IDENTIFICATION OF ALLELOCHEMICAL COMPOUNDS FROM EACH PART OF *Tetracera indica* (L.) MERR

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ABSTRACT

Plant allelochemical is very potential to be developed as an effective and environmentally friendly bioherbicide for controlling weeds. Each part of *Tetracera indica* has potential as an allelochemical compounds bioherbicide. The purpose of this research was to identify the allelochemical content of each part of *Tetracera indica* (L.) Merr. Allelochemical compounds were analyzed using GC-MS (Gas Chromatography-Mass Spectrometer) method. The result of the analysis showed an extract of each part *Tetracera indica* had different type of compounds. Potential allelochemical bioherbicide compounds were fatty acids, flavonoids, and steroids. Flower extract has the highest compound of fatty acids and steroids compared to other parts.

Keywords: Allelopathy, Bioherbicide, Extract, GC-MS, *Tetracera indica*.

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INTRODUCTION

The presence of weeds in agriculture can reduce plant growth and production caused by competition in the use of nutrients, light, and other growing facilities. One effort to save yield losses due to weeds is carried out weed control using synthetic herbicides. The use of synthetic herbicides is preferred because more efficient in terms of energy, time, and application costs compared to other techniques. The continuous use of synthetic herbicides causes several problems including concerns over the evolution of weed resistance, shifting weed species, water and soil surface pollution, and increased production costs.¹

One alternative to overcome the adverse effects of using synthetic herbicides is replacing them with bioherbicides. Bioherbicides can be produced from plant extracts that have allelochemical compounds. Allelochemical compounds are produced by a plant to inhibit the growth of other plants in the vicinity. Allelochemical compounds can be developed in controlling weeds to reduce labor costs and increase efficiency without endangering the environment.²

Tetracera indica is the weed that has known its potential as a bioherbicide based on allelochemical compounds. The extract of *T. Indica*'s canopy at a concentration of 50-250 g.l⁻¹ can suppress the growth of *Ageratum conyzoides*, *Asystasia intrusa*, and *Eleusine indica*. The compound chemical of *T. Indica*'s canopy which is thought to play a role in suppressing weed is 1,2-benzenediol, chloroacetic acid, 2,6-dimethoxy phenol, and pyrocatechol which are phenol group compounds.³

Allelochemical compounds are one type of secondary metabolites produced by plants. The distribution of some secondary metabolites is uneven in plant organs.⁴ This evidence from various research has been carried including research on phenol compounds in *Cyperus rotundus*, plant canopy extracts contain more phenol group compounds than rhizome extracts.⁵ Research on *Clidemia hirta*'s allelopathic activity showed that leaf extract is the most significant compared to root extract and stem extract in inhibiting germination and growth of *Raphanus sativus*.⁶

Different concentrations and types of secondary metabolites may also be present in each part of *T. indica*. So far no research has been carried on this matter. All this time, *T. indica* has been used by Malay people in Malaysia, roots, and leaves used as medicine while the fruit is made of vinegar. The difference in the use of each part *T. indica* reinforces the suspicion of differences compounds in each part will affect its potential

as a bioherbicide. The purpose of this research is to study the content of allelochemical compounds from each part of *T.indica* (L.) Merr. plant.

EXPERIMENTAL

Allelochemical compound analysis was performed using GC-MS (Gas Chromatography-Mass Spectrometer) method. Fresh *Tetracera indica* used to extract material are plants that have entered the generative phase taken from Cikabayan oil palm plantations which are located at an altitude of 206 meters above sea level with latosol soil type and high precipitation. Parts of the plants that were used in this research were leaves, stems, flowers, and roots. Leaves and flowers dried and then blended with a blender. The roots and stems chopped and then dried using an oven at 58°C for 15 hours, then mashed using a hammermill and disk mill. Making *T. indica* extract was carried using the maceration-distillation method with methanol as a solvent. The maceration method that is 1 kg of material soaked in 3 l of methanol for 24 hours, after it was filtered, the remaining pulp was macerated up to 4 times.⁷ The macerate was concentrated using a rotary evaporator so that an extract of each part of *T. indica*.

RESULTS AND DISCUSSION

Results of GC-MS (Gas Chromatography-Mass Spectrometer) analysis showed that were various types of compounds scattered in each section with different compositions and percentages in each part *Tetracera indica*. Compounds 9, 12-Octadecadienoic acid (Z, Z), Flavone 5,7-dihydroxy-8-methoxy (wogonin), and Hexadecanoic acid are three compounds with the largest content and are contained in all parts of *T. indica*. The compound with the highest percent area in all parts of *T. indica* is 9, 12-Octadecadienoic acid (Z, Z) which is a class of fatty acids.

Methanol extract *T. indica* compounds belong to a class of fatty acid, flavonoid, and steroid compounds.⁸ The leaves contain 11 compounds with 4 compounds classified as fatty acids (total % area: 55.53%), 1 flavonoid (24.82%), and 3 steroids (6.34%) (Table-1). *T. indica* leaves were identified to contain flavonoid compounds, 5,7-dihydroxyflavone-O-8-sulphate, techtochrysin, wogonin, norwogonin, kaempferol, and quercetin.⁹ The results of this research indicate that flavonoid compounds identified in the leaves *T. indica* are only wogonin (Flavone, 5,7-dihydroxy-8-methoxy).

T. indica stem contains 12 compounds with 4 fatty acids (53.17%), 1 flavonoid (24.01%), and 2 steroids (4.15%) (Table-2). According to previous research on the stem contain four monoflavonoids namely wogonin, norwogonin, quercetin, and techtochrysin¹⁰, and contain betulinic acid¹¹, of the five compounds the only wogonin was identified in this research.

Table-1: The Compound identified in the *T. indica* Leaf Extract

No.	Compound	Chemical Formula	Class	Percent Area	Retention Time
1	9, 12-Octadecadienoic acid (Z,Z)	C ₁₈ H ₃₂ O ₂	Fatty acid	38.88	30.054
2	Flavone, 5,7-dihydroxy-8-methoxy	C ₁₆ H ₁₂ O ₅	Flavonoids	24.82	33.123
3	Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	Fatty acid	12.56	28.896
4	Stigmastan-3,5-diene	C ₂₉ H ₄₈	Steroids	3.25	36.074
5	9, 12-Octadecadien-1-ol	C ₁₈ H ₃₄ O	Fatty acid	2.97	32.544
6	2-Furancarboxaldehyde,5-(Hydroxymethyl)	C ₆ H ₆ O ₃	Furan compounds, aldehydes	2.71	9.837
7	beta-Monoolein	C ₂₁ H ₄₀ O ₄	Monoglyceride	1.84	31.337
8	Stigmast-5-en-3-ol	C ₂₉ H ₅₀ O	Steroids	1.74	39.370
9	Phytol	C ₂₀ H ₄₀ O	Diterpenoid alcohol	1.37	29.599
10	Cholesta-3,5-Diene	C ₂₇ H ₄₄	Steroids	1.35	35.060
11	Cis-13-Octadecenoic acid, methyl ester	C ₁₉ H ₃₆ O ₂	Fatty acid	1.12	29.489

Table-2: The compound identified in the *T. indica* stem extract

No.	Compound	Chemical Formula	Class	Percent Area	Retention Time
1	9, 12-Octadecadienoic acid (Z,Z)	C ₁₈ H ₃₂ O ₂	Fatty acid	36.47	30.027
2	Flavone, 5,7-dihydroxy-8-methoxy	C ₁₆ H ₁₂ O ₅	Flavonoids	24.01	33.116

3	Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	Fatty acid	13.94	28.889
4	2-Furancarboxaldehyde,5-(Hydroxymethyl)	C ₆ H ₆ O ₃	Furan compounds, aldehydes	4.15	9.982
5	Stigmast-5-en-3-ol	C ₂₉ H ₅₀ O	Steroids	2.58	39.432
6	9-Octadecenoic acid (Z), methyl ester	C ₁₉ H ₃₆ O ₂	Fatty acid	1.73	29.496
7	4H-Pyran-4-on, 2,3-dihydro-3,5-dihydroxy-6-methyl	C ₆ H ₈ O ₄	Pyrone	1.61	6.955
8	Cholest-5-en-3-yl-chloridocarbonate	C ₂₈ H ₄₅ ClO ₂	Steroids	1.57	35.067
9	2,3-Hydro-3,3-Dimethyl-2-Methylenbenzofuran	C ₁₀ H ₁₀ O ₂		1.28	34.619
10	.beta-Monoolein	C ₂₁ H ₄₀ O ₄	Monoglyceride	1.15	31.337
11	2-Hydroxycyclopentadecanone	C ₁₅ H ₂₈ O ₂	Ketone	1.07	32.537
12	13-Tetradecenal	C ₁₄ H ₂₆ O	Fatty acid	1.03	32.330

T. indica root contains the most types of compounds, namely 14 compounds. Compounds that belong to the allelochemical group are also more diverse, namely 4 fatty acid compounds (58.65%), 2 flavonoids (12.51%), and 4 steroids (7.03%). The root has a flavonoid content with a lower percent area than leaves and stems but a higher steroid compound (Table-3).

Table 4 showed the compounds identified in flowers and fruit, there are 10 compounds, 4 compounds including fatty acid (61.43%), 1 flavonoid compound (19.76%), and 4 steroid compounds (9.14%). The flower and fruit parts contain fatty acids and steroids with a higher percentage of the area than the other parts.

Visible differences in percent area of each class compounds including allelochemicals in each part *T. indica*. Percent area of compounds including allelochemical group is high in each part, but the high content does not automatically reflect *T. indica* can be used as a bioherbicide, because even though many compounds are identified including allelochemical groups, not every compound has allelopathic activity and only a small percent is true containing allelochemicals.¹² Identification of compounds including allelochemicals requires further research.

Table-3: The Compound identified in the *T. indica* Root Extract

No.	Compound	Chemical Formula	Class	Percent Area	Retention Time
1	9, 12-Octadecadienoic acid (Z,Z)	C ₁₈ H ₃₂ O ₂	Fatty acid	39.46	30.055
2	Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	Fatty acid	15.26	28.903
3	Flavone, 5,7-dihydroxy-8-methoxy	C ₁₆ H ₁₂ O ₅	Flavonoids	9.45	32.999
4	2-Furancarboxaldehyde,5-(Hydroxymethyl)	C ₆ H ₆ O ₃	Furan compounds, aldehydes	7.38	10.217
5	Peri-Xanthoxanthne-4,10-Dione, 2-Acetyl-8-(1-Methylethyl)			4.87	36.115
6	Stigmast-5-en-3-ol	C ₂₉ H ₅₀ O	Steroids	3.39	39.480
7	3,5-Dihydroxy-7-methoxy-2-(4-Methoxyphenyl)-4H-Chromen-4-One	C ₁₇ H ₁₄ O ₆	Flavonoids	3.06	35.012
8	2,3-Dihydro-3,3-Dimethyl-2-Methylenbenzofuran	C ₁₀ H ₁₀ O ₂		2.7	34.633
9	9-Octadecenoic acid (Z), methyl ester	C ₁₉ H ₃₆ O ₂	Fatty acid	1.98	29.496
10	9,17-Octadecadienal, (Z) -	C ₁₈ H ₃₂ O ₂	Fatty acid	1.95	32.564
11	1,2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester	C ₁₆ H ₂₂ O ₄	Carboxylic acid	1.63	31.696
12	3-Bromocholest-5-ene	C ₂₇ H ₄₅ Br	Steroids	1.35	35.074
13	Stigmasta-5,22-dien-3-ol, acetate, (3β)	C ₃₁ H ₅₀ O ₂	Steroids	1.15	35.398
14	Stigmastan-3,5-diene	C ₂₉ H ₄₈	Steroids	1.14	35.647

Table-4: The Compounds identified in *T. indica* Flower and Fruit Extracts

No.	Compound	Chemical Formula	Class	Percent Area	Retention Time
1	9, 12-Octadecadienoic acid (Z,Z)	C ₁₈ H ₃₂ O ₂	Fatty acid	43.49	29.985
2	Flavone, 5,7-dihydroxy-8-methoxy	C ₁₆ H ₁₂ O ₅	Flavonoid	19.76	32.992
3	Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	Fatty acid	14.67	28.834
4	Stigmastan-3,5-diene	C ₂₉ H ₄₈	Steroids	4.86	36.060
5	9-Octadecenoic acid (Z), methyl ester	C ₁₉ H ₃₆ O ₂	Fatty acid	1.98	29.489
6	Cholest-5-en-3-yl-chloridocarbonate	C ₂₈ H ₄₅ ClO ₂	Steroids	1.83	35.047
7	.gamma-Sitosterol	C ₂₉ H ₅₀ O	Steroids	1.42	39.335
8	.beta-Monoolein	C ₂₁ H ₄₀ O ₄	Monoglyceride	1.38	31.330
9	9,17-Octadecadienal, (Z) -	C ₁₈ H ₃₂ O ₂	Fatty acid	1.29	32.323
10	Ergost-5,8 (14)-dien-3-ol	C ₂₈ H ₄₆ O	Steroids	1.03	34.598

Compounds that are thought to be allelochemicals are Stigmastan-3,5-diene, Stigmast-5-en-3-ol, Stigmasta-5,22-dien-3-ol, acetate, (3 β), Cholesta-3,5-Diene, and Cholest-5-en-3-yl-chloridocarbonate. Stigmastan compounds are compounds that potentially inhibit the germination of *Mimosa pigra*¹³ and *Raphanus sativus*.¹⁴ Cholesta compound is one of the allelochemical candidates in rice because its inhibitory activity is greater than other compounds.¹⁵ In addition to stigmastan and cholesta compounds, Phytol compounds have also been known to be active compounds in *Terminalia catappa*'s leaves that can inhibit the germination and growth of *Cleome rutidosperma*.¹⁶

Identification *T. indica* in this research also showed the content of flavonoid compounds, wogonin, which was identified in all parts of *T. indica* with fairly high content. Flavonoids are known to have a role inhibiting growth which acts as a strong inhibitor of IAA-oxidase, but so far no research on the activity wogonin as an allelochemical.¹⁷ Research⁷ about wogonin compounds are known to have potential as diabetes mellitus drugs.¹¹ Nanoparticle have the potential to be used with active compound in biomedical applications.¹⁸

Presence of 1,2-benzenediol, Chloroacetic acid, Phenol, 2,6-dimethoxy, and Pyrocatechol which are allelopathic compounds, but in this research, the analysis did not show the existence of compounds³. This might be due to differences in the solvents used, the research uses distilled water while this research uses methanol as a solvent³. Different solvents produce different compounds in their solutions such as cyperene and culmorin which are allelopathic compounds in *C. rotundus* can only be identified in fresh *C. rotundus* dissolved in water and not found in formulas dissolved in ethanol.¹⁹ Other factors that might cause differences in the results of the analysis are environmental factors that affect the production of *T. indica* allelochemical, namely state of population differences, life cycle and time of planting, soil and climate, and the presence of biotic and abiotic stresses.²⁰ *T. indica* used are taken during high rainfall so that it may cause quantity and quality of produced allelochemicals to be low because allelopathic compounds will be produced more in nutrient stress and drought.⁷

CONCLUSION

Extract from each part of *Tetracera indica* has different type and content of compounds. Potential compounds as bioherbicides are fatty acids, flavonoids, and steroids which are classified as allelochemical compounds. Flower extract has the highest compound fatty acids and steroids compared to other parts. Purification of each compound indicated as allelochemical and testing of ability as bioherbicide needed to ascertain the compound allelochemical in *T. indica*.

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