

## Herbicidal activity of *Ageratum conyzoides* L. on *Amaranthus spinosus* L. and GC-MS profile of *n*-hexane extract

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### ABSTRACT

We evaluated the herbicidal activity of sub-subfractions recovered from *n*-hexane leaf extract of *Ageratum conyzoides* L. on *Amaranthus spinosus* L. growth. The column chromatography was used to separate the *n*-hexane extract into its subfractions (A) and rechromatograph into its two sub-fractions, sub-subfractions A1 and sub-subfractions A2. In pot culture, these sub-subfractions were applied to *A. spinosus* at 2 %, 4 %, 6 % and 8 % concentrations and distilled water as control. The sub-subfractions showed phytotoxic activity one day after application. All sub-subfractions had promising herbicidal effects on *A. spinosus* as they suppressed *A. spinosus* growth by 100 % at 2 % concentration or higher in 1 day after application (DAA). The compounds detected by GC-MS in sub-subfraction A1 were 2H-1-benzopyran, 6,7-dimethoxy-2,2-dimethyl- (11.01 %), caryophyllene oxide (10.27 %), phytol (8.79 %), cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-, [S-(R\*,S\*)]-(7.32 %), caryophyllene (7.23 %), phenol, 2,6-bis(1,1-dimethylethyl)-4-ethyl- (5.56 %), 1-octadecanethiol (CAS) octadecanethiol (4.71 %), squalene (4.5 %), phytol, acetate (4.17 %), 1-heptadecene (3.47 %), octadecyl trifluoroacetate (3.03 %). Additionally, GC-MS detected the presence of compounds in sub-subfraction A2 which were 9,12,15-octadecatrienoic acid, (Z,Z,Z)- (22.21 %), 2-propenoic acid, 3-(2-hydroxyphenyl)-, (E)- (22.06 %), n-hexadecanoic acid (18.89 %), stigmasterol (10.36 %), ?-sitosterol (7.05 %), 1H-inden-1-one, 7-(1,1-dimethylethyl)-2,3- dihydro-3,3-dimethyl (5.15 %), stigmast-7-en-3-ol, (3 $\beta$ ,5 $\alpha$ )- (4.48 %), 2H-1-benzopyran, 6,7-dimethoxy-2,2- dimethyl- (3.13 %).

**Keywords:** *Ageratum conyzoides*, *Amaranthus spinosus*, billygoat weed, column chromatography, GC-MS, herbicidal, *n*-hexane, sub-subfraction.

### INTRODUCTION

Weeds are commonly known as source of serious problems in crop cultivation because they can reduce yields both in quantity and quality (44). The presence of weeds causes competition for water, light and space, releases chemical compounds that can affect the growth of other plants in the vicinity and can become alternative hosts for pests and plant diseases. The losses caused by weeds average 34 % for each crop, exceeding the losses caused by pests and disease (25). Therefore, the presence of weeds in the cultivation of a crop requires continuous control.

There are several methods that can be used to control weeds include mechanical, technical culture, biological and chemical methods. Unfortunately, the most common method applied in the field is by using synthetic herbicides. The excessive and continuous use of synthetic herbicides causes many harmful side effects such as the of herbicide-resistant weeds (4), adverse health effects, water and soil contamination (24) and killing decomposer organisms (2,22,35,40). Therefore, there is a need for alternative methods of weed control that are environmentally friendly, one of which is to explore the potential of

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plant-derived compounds (allelochemicals) known as allelopathy to be utilized as bioherbicides (6,7).

There are 10-plant species chosen to conduct the screening for their bioherbicidal potential. Five of them are annual plants; (*Pinus merkusii* Jungh. et de Vriese, *Acacia mangium* Willd., *Jatropha curcas* L., *Tectona grandis* L., *Terminalia catappa* L.) and the rest of five are weed species; (*Imperata cylindrica* L., *Chromolaena odorata* L., *Ageratum conyzoides* L., *Cyperus rotundus* L., and *Axonopus compressus* (Swartz) Beauv) against the growth of *Amaranthus spinosus* L. By using the bio-guided fractionation method, it showed that *A. conyzoides* has the greatest potential as a bioherbicide (13,14). Methanol extract of *A. conyzoides* at 20 % concentration can inhibit 100 % growth of *A. spinosus* at 7 Days After Application (DAA) and this effect is not different from the application of synthetic herbicide 2,4-D (dichlorophenoxy-acetic acid) at 0.686 kg b.a ha<sup>-1</sup> (13).

Billygoat weed (*A. conyzoides* L.) (Family Asteraceae) (Figure 1) is a tropical plant, found in various countries such as Africa, Asia and South America. In Indonesia, it is commonly found in cultivated plants, yards, roadsides, and riverbanks (19). This plant is known as a plant that has a smell like a male goat, has many secondary metabolites with various biological activities. The results of the study showed that methanol extract of *A. conyzoides* leaves inhibited the growth of soybean plants (*Glycine max* L.) (28). Extracts using aqueous solvents from *A. conyzoides* leaves, roots, flowers and stems can inhibit the germination and growth of *Parthenium hysterophorus* L. (26), and inhibit the growth of *Sesamum indicum* (23).

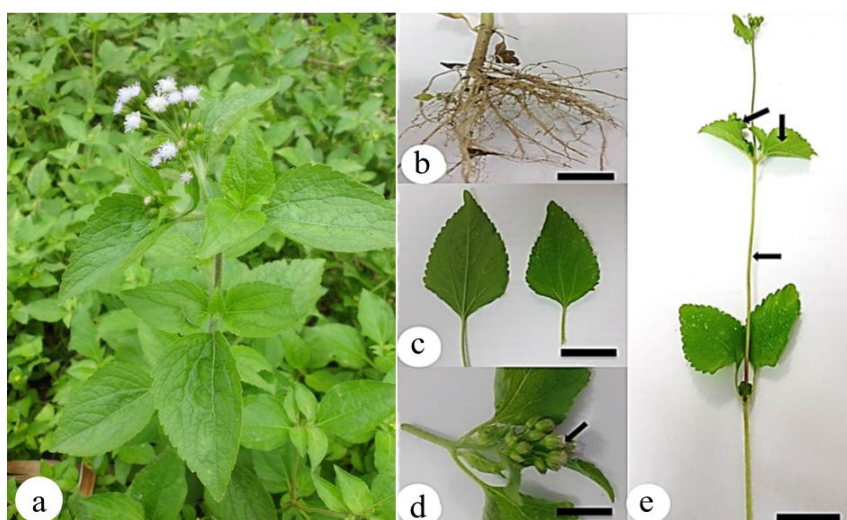


Figure 1. *Ageratum conyzoides* (a). plant population; (b). root; (c). leaves; (d). flower; (e). Stem.

It is important to select solvents based on the characteristics of the target compound to efficiently extract allelochemicals. The solvent's polarity plays a key role in

determining both the quantity and type of allelochemicals obtained (20). Isolating and identifying those responsible for allelopathic activity were achieved through bioassay-guided fractionation, where each step involves evaluating activity and selecting the most potent fractions for further separation (20,27). Research utilizing bio-guided fractionation has shown *A. conyzoides* leaves underwent maceration followed by fractionation with solvents of different polarities, including *n*-hexane, ethyl acetate, and methanol that 15 % concentration of *A. conyzoides n*-hexane extract can inhibit the growth of *A. spinosus* weed by 70 %, not significantly different from the application of 2,4-D at 0.686 kg a.i. ha<sup>-1</sup> at 21 DAA (15).

In a research, with bioassay-guided isolation, sunflower leaf extract was fractionated via high performance liquid chromatography (HPLC), and the fractions were tested against *Chenopodium album* and *Rumex dentatus*. The active fractions tested were re-fractionated, revealing compounds such as phenolics and bioactive terpenoids confirmed through mass spectroscopy and nuclear magnetic resonance spectroscopy (3).

In another research, bio-assay with four fractions of *Lantana camara* leaves extract at 500 ppm, 1000 ppm and 10,000 ppm indicated that chloroform fraction to be most potent phytotoxic against *Phalaris minor*, *Avena fatua*, *Chenopodium album*, and *Rumex dentatus* weeds. Further chromatography and bioassays identified subfraction (iii) of fraction 23 as the most inhibitory (36).

The results of our previous study showed that sub-fractions A (8 %) of *A. conyzoides n*-hexane extract were able to suppressed *A. spinosus* weed at 7 DAA. Gas Chromatography-Mass Spectroscopy results showed that the compounds present in sub-fraction A: 2h-1-benzopyran, 6,7-dimethoxy-2,2- dimethyl (13.85 %), caryophyllene oxide (12.69 %), caryophyllene (12.20 %) (16).

Based on the result, we want to isolate pure compounds from the *n*-hexane extract from *A. conyzoides* in various subfractions and test its activity as a bioherbicide against the growth of *A. spinosus*. In this study, *A. spinosus* was used as a target plant because of its uniform and rapid germination, high sensitivity to allelochemicals and acts as an active competitor (1,34).

## MATERIALS AND METHODS

The research was carried out at Syiah Kuala University (USK), Province of Aceh, Indonesia, from May to November 2024 in the Laboratory of Biology, Organic Chemistry and Weed Science. Pot studies in screen house were conducted in Experimental Farm, Faculty of Agriculture, Syiah Kuala University (USK) (95°22'34, 49°T longitude, 5°34'3,44°U latitudes), altitude: 3 m above sea level, Annual rainfall: 1763.5 mm, max temp: 32.81 °C, minimum temp: 23.67 °C, and relative humidity: 79,81 %.

### Experimental

The experimental treatments consisted of 2 Factors: (i) *A. conyzoides n*-hexane extracts sub-subfractions A1 and A2, (ii) Concentrations of 4 (2, 4, 6, 8 %) and distilled

water as control. The treatments were replicated thrice in completely randomized design (CRD). The seeds of *A. spinosus* were gathered in Blang Bintang, Aceh Besar. Dr. Saida Rasnovi, the botanist, identified the plants.

### Fractionation

The *A. conyzoides* leaves were air dried for two weeks at room temperature and then grinded. The 25 kg grinded leaves were placed in ammonia for 1.0 h. The ammonia used for total basifying process was 4 L. The basified grinded leaves were then macerated 6-times using *n*-hexane. Each extraction took three days and used 20 L of solvent. Thus, total solvent used in this process were 120 L. The organic fractions recovered after extraction with each solvent were filtered and dried in a rotary evaporator (20). We obtained 250 g of *n*-hexane extract.

### Isolation

The *n*-hexane extract was fractionated by column chromatography. The size of the column chromatography used is 5 cm in diameter and 73 cm in length (1.4 L volume). Cotton was put into the bottom column and sand was heated and sieved using 12 mesh sieve. Then, 250 g silica gel was added after soaking for about 24 h with *n*-hexane. Sand was added with 1,5 cm thickness on the silica gel and finally on the top is the extract (50 g). Then, the column was eluted with *n*-hexane: ethyl acetate at a ratio of 9:1, v/v by keeping no air bubbles in the static phase. Then, the column faucet was slowly opened so that the eluent flowed by 15 drops per min. Fractions were accommodated in glass bottles (100 mL of each). The column was further eluted with mobile phases as of *n*-hexane:ethyl acetate (9:1; 8:2; 7:3; 6:4; 5:5; 4:6, 3:7 and 2:8, v/v). This process replicated 4 times to obtained enough subfraction for re-column chromatography. Therefore, in this process, total extract used were 200 g, solvent used were 7.4 L of *n*-hexane, and 3.6 L of ethyl acetate. According to their thin layer chromatography patterns with *n*-hexane and ethyl acetate eluents (9:1), pools of fractions were created (20). The subfractions that were obtained from this process were subfraction A (72 g), B (42 g), dan C (6 g).

The subfraction A obtained at the subfraction level was re-column chromatographed. The size of the column chromatography used is 3 cm in diameter and 65 cm in length (0.4 L volume). Cotton and sand were put into the bottom column. Then, 100 g silica gel was added after soaking for about 24 h with *n*-hexane. Sand was added on the silica gel and finally on the top is the extract (50 g). The *n*-hexane extract was isolated by column chromatography using silica gel G 60 F stationary phase by gradient elution. The eluent ratios used were *n*-hexane and ethyl acetate (9:1; 8:2; 7:3; 6:4 and 5:5, v/v). In this process, total solvent used were 1.7 L of *n*-hexane, and 0.3 L of ethyl acetate. Fractions were collected every 50 mL and each fraction was subjected to thin layer chromatography with *n*-hexane and ethyl acetate eluents (9:1). The sub-subfractions that were obtained by this process were A1, A2, A3 and A4. In this study, sub-subfraction A1 and A2 obtained from re-column chromatography process were further tested through field application and GC-MS analysis.

### Pot culture

We collected soil up to 20 cm depth from the Lampakuk Village, Aceh Besar. The soil was dried for 7 days and then sieved to remove the plant residue. One kg soil was placed in each plastic pot (16 cm dia and 13 cm depth). On October 24, 2024, 5-unsterilized of *A. spinosus* seeds were immersed in water for 2.0 h and sown in each pot at 2 cm depth. When the seedlings were 14 days old (November 7, 2024), the plants were foliar sprayed (4 ml per pot) with either water or plant extract as per treatments. Two hundred ml tap water was used to irrigate the pots twice a day. Weed control (%) was determined everyday (per 24 h.) at 6 PM for 7 days. Using a 0-100 rating scale, the *A. spinosus* weed control (%) was evaluated based on 5 observations (Table 1).

Table 1. Rating system used to assess weed control

Effects	Rating	Effects Description
No effect	0	No weed control, No crop reduction or injury
Slight	10	Very poor weed control, slight crop discolouration or Stunting
	20	Poor to deficient weed control, slight crop discolouration, stunting. or stand loss,
	30	Crop injury more pronounced, but not lasting
Moderate	40	Deficient weed control, Moderate injury, crop usually recovers
	50	Deficient to moderate weed control, Crop injury more lasting, recovery doubtful
	60	Moderate weed control, Lasting crop injury no recovery
Severe	70	Weed control less than satisfactory, Heavy crop injury and stand loss
	80	Satisfactory to good weed control, Crop nearly destroyed- A few surviving plants
	90	Very good to excellent weed control, only occasional live crop plants left
Complete effect	100	Complete weed destruction, Complete crop destruction

Source : (17)

### GC-MS analysis

GC-MS analysis was carried out using the Thermo Scientific ISQ 7000 Single Quadrupole GC-MS system, equipped with a triple off-axis Thermo Scientific DynaMax XR detector featuring an electronic dynamic range of  $>10^9$ . The system utilizes a dual mass filter with an off-axis ion guide pre-filter. The sample was injected in a 1.00  $\mu$ L volume using a screening method on a TG-5MS column. The analysis was carried out in Total Ion Chromatogram (TIC) mode with a run time of 63.99 min. The injection was classified as a blank type for screening purposes. Compounds were identified by comparing their mass spectra and retention times with those in the Thermo scientific Chromeleon 7.2. Chromatography Data System (CDS) and quantified based on their relative peak areas.

### Statistical analysis

At a 5 % probability level, Duncan's new multiple range test and analysis of variance (F test) were applied to all data. The SPSS version 23 programme (SPSS Inc., Chicago, IL) was used to conduct the analysis.

## RESULTS AND DISCUSSION

### Weed Control

The application of *n*-hexane extracts from *A. conyzoides* sub-subfractions A1 and A2 showed a strong inhibitory effect on *A. spinosus* weed growth. All tested concentrations (2 %, 4 %, 6 % and 8 %) of both A1 and A2 resulted in weed control percentages of 100 % at 1 day after application (DDA) (Figure 2). This shows a better and faster effect compared to weed control with subfraction A of *n*-hexane extract of *A. conyzoides* (16). Statistical analysis revealed no significant differences ( $p > 0.05$ ) among the various concentrations within each sub-subfraction. This suggests that even at the lowest tested concentration (2 %), both sub-subfractions exerted a maximal herbicidal effect, effectively saturating the response. In contrast, the distilled water control exhibited a significantly lower weed control effect.

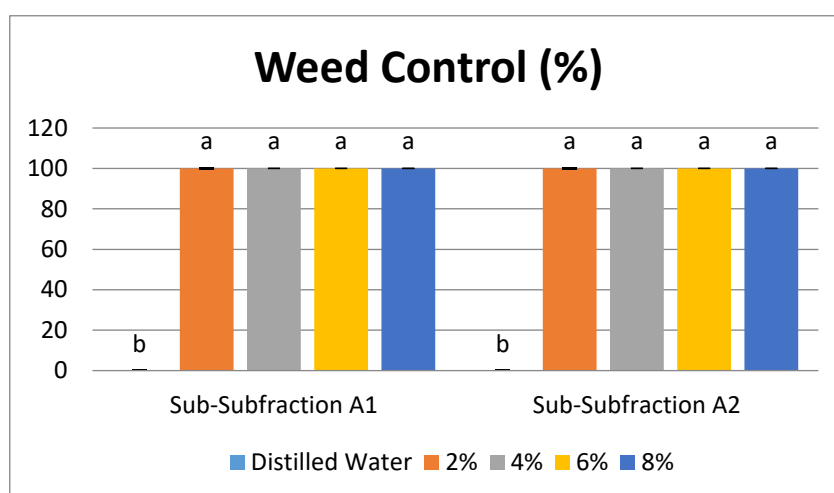


Figure 2. Effects of *n*-hexane extracts of *A. conyzoides* sub-subfraction A1 and A2 concentrations on control of *A. spinosus* weed at 1 DAA.

The *A. spinosus* weed showed symptoms of shoot withering at 1 DAA due to application of *Ageratum conyzoides* sub-subfractions. There was damage to the stems and leaves of the shoots indicated by the reduced size of the stems and curled leaves. In the next observation, the weeds showed symptoms of collapsing and drying, the stem size decreased, dried up, and turned yellow, while the leaves dried up, curled up and turned brown. These symptoms were seen in each treatment tested.

**GCMS Analysis****Sub-Subfraction A1**

In the *n*-hexane extract of *A. conyzoides* sub-subfraction A1, a total of 52 types of compounds were found (Table 2). Based on relative area (%), there were 11 main compounds (Table 3) that expected as the active compounds. At a retention time of 18,73 min., the compound 2H-1-benzopyran, 6,7-dimethoxy-2,2-dimethyl- (11.01 %) was obtained. At a retention time of 17,43 min., caryophyllene oxide (10.27 %) was found. At a retention time of 22,47 min., phytol (8.79 %) was obtained.

Table 2. All constituents in the *n*-hexane *A. conyzoides* extract sub-subfraction A1

No.	R.Time	Name	Area %
1	3.092	Cyclohexane	0.28
2	7.526	(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	0.06
3	7.92	2-Butenoic acid, 2-methyl- (CAS) 2-methyl-2-butenoic acid	0.37
4	8.304	Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1S)-	0.11
5	9.215	D-Limonene	0.62
6	10.429	1,6-Octadien-3-ol, 3,7-dimethyl-	0.69
7	14.146	Alpha.-cubebene	0.09
8	14.225	Alpha.-longipinene	0.09
9	14.33	Phenol, 2-methoxy-4-(2-propenyl)- (CAS) Eugenol	0.29
10	14.627	1-Tetradecene	0.30
11	14.679	Beta. bourbonene	0.04
12	14.735	Cedrene	0.21
13	15.263	Caryophyllene	7.23
14	15.305	Bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6-(4-methyl-3-pentenyl)-	0.77
15	15.393	Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-, [S-(R*,S*)]-	0.60
16	15.508	Cis.-beta.-farnesene	0.72
17	15.65	Alpha.-Humulene	0.75
18	16.016	Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-, [S-(R*,S*)]-	1.93
19	16.18	Cubedol	0.92
20	16.238	Beta.-Bisabolene	0.69
21	16.523	Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-, [S-(R*,S*)]-	7.32
22	16.734	Cis-limonene oxide	0.47
23	16.949	Nerolidol	1.41
24	17.209	1-Heptadecene	1.93
25	17.436	Caryophyllene oxide	10.27
26	17.658	Humulene oxide	1.03
27	18.737	2H-1-Benzopyran, 6,7-dimethoxy-2,2-dimethyl-	11.01
28	18.851	(-)-Caryophyllene oxide	0.71
29	19.06	Phenol, 4-nonyl- (CAS) p-nonylphenol	0.32
30	19.309	Phenol, 2,4,6-tris(1,1-dimethylethyl)-	0.44
31	19.504	1-Heptadecene	3.47
32	19.97	Phenol, 2,6-bis(1,1-dimethylethyl)-4-ethyl-	5.56
33	20.095	2-Pentadecanone, 6,10,14-trimethyl-	1.88

34	20.41	E-6-Octadecen-1-ol acetate	1.14
35	20.525	P-Octylacetophenone	0.58
36	20.828	Hexadecanoic acid, methyl ester	0.86
37	21.498	1-Octadecanethiol (CAS) octadecanethiol	4.71
38	21.908	Geranyl linalool isomer B	0.60
39	22.479	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	0.92
40	22.561	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	1.85
41	22.743	Phytol	8.79
42	22.958	Palmitaldehyde, diallyl acetal (CAS) 1,1-diprop-2-enoxy hexadecane	0.47
43	23.097	Linoleic acid ethyl ester	0.62
44	23.17	Ethyl linoleolate	1.17
45	23.352	Octadecyl trifluoroacetate	3.03
46	23.604	Phytol, acetate	4.17
47	24.349	2-Nonadecanone	0.31
48	25.042	n-Tetracosanol-1	2.00
49	25.854	Pentatriacontane	0.23
50	26.611	Octacosanol	0.97
51	28.408	Squalene	4.50
52	30.343	Hexatriacontane	0.49

Table 3. Main constituents in the *n*-hexane *A. conyzoides* extract sub-subfraction A1.

No.	R.Time	Name	Area %
1	18.737	2H-1-Benzopyran, 6,7-dimethoxy-2,2-dimethyl-	11.01
2	17.436	Caryophyllene oxide	10.27
3	22.743	Phytol	8.79
4	16.523	Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-, [S-{R*,S*}]	7.32
5	15.263	Caryophyllene	7.23
6	19.97	Phenol, 2,6-bis(1,1-dimethylethyl)-4-ethyl-	5.56
7	21.498	1-Octadecanethiol (CAS) Octadecanethiol	4.71
8	28.408	Squalene	4.5
9	23.604	Phytol, acetate	4.17
10	19.504	1-Heptadecene	3.47
11	23.352	Octadecyl trifluoroacetate	3.03

The other main constituents were obtained were cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-, [S-(R\*,S\*)]- (7.32 %), at retention time of 16.52 min., followed by caryophyllene (7.23 %), which found at a retention time of 15.26 min. Phenol, 2,6-bis(1,1-dimethylethyl)-4-ethyl- (5.56 %) was obtained at a retention time of 19.97 min. The compound 1-octadecanethiol (CAS) octadecanethiol (4.71 %) was found at a retention time of 21.49 min. At a retention time of 28.40 min., squalene (4.5 %) was obtained, followed by phytol, acetate (4.17 %) at retention time of 23.60 min. At a retention time of 19.50 min., there was 1-heptadecene (3.47 %), and at a retention time of 23.35 min., octadecyl trifluoroacetate (3.03 %) was obtained.

The compound 2H-1-benzopyran, 6,7-dimethoxy-2,2-dimethyl- has previously been identified in the *n*-hexane extract fraction of *Ageratum conyzoides* (15) as well as in subfraction A of the *n*-hexane extract of *A. conyzoides* (16). 2H-1-benzopyran, 6,7-dimethoxy-2,2-dimethyl- was reported found in high concentration in the essential oils from the aerial parts of of *A. conyzoides* (8). It was known for its wide spectrum as antifungal agent, with allatocidal and insect-growth regulator activities (31). The phytotoxic effect of 2H-1-benzopyran, 6,7-dimethoxy-2,2- dimethyl- was reported on radish, mungbean, tomato, and ryegrass seedlings (30). It's phytotoxicity was reported on radish, mung bean, tomato and ryegrass seedlings. The specific compound 2H-1-benzopyran, 6,7-dimethoxy-2,2-dimethyl-, which is exclusively found in resistant plant varieties after inoculation, has been confirmed to possess allelochemical properties. It exhibits strong allelopathic effects as well as significant synergy, suggesting its potential role in plant defence mechanisms and ecological interactions (43).

Phytol was reported obtained in the subfraction A of *n*-hexane extract of *A. conyzoides* (16). Phytol was reported had inhibitory effect to germination, shoot length, and fresh weight of cowpea (*Vigna unguiculata*) (32). Phytol was found to be able to interact and damage the structure of the phospholipid bilayer of the cell membrane (9). It was previously reported that phytol was obtained in *Cassia italica* methanol leaf extract and had antimicrobial, anticancer, anti-inflammatory, and diuretic agent (39). Other studies also showed that phytol induced a defence response in host plants, inhibiting Root-Knot Nematodes (RKN) penetration (18).

The compound Caryophyllene has previously been identified in the fraction of *n*-hexane extract fraction of *Ageratum conyzoides* (15) as well as in subfraction A of the *n*-hexane extract of *A. conyzoides* (16). Two sesquiterpenes,  $\beta$ -caryophyllene and caryophyllene oxide, have been identified as key compounds in *Senecio salignus* extract that affect photosynthesis in *Physalis ixocarpa* and *E. crus-galli*. In *P. ixocarpa*, 100  $\mu$ g/mL of  $\beta$ -caryophyllene significantly disrupted photosynthesis by inhibiting photosystem II, turning active centres into "heat sinks" that could not reduce QA. Additionally,  $\beta$ -caryophyllene caused chlorosis in treated leaves (38). Another study indicated that the sesquiterpenes  $\beta$ -caryophyllene and caryophyllene oxide reduced the dry biomass of *Physalis ixocarpa* plants, with  $\beta$ -caryophyllene exerting a stronger influence. Additionally,  $\beta$ -caryophyllene has been appeared to restrain root prolongation in *P. ixocarpa* and *Echinochloa crus-galli* seedlings (42).

$\beta$ -caryophyllene is also known as a key component in the essential oil of *Artemisia lavandulafolia*, which has been reported to inhibit the seedling growth of *Achyranthes japonica* (29). In another study, the application of *Cullen plicata* essential oil resulted a reduction in germination of *Bidens pilosa* and *Urosermum picroides*. This effect is associated with the high content of Caryophyllene oxide, which is likely responsible for its allelopathic activity, inhibiting seed germination and potentially affecting seedling development (11). The caryophyllene compound obtained from *Cleome amblyocarpa* exhibit substantial allelopathic inhibition activity against the weed *Dactyloctenium aegyptium* (12).

Squalene, important constituent found in the leaves and bark of *M. concanensis* and was known for its antioxidant activity. Additionally, studies have shown that squalene

exhibits chemopreventive activity against colon carcinogenesis, highlighting its potential therapeutic applications (5). It was reported that the major compounds (squalene, stigmasterol, linoleic acid, palmitic acid, stearic acid, lupeol and phytol) in *Excoecaria agallocha* leaf leachates were inhibitory to seeds germination and seedlings growth of *Eleusine coracana* (10).

Chromatogram of *n*-hexane extract of *A. conyzoides* sub-subfraction A1 identified by GC-MS were shown in Figure 3.

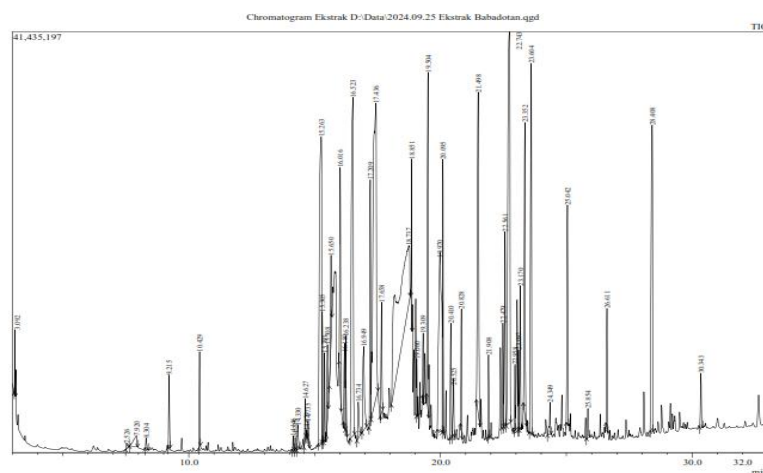


Table 4. All constituents in the *n*-hexane *A. conyzoides* extract sub-subfraction A2

No.	R.Time	Name	Area %
1	15.785	2-propenoic acid, 3-(2-hydroxyphenyl)-, (E)-	22.06
2	17.506	2(4H)-benzofuranone, 5,6,7,7a-tetrahydro- 4,4,7a-trimethyl-, (R)-	1.61
3	19.278	Demethoxyencecalinol	1.26
4	19.904	2,4,5,5,8a-pentamethyl-6,7,8,8a-tetrahydro- 5H-chromene	0.43
5	20.632	2H-1-benzopyran, 6,7-dimethoxy-2,2- dimethyl-	3.13
6	20.948	1H-inden-1-one, 7-(1,1-dimethylethyl)-2,3- dihydro-3,3-dimethyl-	5.15
7	21.901	Ethanone, 1-(7-hydroxy-5-methoxy-2,2- dimethyl-2H-1-benzopyran-8-yl)-	0.51
8	22.727	8-acetyl-5,5-dimethyl-nona-2,3,8-trienoic acid, methyl ester	1.03
9	23.071	Benzoic acid, 2-bromo-a,a-dimethyl-	0.62
10	23.264	Dodecanoic acid, tetradecyl ester	0.49
11	23.809	6-(1'-Hydroxyethyl)-7-methoxy-2,2- dimethylchromene	2.27
12	24.176	13-heptadecyn-1-ol	1.07
13	25.876	1-heptatriacotanol	1.05
14	26.533	l-(+)-ascorbic acid 2,6-dihexadecanoate	0.61
15	27.329	n-hexadecanoic acid	18.89
16	28.665	7-methyl-Z-tetradecen-1-ol acetate	0.38
17	29.349	Phytol	1.04
18	30.475	9,12,15-octadecatrienoic acid, (Z,Z,Z)-	22.21
19	33.991	Tetracosan-10-yl acetate	0.58
20	36.733	Docosanoic acid	0.81
21	37.022	9-Hexacosene	0.45
22	44.344	4-Norlanosta-17(20),24-diene-11,16-diol-21-oic acid, 3-oxo-16,21-lactone	1.36
23	44.963	5-Cholestene-3-ol, 24-methyl-	0.69
24	45.463	Stigmasterol	10.36
25	46.120	?-sitosterol	7.05
26	46.722	Stigmast-7-en-3-ol, (3 $\beta$ ,5 $\alpha$ )-	4.48
27	49.790	Androst-7-ene-6,17-dione, 2,3,14-trihydroxy-, (2 $\beta$ ,3 $\beta$ ,5 $\alpha$ )-	1.83

Table 5. Main constituents constituents in the *n*-hexane *A. conyzoides* extract sub-subfraction A2

No.	R.Time	Name	Area %
1	30.475	9,12,15-octadecatrienoic acid, (Z,Z,Z)-	22.21
2	15.785	2-propenoic acid, 3-(2-hydroxyphenyl)-, (E)-	22.06
3	27.329	n-hexadecanoic acid	18.89
4	45.463	Stigmasterol	10.36
5	46.120	?-sitosterol	7.05
6	20.948	1H-inden-1-one, 7-(1,1-dimethylethyl)-2,3- dihydro-3,3-imethyl-	5.15
7	46.722	Stigmast-7-en-3-ol, (3 $\beta$ ,5 $\alpha$ )-	4.48
8	20.632	2H-1-benzopyran, 6,7-dimethoxy-2,2- dimethyl-	3.13

The compound n-hexadecanoic acid is a Palmitic acid known for its biological activity as antioxidant, hypocholesterolemic, nematicide, pesticide, lubricant, antiandrogenic, flavour, hemolytic, and 5-alpha reductase inhibitor (39). This compounds also found in *Azolla pinata* and has been reported to have strong larvicidal effects (37).

Chromatogram of *n*-hexane extract of *A. conyzoides* sub-subfraction A2 identified by GC-MS were shown in Figure 4.

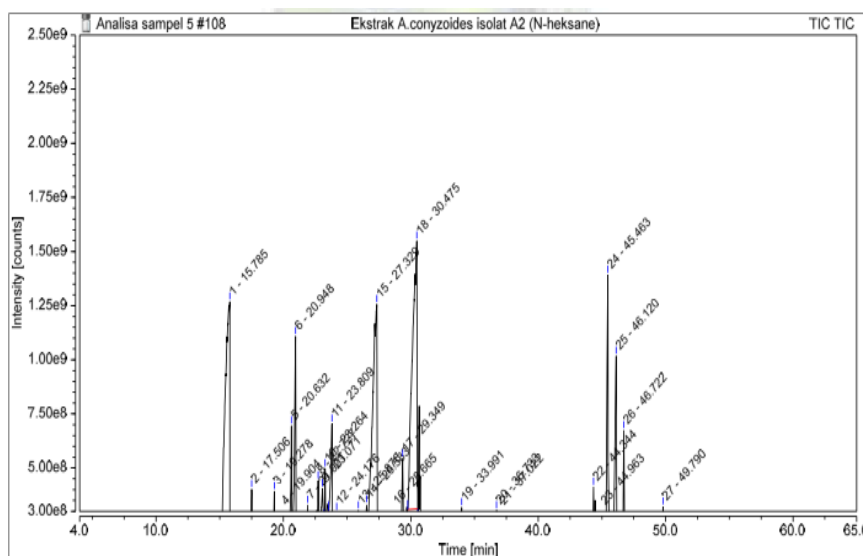


Fig. 4. Chromatogram of *n*-hexane extract *A. conyzoides* sub-subfraction A2 identified by GC-MS

Stigmasterol has structural similarity to  $\beta$ -sitosterol but differs in the presence of a trans- oriented double bond in the side chain. Some sterols, especially  $\beta$ -sitosterol, showed antioxidant properties. Biosynthetically, stigmasterol is produced by the desaturation of  $\beta$ -sitosterol (41). It was suggested that compound Stigmasterol had role in plant biotic and abiotic stress tolerance mechanism, such as pathogen infection, salinization, unfavourable temperatures, drought, UV radiation, heavy metals and stress phytohormones. It was also reported to has activities as anti-osteoarthritis, anti-inflammatory, anti-diabetic and neuroprotective (41). In another research, the phytosterols campesterol and Stigmasterol were identified as compounds that reduce the content of chlorophyll in sclareol-treated *Arabidopsis* plants. The exogenous application of campesterol or stigmasterol dose-dependently reduced the content of chlorophyll in *Arabidopsis* leaves (21).

## CONCLUSIONS

Analysis of weed control percentage indicated that all sub-subfractions tested of *A. conyzoides* *n*-hexane extract suppressed *A. spinosus* growth by 100 % at concentration of 2 % or higher by 1 DAA. Hence, the sub-subfractions of *A. conyzoides* *n*-hexane extract may be developed as a promising herbicide against *A. spinosus*. The higher purity level of the compound, leads to greater effects of sub-subfractions of *n*-hexane extract of *A. conyzoides* on the growth of *A. spinosus* weed. The major compounds detected by GC-MS in sub-subfraction A1 were: 2H-1-benzopyran, 6,7-dimethoxy-2,2-dimethyl- (11.01 %), caryophyllene oxide (10.27 %) and phytol (8.79 %), while in sub-

subfraction A2, there were 9,12,15-octadecatrienoic acid, (Z,Z,Z)- (22.21 %), 2-propenoic acid, 3-(2-hydroxyphenyl)-, (E)- (22.06 %) and n-hexadecanoic acid (18.89 %).

### ACKNOWLEDGMENTS

The authors would like to thank the Syiah Kuala University, Ministry of Education, Culture, Research and Technology Contract number 211/UN11.2.1/PG.01.03/SPK/PTNBH/2024 for research funding, Mariah Nasrina Mardhatillah, Pretty Amara Dinili, Elfi Yufianda, Riska Rahmawati Fadillah, and Raja Samudra for experimental assistance in the field and laboratory.

### DECLARATION

We declare that all authors of this Ms have made substantial contributions. We have not excluded any author that substantially contributed to this Ms. We have followed our ethical norms established by our respective institutions

### AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration with all authors. All authors finally approved and drafted the manuscript.

### CONFLICT OF INTEREST

The authors announce that they have no conflict of interest.

### ETHICAL APPROVAL

The authors declare that the study was carried out following scientific ethics and conduct. However, this study did not involve any use of animals, hence no ethical approval has been obtained from the concerned committee.

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