human body via certain animals, which eat those leaves or fruits. Thus, the compound can affect the body's systems within the human body, such as nervous systems and others. This infected nervous system can cause mental retardation [19]. Therefore, the research about antifeedant activity from the compounds derived from neem root skin against the bioindicator of Hong Kong caterpillar (*T. molitor*) is necessary to be carried out.

■ EXPERIMENTAL SECTION

Materials

The root bark of *A. indica* A. Juss was collected from the Darussalam area, a part of Syiah Kuala sub-district Banda Aceh, in 2017. Dr. Saida Rasnovi identified the sample, number: 393/11.1.28.1/DT/UN 2017 and subjected it as the herbarium to the Biology Department, Faculty of Mathematics and Natural Sciences, Universitas Syiah Kuala.

Instrumentation

Mass spectra were measured using a Shimadzu GC-MS QP 2010 Ultra. In addition, the 1D spectra of ^{1}H- and ^{13}C-NMR were measured in $CDCl_3$ solvent using an ECA JEOL 400 MHz spectrophotometer. Furthermore, column chromatography was conducted on silica gel 60 (70-230 mesh, Merck), and the TLC analysis was carried out using precoated silica gel plates (Merck).

The methodology used for phytochemicals testing could be found in the Phytochemical Methods, Simplified Determination Method to Analyze Plants [20].

Procedure

Extraction of A. indica *A. Juss root bark*

About 2.2 kg root bark of *A. indica* A. Juss was dried as preparation for the maceration process. First, the root bark was macerated with *n*-hexane for 72 h; then, it was filtered to collect the residue. The obtained residue was soaked into the semi-polar solvent, ethyl acetate, for 72 h, then it was filtered for obtaining further residue. The residue obtained was macerated with methanol solvent for getting the next residue. Then, each obtained residue was evaporated by using the rotary evaporator. There were about 25.314 g (1.13%) collected residue concentrated from the *n*-hexane extract, 28.315 g (1.24%) from ethyl acetate extract, and 214 g (9.41%) from the methanol extract. All residues were subjected to identification of antifeedant activity. Then, the residue derived from ethyl acetate was determined as the most active antifeedant against *T. molitor* instar III. Therefore, the fractionation was conducted with the ethyl acetate extract, and its chemical composition was characterized by Gas Chromatography-Mass Spectrometry (GC-MS).

Fractionation of ethyl acetate extract

About 16 g of the ethyl acetate extract was fractionated using gravity column chromatography. The eluent system was using *n*-hexane: ethyl acetate with the ratio of *n*-hexane 100%, *n*-hexane:ethyl acetate (98:2); (95:5); (92:8); (90:10); (85:15); (80:20); (70:30); (60:40); (55:45); (50:50); *n*-hexane:ethyl acetate (40:60); 100% of ethyl acetate and 100% of methanol. The separation result showed 267 fractions (each 60 mL) obtained after being monitored by thin-layer chromatography (TLC). The same stain pattern was combined to form five fraction groups: A, B, C, D, and E. Fractions A and B contain terpenoids with the weight of 0.73 and 0.80 g, respectively. Fraction C, as brownish-black residue

contains alkaloids and terpenoids, resulted in 0.80 g. Fraction D, weighing 3.27 g as brownish-black, contains terpenoids and saponins, while fraction E, as brownish-black residue in 7.27 g weight, contains terpenoids. Then, those five fraction groups were subjected to the identification of antifeedant activity. The result determined that fractions A and B had the highest antifeedant activity. Furthermore, the separation of fraction A was relatively clean, and this group belongs to the active antifeedant fraction group (%AI = 62.03% at 0.1% concentration).

The fixed amount of 0.80 g of fraction A was addressed for gravity column chromatography with the eluent system of *n*-hexane:ethyl acetate (90:10). The 81 fractions were obtained with fractions 48 and 49 (A3) contained 0.04 g in each. Next, fraction A3 was subjected to further separation using *n*-hexane:ethyl acetate (90:10) eluent system. Another 19 fractions were obtained, whereas fractions 11-13 ($A3_2$) were determined relatively pure after TLC analysis performed with three different eluent systems. Furthermore, the $A3_2$ compound was characterized by ^{1}H-NMR, ^{13}C-NMR, infrared spectrometers and MS.

Preparation of bioindicators

The *T. molitor* instar III was used as bioindicators in this research. It was bought from mealworm breeders in Keutapang, Aceh Besar, and it fasted for 24 h before the antifeedant activity test.

Preparation of test solution

The test solution was generated in 10% wt/v concentration. Ten grams of methanol extract were dissolving into 100 mL of methanol. The solution was diluted into the 5, 1, and 0.5% of final concentrations. Moreover, the same treatment was repeated for the ethyl acetate and *n*-hexane extracts.

Test media preparation

A plastic with a 15 × 10 cm^2 size was prepared and covered with sterile gauze moistened by distilled water.

Antifeedant activity assay

Fresh spinach leaves were prepared and put into the crude extract of methanol (0.5%) for a minute. After that, it was left to dry for about 15 min, and the weight gain (x_1) was measured. The spinach leaves were placed into the test medium within a plastic container, which had been prepared previously. Furthermore, the 10 larvae of *T. molitor* instar III were placed into the container, covered with a cloth, and tied with a rubber band for 24 h. Then, the weight of spinach leaves was measured to calculate the weight lost (x_2), while the weight of leaves was found different between x_1 and x_2. The test was repeated 3 times. The negative control was fresh spinach leaves which were dipped into the extracting solvent used. The same treatment was carried out for concentrations of 1, 5, and 10% of ethyl acetate and *n*-hexane extracts, with 3 replications for each. All of the tests were repeated three times. The antifeedant percentage was calculated using the formula [21]. The standard deviation was obtained from the repetition of antifeedant activity assays, which have been carried out three times.

$$\text{Antifeedant Index (AI)} = \frac{C(x_1 - x_2) - T(x_1 - x_2)}{C(x_1 - x_2) + T(x_1 - x_2)} \times 100\%$$

whereas AI = Antifeedant Index; C = Control; x_1 = weight of leaf which was put into the crude extract as a control; x_2 = weight of leaf which was consumed by larvae as a control; and T = Treatment.

Statistical analysis

Statistical analysis was conducted using the SPSS program and Origin Pro software to create the different curves for the antifeedant activity.

■ RESULTS AND DISCUSSION

The antifeedant activity was identified among *n*-hexane, ethyl acetate, and methanol extract derived from *A. indica* A. Juss root bark. The ethyl acetate extract demonstrates the highest antifeedant activity (%AI = 71.78%) against *T. molitor* instar III compared to *n*-hexane (6.71%) and methanol extract (40.14%). Therefore, the isolation and activity test focused on the ethyl acetate extract. First, the ethyl acetate extract was separated by gravity column chromatography to obtain five subfractions, i.e., fraction A (0.8 g), B (0.73 g), C (0.8 g), D (3.27 g), and E (7.27 g). Then, the measurement of antifeedant activity for those five groups of fractions was applied to *T. molitor* instar III. The results of the antifeedant activity test of ethyl acetate

extract and the fraction groups (A, B, C, D, and E) are listed in Table 1.

Based on Table 1, the antifeedant activity test results showed that the %AI for various fraction groups is more than 50% at 0.1 and 0.5% of concentration. This antifeedant observation showed that fraction B is the most active antifeedant from ethyl acetate extracts and the various fraction groups, followed by fraction D, fraction E, fraction A, and fraction C. The relationship curve between %AI and the concentration of each extract is presented in Fig. 1. The obtained %AI is directly affected by the concentration of each fraction contained in the ethyl acetate extract.

The ethyl acetate extract of *A. indica* A. Juss root bark was characterized by a gas chromatography-mass spectrometry (GC-MS) to identify the composition of the compounds. The chromatogram is shown in Fig. 2. Several compounds are contained in the ethyl acetate extract derived from the root bark of *A. indica* A. Juss, and the result from GC-MS analysis are listed in Table 2. Nineteen compounds have above 70% of similarities. The two major compounds, methyl 8-octadecenoate (26.33%) and methyl hexadecanoate (14.18%) have 96% similarities. Therefore, we can conclude that ethyl acetate extract contains antifeedant compounds, like nimbin, patchouli alcohol, *p*-methoxycinnamate, terpenoid, and monoterpene. These compounds work simultaneously to give the highest antifeedant activity of ethyl acetate extract. Nimbin has been reported as an anti-microorganism, which is very useful in controlling plant disease [22]. In this experiment, the nimbin was found as a Nimbiol, also classified as a terpenoid. Terpenoid compounds could affect the feeding selection of insects. This finding is in good agreement with Wei et al. [15],

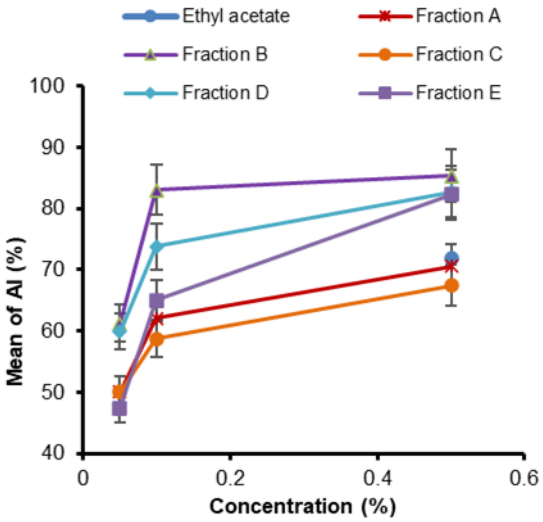

Fig 1. The differences antifeedant activity of ethyl acetate extracts and their fraction groups

Table 1. The antifeedant activity of ethyl acetate extract and fraction group A-E against *T. Molitor*

Conc. (%)	Mean of %AI ± Standard Deviation					
	Ethyl Acetate Extract	Fraction A	Fraction B	Fraction C	Fraction D	Fraction E
0.05		50.03 ± 2.97	61.25 ± 2.97	50.05 ± 18.44	59.9 ± 16.59	47.36 ± 13.56
0.1		62.03 ± 1.30	83.02 ± 6.05	58.72 ± 9.22	73.75 ± 15.43	65.01 ± 16.95
0.5	71.78 ± 19.99	70.55 ± 1.98	85.29 ± 5.36	67.4 ± 1.85	82.71 ± 4.53	82.22 ± 9.45

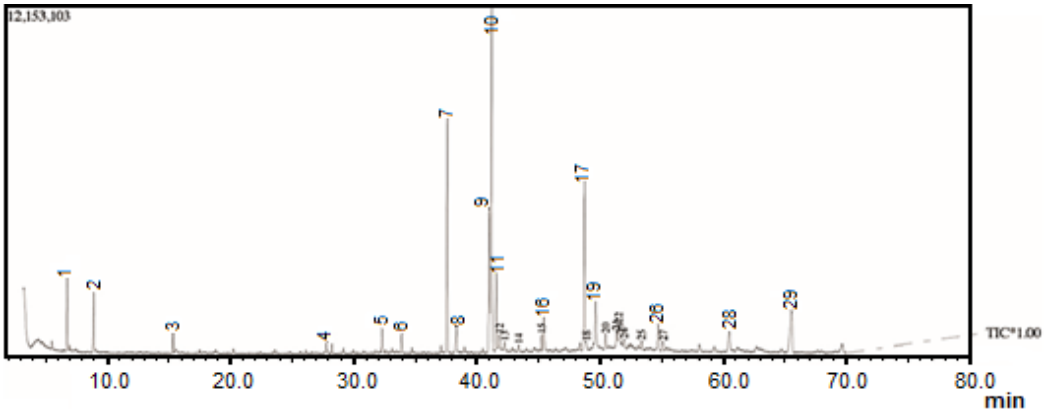

Fig 2. The chromatogram of the ethyl acetate extract derived from the root bark of *A. Indica* A. Juss

Table 2. Characterization results of the compounds contained in the ethyl acetate extract derived from *A. indica* A. Juss root bark by GC-MS (all of the structure should be rewritten and give the correct IUPAC name of the compounds)

No	Retention Time (min)	Area (%)	Compound	SI (%)
1	6.806	3.49	4-hydroxy-4-methyl-2-Pentanone	95
2	8.963	3.03	2-butoxy ethanol	98
3	15.366	0.75	2-Butoxyethyl acetate	94
4	27.832	0.47	2,4-bis(1,1-dimethylethyl) phenol	91
5	32.308	1.25	1,6-Methanonaphtalen-1(2H)-ol, octahydro-4,8a,9,9-tetramethyl-, [1R-(1.alpha,4.beta,4a.alpha,6.beta, 8a.alpha)]	95
6	33.928	0.98	Ethyl *p*-methoxycinnamate	71
7	37.630	14.18	Methyl hexadecanoate	96
8	38.400	1.48	Hexadecanoic acid	95
9	40.995	9.01	Methyl cis-9 cis-12-Octadecadienoate	94
10	41.212	26.33	Methyl Octadec 8-Enoate	96
11	41.602	3.70	Methyl n-octadecanoate	96
12	41.866	1.22	9Z,12Z-Octadecadienoic acid	88
13	45.243	0.69	Arachidic acid methyl ester	96
14	45.439	2.40	Podocarpa-6,8,11,13-tetraen-12-ol, 13-isopropil-, acetate	83
15	48.785	9.94	Bis(2-ethylhexyl) 1,2-Benzenedicarboxylate	97
16	48.900	0.53	Nimbiol	70
17	49.639	3.63	Podocarpa-8,11,13-triene-7beta, 13-diol,14-Isopropyl	74
18	50.469	0.87	7-isopropyl-1,1,4a,6-tetramethyl-2,3,4,4a,10,10a-hexahydrophenanthren-9(1H)-one	84
19	54.755	1.84	Piperidine, 1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2,4-pentadienyl]-,(E,E)	90

who reported that terpenoids are potent for control insects.

Fraction A was addressed to further purification using chromatography to produce $A3_2$ isolate. It is due to the amounts of other fractions was too small. Even though the antifeedant activity of fraction A is lower than other fractions, it still has a reasonable antifeedant activity to be applied (~70%).

Based on Fig. 3, there are 12 carbon atoms present in the $A3_2$ isolate. The carbon at C-11 as a carbonyl ester appeared at δ_C 167.5 ppm and C-8 for OCH_3 at δ_C 55.5 ppm. The C-14 and C-15 at δ_C 60.4 and 14.5 ppm belong to CH_2 and CH_3. In addition, and alkene carbons of C-9 and C-10 were detected at δ_C 144.4 and 115.8 ppm. Atom C of C-1 to C-6 were aromatic carbons with δ_C of 127.3; 129.8; 114.4; 161.4; 114.4 and 129.8 ppm, respectively. According to previous research, the ^{13}C-NMR spectrum of the $A3_2$ isolate is very similar to ethyl *p*-methoxy cinnamate that has been reported [23].

The comparison of ^{1}H-NMR and ^{13}C-NMR spectra data of $A3_2$ isolate with ethyl *p*-methoxycinnamate compounds are listed in Table 3. Ethyl *p*-methoxycinnamate has various pharmacological activities, including anti-inflammatory [24] anti-hyperglycemic [25], and antibacterial [26]. The ethyl *p*-methoxycinnamate compound has been found in the chloroform extract of the leaves of *A. indica* A. Juss as an anti-inflammatory [27].

The ^{1}H-NMR spectrum of the $A3_2$ isolate showed in Fig. 4. The ^{1}H-NMR spectrum showed three aliphatic protons bound to H-15, which appeared as triplets at δ_H 1.33 ppm, and two aliphatic protons bound to H-14 showed as quartets at δ_H 4.25 ppm. This pattern happens because the protons attached to the H-14 atom are

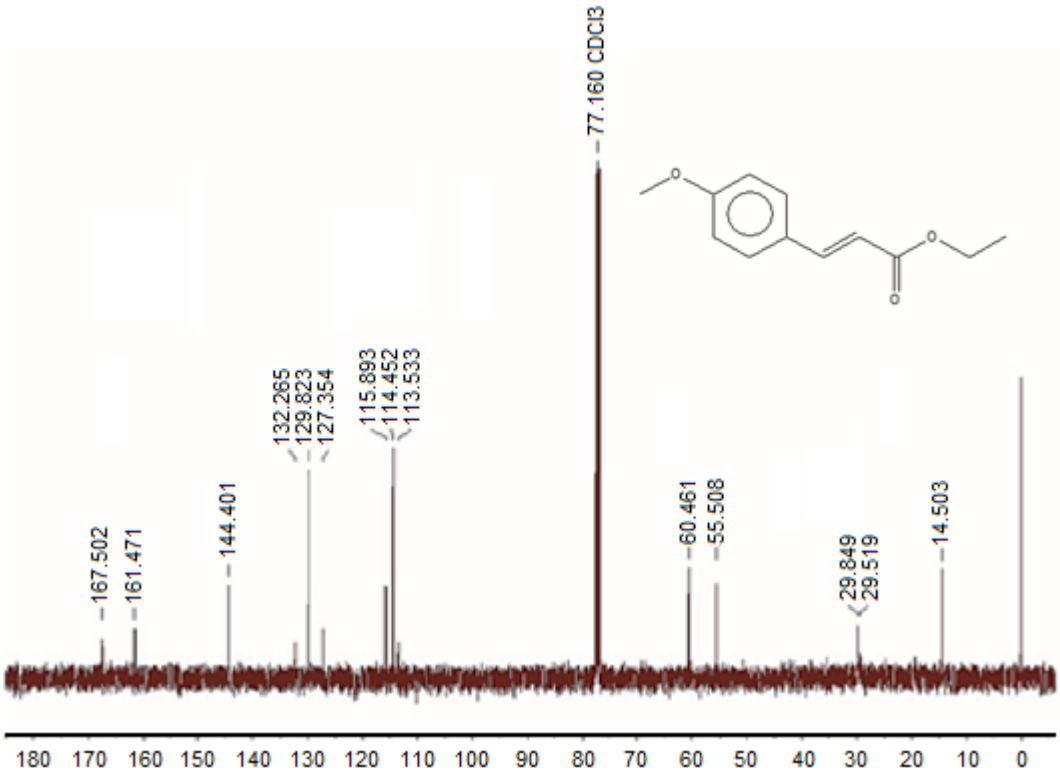

Fig 3. The ^{13}C-NMR spectra of the A3$_2$ isolate

Table 3. The comparison of ^{1}H-NMR and ^{13}C-NMR spectra data of A3$_2$ isolate with ethyl *p*-methoxycinnamate compounds

Position	Isolate A3$_2$	Ethyl*p*-methoxycinnamate	Isolate A3$_2$	Ethyl*p*-methoxycinnamate
	δ_H,ppm	δ_H, ppm	δ_C, ppm	δ_C, ppm
1	-	-	127.3	127.3
2	7.47 (*d*,2H)	7.42 (*d*,2H)	129.8	129.7
3	6.90 (*d*,2H)	6.90 (*d*,2H)	114.4	114.3
4	-	-	161.4	161.3
5	6.90 (*d*,2H)	6.90 (*d*,2H)	114.4	114.3
6	7.47 (*d*,2H)	7.42 (*d*,2H)	129.8	129.7
7	-	-	-	-
8	3.83 (*s*,3H)	3.82 (*s*,3H)	55.5	55.3
9	7.64 (*d*,1H)	7.65 (*d*,1H)	144.4	144.2
10	6.30 (*d*,1H)	6.31 (*d*,1H)	115.8	115.7
11	-	-	167.5	167.3
12	-	-	-	-
13	-	-	-	-
14	4.25 (*q*,2H)	4.25 (*q*,2H)	60.4	60.3
15	1.33 (*t*,3H)	1.32 (*t*,3H)	14.5	14.5

affected by the O atom's electronegativity, which causes a significant chemical shift value. Atom H on H-8 is a singlet signal defined as a methoxy proton with δ_H 3.3 ppm. The singlet signal indicates that the methoxy proton has no neighbor. The chemical shift values of H-9 and H-10 as alkene were signaled at δ_H 7.64 ppm (1H, d)

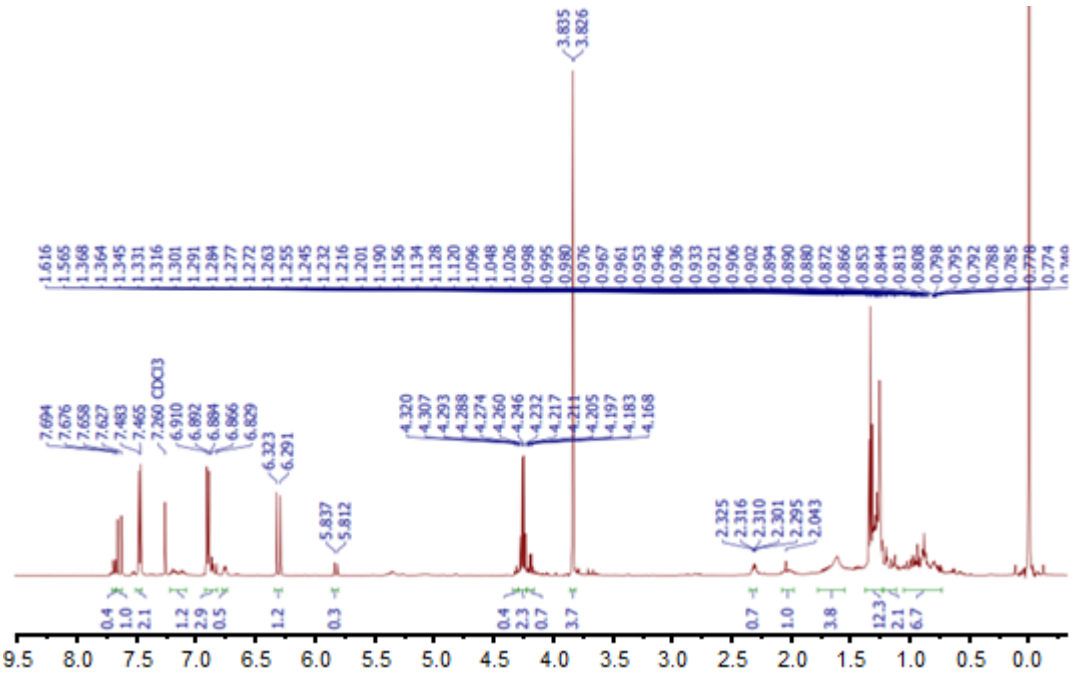

Fig 4. The ^{1}H-NMR spectrum of $A3_2$ isolate

and 6.30 ppm (1H, d), respectively. In addition, the presence of aromatic protons of H-2 and H-3 atoms were appeared at δ_H 7.47 ppm (2H, d) and 6.90 ppm (2H, d), respectively.

The FT-IR spectrum supported the $A3_2$ isolate as ethyl *p*-methoxycinnamate, which shows a carbonyl group (C=O) ester at the wavenumber 1738 cm^{-1}. The aliphatic C–H vibration at the wavenumbers of 2925 and 2854 cm^{-1} showed that the compound contains methyl (CH_3), methylene (CH_2), and methine (–CH) groups. Moreover, the absorption in the area 1634–1512 cm^{-1} indicated the presence of aromatic carbon-carbon double bonds (–C=C–), then an absorption at 829 cm^{-1} indicated the para-substitution. While the absorption at 1372 cm^{-1} showed the existence of stretch vibration (–C–H). Absorption at 1170 cm^{-1} indicated the presence of ether (–C–O) groups. These groups are relatively reactive and react with other compounds to form antifeedant substances, causing the AI to increase. In addition, the presence of conjugated double bonds in some compounds is responsible for their properties, which is easily evaporate, i.e., essential oil properties.

The identification of $A3_2$ isolate as ethyl *p*-methoxycinnamate is supported by MS data, as shown in Fig. 5. The fragmentation of the ethyl *p*-methoxycinnamate molecule occurs at m/z 206, 178, 161, 147, 134, and 118. The characteristic of the ethyl *p*-methoxycinnamate compound is found in the fragment of m/z 161 as a base peak [28]. Furthermore, it also reported the fragmentation pattern of ethyl *p*-methoxycinnamate, which is similar to the $A3_2$ isolate [28]. Based on the data above, the $A3_2$ isolate has a high similarity with the ethyl *p*-methoxycinnamate compound, so that the structure of the $A3_2$ isolate is determined to be an ethyl *p*-methoxycinnamate compound.

The *n*-hexane extract from *A. indica* A. Juss root bark contains alkaloids, steroids, terpenoids, and saponins, while the ethyl acetate extract contains alkaloids, steroids, terpenoids saponins, and phenolics. In comparison, the methanol extract contains steroids,

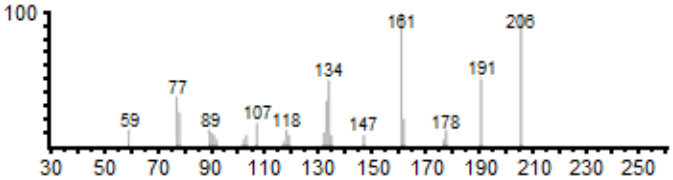

Fig 5. The MS spectrum of $A3_2$ isolate (from GC-MS)

terpenoids, saponins, flavonoids, and phenolics. The presence of terpenoids and steroids is commonly found in plants since terpenoids are known as the basic of steroid biosynthesis [29]. In addition, several secondary metabolites were found in *A. indica* A. Juss, such as terpenoids, steroids, and flavonoids [14]. Thus, it corresponds with the previous research, which exhibited that the ethyl acetate extract of *A. indica* A. Juss leaves contain an alkaloid, terpenoids, steroid, and phenolic metabolites [30].

While the identification result of secondary metabolite content showed no alkaloids detected in the methanol extract, it is appropriate with the study results [31], which reported that the methanol extract of *A. indica* A. Juss leaves contained terpenoids, flavonoids, and saponins. Thus, the differences between the chemical compounds derived from the extracts are due to the sample used (root, bark, or leaves) and the solvents used (nonpolar, semi-polar, and polar).

The antifeedant nature is suspected in *A. indica* A. Juss since the chemical compounds contained in the fraction group of ethyl acetate extract of are consist of alkaloids, such as piperidine, 1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2,4-pentadienyl]-,(E,E), terpenoids, such aspodocarpa-6,8,11,13-tetraen-12-ol-13-isopropyl acetate, Nimbiol, podocarpa-8,11,13-triene-7beta, 13-diol,14-isopropyl and, 7-isopropyl-1,1,4a,6-tetramethyl-2,3,4,4a, 10,10a-hexa hydrophenanthren-9(1H)-one, according to GC-MS result. The content of these chemical compounds significantly affects the behavioral eating of insects; for instance, the alkaloid compounds exhibited a bitter taste to inhibit the antifeedant activity [20]. Moreover, the terpenoid compounds may affect insect feeding. This finding corresponds to the previous research, which reported that terpenoids could control insects by acting as antifeedants [15]. Furthermore, the saponins also could reduce the absorption of food within the gut of insects and inhibit the absorption of food in the intestine [32]. These saponins may also have an antifeedant activity to the other insects, such as *Spodopteralitura* [33].

According to the result of GS-MS analysis, two major compounds were detected, methyl hexadecanoate and methyl 8-octadecenoate determined to have an antioxidant, antibacterial, and anticancer activity, which can inhibit microbial growth [34]. In addition, nimbiol was detected, and it exhibited antibacterial and antifungal activity [22]. Moreover, this compound is commonly found in the *A. indica* A. Juss. Further research is required to observe the matrix in this field.

■ CONCLUSION

The results of the phytochemical test showed that the root bark extract of the plant *A. indica* A. Juss contained secondary metabolites of alkaloids, terpenoids, steroids, flavonoids, saponins, and phenolic compounds. *A. indica* A. Juss root bark has better antifeedant activity in ethyl acetate extract, and its subfraction, i.e., $A3_2$ was confirmed as ethyl *p*-methoxycinnamate from ^{13}C- and ^{1}H-NMR, FTIR, and MS spectra. This compound is responsible for the antifeedant activity of *A. indica* A. root extract. Therefore, *A. indica* root is the potential to be used as *T. molitor* instar III antifeedants.

■ ACKNOWLEDGMENTS

The authors acknowledge Universitas Syiah Kuala for providing financial support for this research.

■ AUTHOR CONTRIBUTIONS

RN and CNC conducted the experiment; NS conducted the calculations; MB and M wrote and revised the manuscript. All authors agreed to the final version of this manuscript.

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