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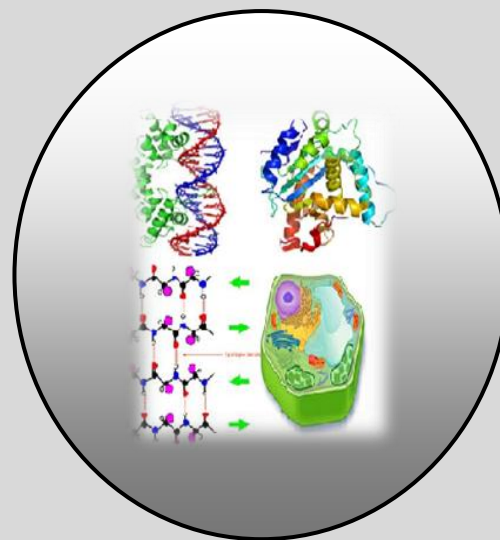
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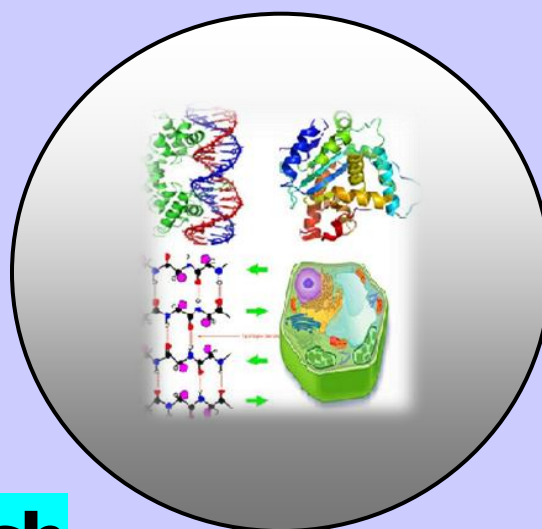
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RESEARCH PAPER

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Compound Analysis of *Tithonia diversifolia* Leaves

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ABSTRACT

This study aimed of compounds analysis of Tithonia diversifolia leaves using UV-Vis spectroscopy, IR and GC-MS analyses.

Extract of Tithonia diversifolia leaves were macerated with methanol. Methanol extract obtained from the maceration was then evaporated and partitioned with ethylacetat. Ethyl acetat fraction was further evaporated and separated using GCC guided by TLC. The compounds obtained from GCC were identified by using TLC with 3 different types of eluent mixture.

They showed that compound analysis using UV-Vis spectroscopy gave the maximum wavelength (λ_{maks}) at 220.80 nm and 400.20 nm. IR spectrum showed functional groups C=O carbonyl, -OH carboxylate, C-O ester, and C-H aliphatic. The result of GC-MS analysis showed that secondary metabolite compound which was isolated has a similarity index (SI) to 17 β -(acetyloxy)-2 α -methyl-5 α -estran-3-one of 75 with a purity of 69.53%.

Keywords: Compound, Analysis, UV-Vis Spectroscopy, IR and GC-MS Analyses and Tithonia diversifolia leaves.

INTRODUCTION

As a tropical country, Indonesia has a large amount of plant species thriving in its region. Not only plants used for food and for industrial export, but also plants that can be used in the field of medicine for natural treatment. Thus, today many alternative treatments using various types of herbs are found in Indonesia.

Medical treatment with natural ingredients has existed for hundreds of year's and cannot be removed from the daily life of Indonesian people. One of the competitive advantages of this treatment is that the side effects are relatively small compared to the chemical treatment. In addition, traditional medicines are easy to obtain and can be processed easily by almost everyone. This suggests that the treatment through natural

ingredients can still compete at present time despite the rapid advances in science and technology. Furthermore, with the great advancement of technology many scientists start optimizing the utilization of various medicinal plants for more efficient treatments. One of the natural plants which is often prescribed in traditional medicine is *Tithonia diversifolia*. This plant usually grows wild in steep places, such as on cliffs, river banks, and ditches. In addition, this annual plant can grow well in places with high light-intensity at the height of 5-1500meters above sea level. *Tithonia diversifolia* has a straight-erected stems, three-branched leaves with green main veins and others branching off, yellow flowers, and the roots are in the form of a taproot (Hutapea, et.al., 1994).

Based on various studies it is known that the leaves *Tithonia diversifolia* can be used to treat stomach-ache, bloating, bloody injury, and as anti-inflammatory and antidiabetic. Further research reveals that the leaves of this plant contain alkaloids, terpenoids, flavonoids, saponin, tannins, and polyphenols. These compounds are included in the class of secondary metabolites, thus allowing these plants to be used for medical treatment.

This study was carried out to isolate and identify secondary metabolites from *ethyl acetate* fraction of *Tithonia diversifolia* leaves. The extraction method used in this study was maceration. This method was chosen because it has several advantages, i.e. the required tools are relatively simple; it can be used for extraction in large quantities, the cost is relatively low, and it can be performed at room temperature without heating. This process technically used methanol solvent and the result was then partitioned using n-hexane. Methanol was used as a solvent because it can dissolve almost all kinds of secondary metabolites. The process of compound separation was conducted using Gravity Column Chromatography (GCC) method. To determine the purity of the compound and the GCC eluent a Thin Layer Chromatography (TLC) test was conducted. To determine the characteristics of the structure, a further analysis of pure compounds resulted from the isolation was conducted using UV-Vis spectrophotometer, infrared (IR) spectroscopy and GC-MS spectroscopy. The result of this study is expected to optimize the use of the *Tithonia diversifolia* as traditional and modern medicine.

The study aims to:

1. Compound analysis of *Tithonia diversifolia* leaves using Thin-layer Chromatography (TLC) and Gravity Column Chromatography (GCC), UV-Vis (Ultraviolet-Visible) spectrophotometer, IR (infrared) spectroscopy, and GC-MS Spectroscopy; and
2. Reveal the type of compound in the ethyl-acetate fraction of *Tithonia diversifolia* leaves by maceration method and separation by chromatography.

The benefits that can be drawn are, among others:

1. to provide information on how to compound analysis of *Tithonia diversifolia* leaves;
2. to contribute in developing science in chemistry, especially organic chemistry and pharmaceuticals.

REVIEW

Tithonia diversifolia flower can be classified as follows (Hutapea, 1997:297):

Division: Spermatophyta Sub Division: Angiospermae Class : Dicotyledoneae Ordo: Asterales
Family : Asteraceae Genus : *Tithonia* Species: *Tithonia diversifolia*

Tithonia diversifolia is an upright shrub that can reach a height of 3 meters, have sprouts, and crept on the ground. Generally, this plant grows wild on steep places, for example on the cliffs, river banks, and ditches.

This plant grows easily in places with a height of 5-1500 meters above sea level, and is also an annual plant that likes bright places and grows well in a place exposed to direct sunlight (Sulistijawati and Didik, 2001).

According Taofik, et.al. (2010), the extract of *Tithonia diversifolia* leaves contains an active ingredient that can kill the *eriophyidae* (mites that usually infest fowl). Based on his study, it is known that the extract of *Tithonia diversifolia* leaves has a toxicity level for *eriophyidae* mites at 2.2922 ppm during a 72-hour treatment. Using the phytochemical and High Performance Liquid Chromatography (HPLC) analysis, the study has shown that the extract contains flavonoids, alkaloids, and tannins. From this result it is known that *Tithonia diversifolia* plants have a potential to be utilized as botanical insecticide which is very economical.

Tithonia diversifolia is generally utilized traditionally for its leaves. The leaves contain active substances that can be used in medical treatment. Apart from being a botanical insecticide, a lot of research has also proven that the content of the active compounds in the leaves *Tithonia diversifolia* can be used as medicine to treat antidiabetic, anti-virus, anti-malaria, liver, and strep throat. The plant of *Tithonia diversifolia* is shown in Figure 1.



Figure 1. *Tithonia diversifolia*
(Source: personal documents).

RESULTS AND DISCUSSION

1. Separating *Tithonia diversifolia* Leaves by Maceration and Partition

The result of the separation and partition leaf maceration *Tithonia diversifolia* are shown in Table 1.

Table 1. Results of Separation by Maceration and Partitions.

Material	Separation method	Solvent	Extract Volume concentrated
0.5 kg dried powder of <i>Tithonia diversifolia</i> leaf	Maceration	Methanol	115 mL
	Partition	ethyl acetate	50.6 mL

2. Separation of Ethyl acetate with Chromatography

Separation technique performed by using Vacuum Liquid Chromatography is the first process to obtain secondary metabolites.

In this technique a column of 9.5cm diameter and 5 cm was used with tools such as a vacuum pump. Previously, the concentrated *ethyl acetate* extracts using silica gel Merck 60 (mesh). Eluent used in this process was 100% n-hexane, n-hexane: *ethyl acetate* in the ratio of n-hexane : ethyl acetate = 6 : 4 Rf 0.4 ; acetone : ethyl acetate= 4 : 1 Rf0.875; and dichloromethane : acetone= 9 : 1 Rf 0.425.

The subsequent separation process was conducted the gravity column chromatography. Fraction I results which have been concentrated and then impregnated with silica gel Merck 60 (200-400 mesh). The column used for chromatography is 30 cm in length with a diameter of 2.5 cm. Eluent used in this process a mixture of n-hexane: *ethyl acetate* in a ratio of 6:4.

The generated eluent was then collected in a 20 mL bottle fraction. This process resulted in 60 bottles of fractions, and each fraction was then identified using Thin Layer Chromatography (TLC) with an interval of 3 bottles. Fractions were grouped by determining the similar value of Rf; thus 6 groups of fractions, i.e. F1 (3-7), F2 (8-12), F3 (13-17), F4 (18-20), F5 (21-40), and F6 (41-60) were obtained. Of the six groups of the fraction, fraction F6 (41-60) was concentrated.

Fraction F6 (bottles 41-60) was concentrated further separation by TLC because the results have not shown single spot. The fraction of silica gel was impregnated using Merck 60 (mesh). The separation was carried out using n-hexane eluent: *ethyl acetate* in the ratio of 6: 4. The generated eluent was accommodated within \pm 5 mL bottle fractions and fractions obtained 32 bottles. Each fraction was further identified using Thin Layer Chromatography (TLC) with the interval of one bottle.

Based on the similarity value of Rf, all fractions were grouped into two groups, namely F(6A) fraction (no. 1-12) and F(6B) (no. 13-32). At TLC chromatogram, F6-A (1-12) showed a single spot so that the group of this fraction was further concentrated for the purity test.

The process of gravity column chromatography was carried out to obtain the components of secondary metabolite compounds in chloroform fraction of *Tithonia diversifolia* leaf which is simpler. The column used here was with a diameter of 2 cm. A total of 1.00 g concentrated extract chloroform of *Tithonia diversifolia* leaf that had been diluted with a little mixture of eluent n-hexane: ethyl acetate (6:4) and the stationary phase in the form of a mixture of silica gel Merck 60 (200-400 Mesh), resulting in 60 bottles of fractions.

The separation pattern of the whole fraction bottles was observed using TLC under UV light using the same eluent mixture. Bottles with the same separation pattern and Rf value can be combined and grouped, thus resulting in three groups of fractions.

Based on the TLC chromatogram purity test, the results showed a single stain on every kind of eluent (Table 2).

Table 2. Eluent and Rf Value.

Figure	Eluent	Rf value
A	n-hexane : ethyl acetate (6 : 4)	0.4
B	acetone : ethyl acetate (4 : 1)	0.875
C	dichloromethane : acetone (9 : 1)	0.425

From the results it can be concluded that compound 2 separation results in a relatively high purity that can be further analyzed by UV-Vis, IR spectrophotometer, and GC-MS spectrometers.

3. Data Analysis

UV-Vis Spectrophotometer

UV-Vis spectrophotometer used to determine the maximum wavelength of compounds under analysis. The results of measurements of these compounds in methanol showed the maximum absorption of wavelength at 220.80 nm and 400.20 nm.

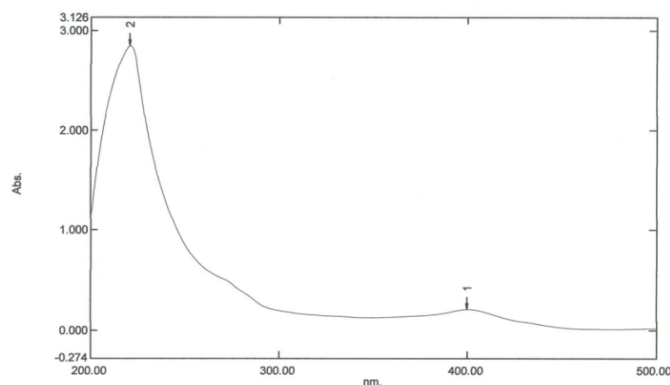


Figure 2. UV-Vis spectra of isolated compounds.

IR Spectrometer

Infrared spectroscopy (IR) used to determine the presence of functional groups contained in the isolated compounds. The analysis of IR spectra of isolated compounds are shown in Table 3.

Table 3. Results of the analysis of the IR spectra.

Wavelength(cm^{-1})	Estimated Group
3464.15	O-H Carboxyl
2970.38 ; 2931.80 and 2877.79	C-H aliphatic
1743.65	C=O Carbonyl
1458.18 and 1381.03	C-H aliphatic
1265.30	C-O ester

GC-MS Spectroscopy

The GC-MS spectroscopy method was used to determine the molecular weight and structure of the isolated compounds. The result of analysis with GC-MS spectroscopy is shown in Figure 3, while the GC-MS chromatogram data of isolated compounds are presented in Table 4.

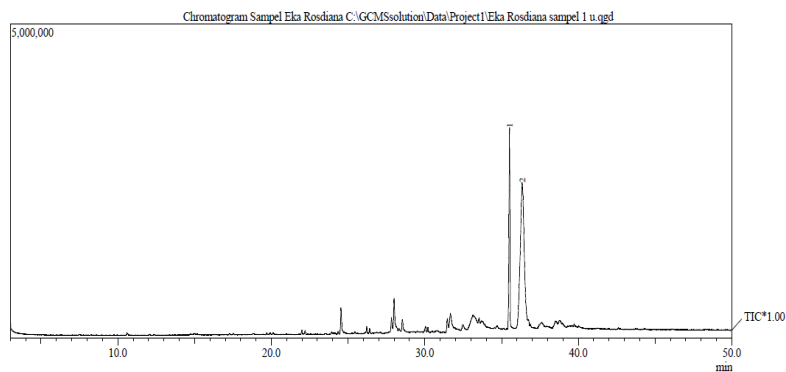
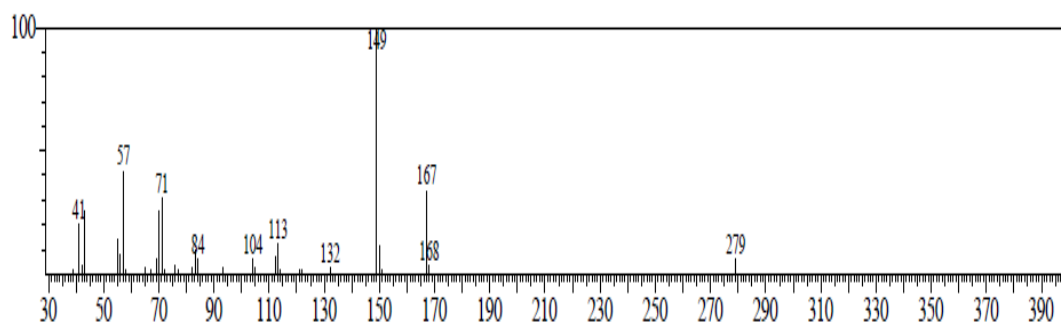


Figure 3. GC-MS Spectroscopy of isolated compounds.

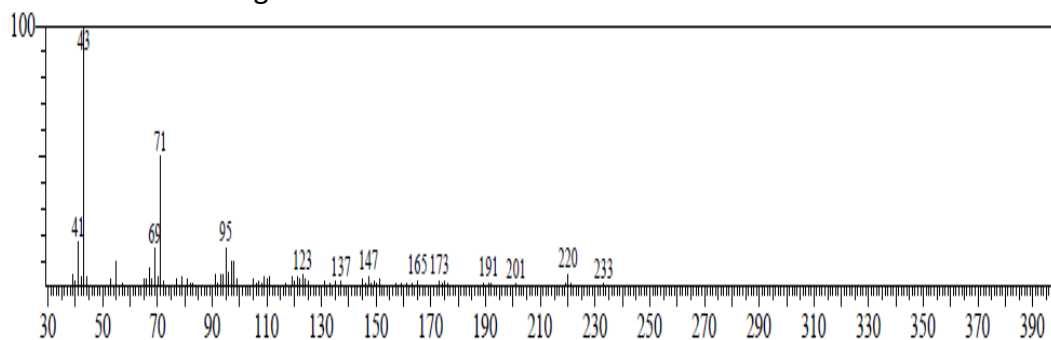
Table 4. GC-MS Chromatogram Data of isolated compounds.

Peak no	Rt (second)	% Area	Estimated compound	SI
1	35.520	30.47	bis (2-ethyl-hexyl) phthalate	95
2	36.347	69.53	estran-3-one, 17-(acetyloxy)-2-methyl-(2 α .,5 α .,17 β)	75

Based on the above chromatogram, the mass spectra of peak 1 has a base peak of 149 with a molecular weight of 390 and indicates the similarity index (SI) with the fragmentation patterns of bis (2-ethyl-hexyl) phthalate compound of 95. The mass spectrum of the isolated compound is shown in Figure 4.

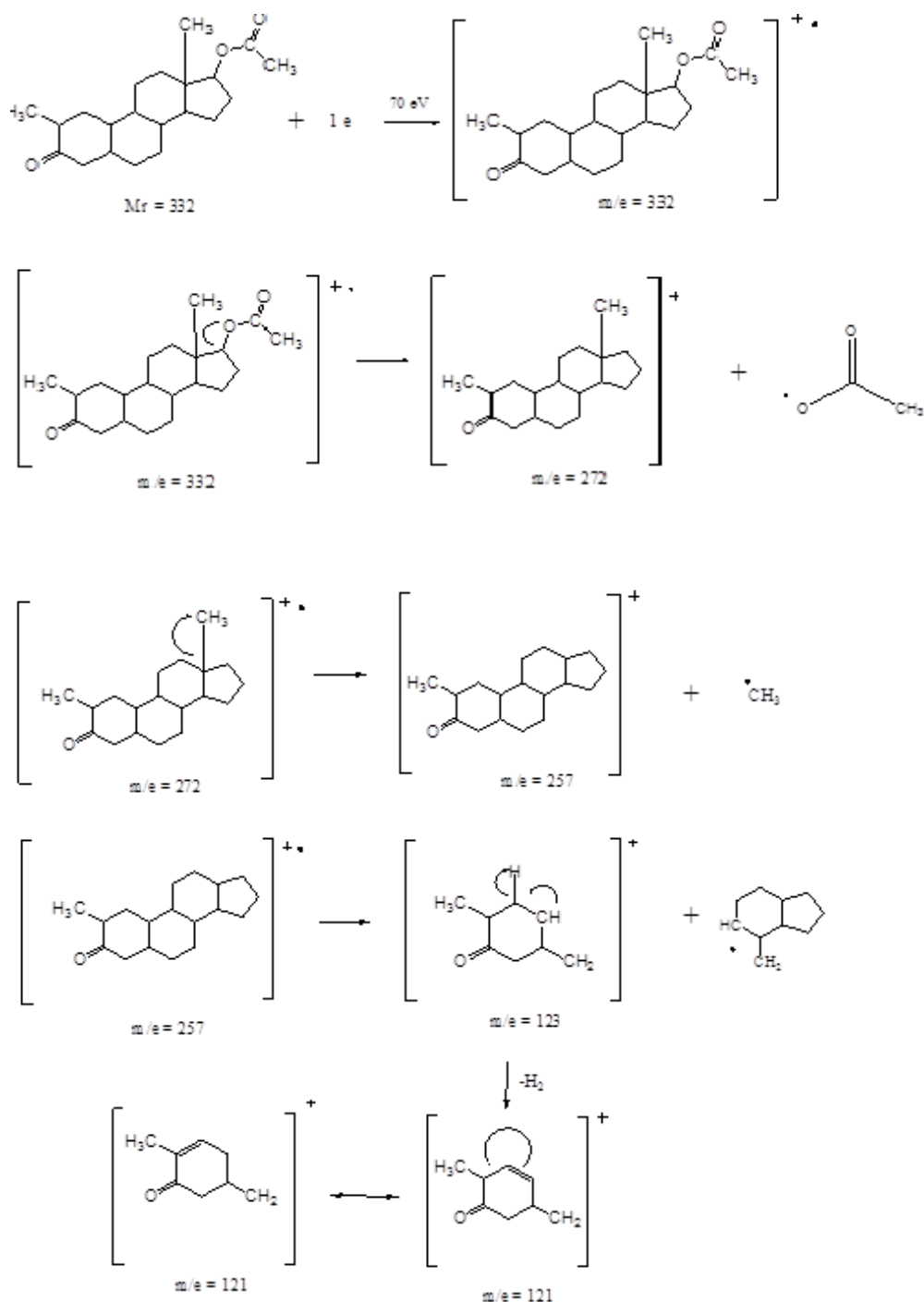
**Figure 4. Mass Spectrum of Peak 1 of the isolated compound.**

The mass spectrum of Peak 2 has a base peak of 43 with a molecular weight of 332 and indicates the similarity index (SI) with the fragmentation patterns of estran-3-one, 17-(acetyloxy)-2-methyl-(2 α .,5 α .,17 β) compound of 75. The mass spectrum of the isolated compound is shown in Figure 5.

**Figure 5. Mass Spectrum of Peak 2 of the isolated compound.**

The aim of this analysis with GC-MS spectroscopy is to determine the purity of isolated compound and determine the molecular weight fragment of its functional group. GC-MS spectroscopic data generated from the isolated compound two peaks. The highest peak is the Peak 1 with an abundance of 30.47% and a retention time of 35.520 minutes and has the m/e of 390. Peak 2 has an abundance of 69.53% with a retention time of 36.347 minutes and has the m/e of 332. Peak 2 is then the dominant compound because peak 2 has a greater abundance percent age than Peak 1.

Fragmentation Patterns of Peak 2



Regarding the mass spectra (M^+) 390 in Peak 1 and (M^+) 332 in Peak 2, there is no peak in mass spectra; this is because the particles had shorter life time and had no time to reach the ion collector so that molecule could not be detected and only the products of fragmentation showed the peak in mass spectra (Fessenden and Fessenden, 1986).

Peak 2, with an abundance of 69.53% and m/e of 332, has similarities with 17 β -(acetyloxy)-2 α -methyl-5 α -estran-3-one. This is supported by the fact that SI of 75 as well as the fragmentation pattern and m/e mass spectra which appeared were similar to the fragmentation pattern and m/e mass spectra of 17 β -(acetyloxy)-2 α -methyl-5 α -estran-3-one. However, in this case, the similarity index was not very big.

Peak 1, with an abundance of 30.47% and m/e of 390, has similarities with bis (2-ethyl-hexyl) phthalate. This is supported by the fact that SI of 95 as well as the fragmentation pattern and m/e mass spectra which appeared were similar to the pattern of fragmentation and m/e mass spectra of bis (2-ethyl-hexyl) phthalate.

The fragmentation patterns of isolated compounds found in the analysis were 17 β -(acetyloxy)-2 α -methyl-5 α -estran-3-one and bis (2-ethyl-hexyl) phthalate. The fragmentation patterns of 17 β -(acetyloxy)-2 α -methyl-5 α -estran-3-one are 332 (M^+), 272, 257, 121, 94, 81, and 43. The fragmentation patterns of 17 β -(acetyloxy)-2 α -methyl-5 α -estran-3-one are shown in Figure 6.

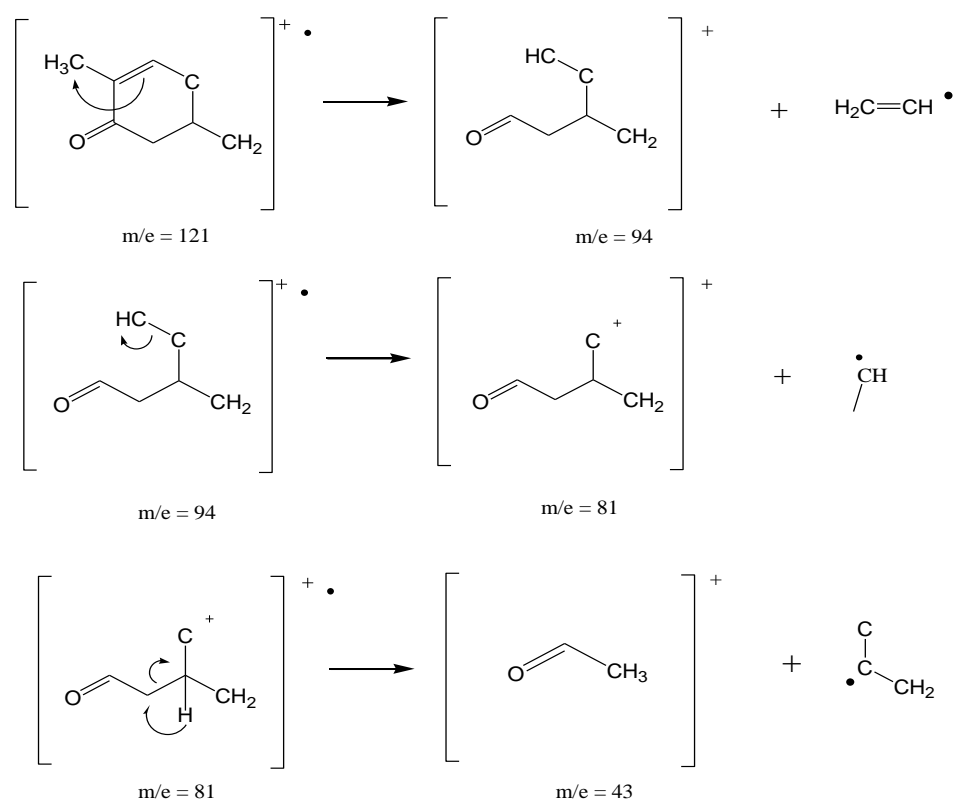


Figure 6. Fragmentation Patterns of 17 β -(acetyloxy)-2 α -methyl-5 α -estran-3-one.

Meanwhile, the fragmentation patterns of bis (2-ethyl-hexyl) phthalate are 390 (M^+), 279, 168, and 149. The fragmentation patterns of bis (2-ethyl-hexyl) phthalate are shown in Figure 7.

Fragmentation patterns of Peak 1

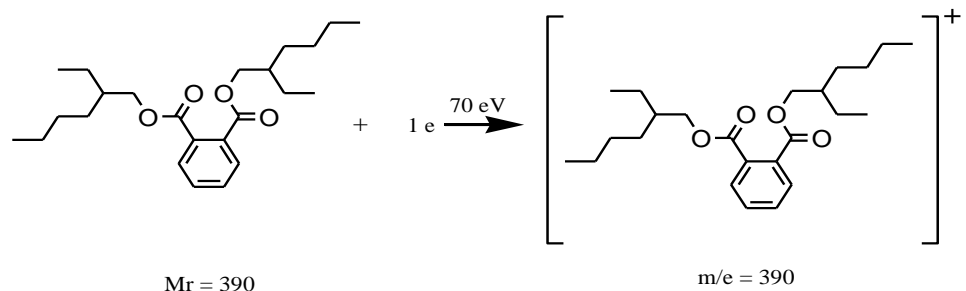


Figure 7. Fragmentation of Peak 1.

The extraction method in this study is macerated by dissolving the powder with methanol solvent. Methanol was chosen to be the solvent because it is widely used in the extraction process and is able to dissolve all the preliminary and secondary metabolites. In addition, methanol also has a low boiling point of 64.7°C, so that it will be easily evaporated.

As much as 0.5 kg *Tithonia diversifolia* leaf powder was macerated using approximately 1 liter technical methanol for 2 x24 hours long, while being shaken occasionally. The powder that has been macerated remaserasi process is done only once at the same time. The methanol extract was then separated from *Tithonia diversifolia* leaf powder and concentrated using Buchii evaporator to obtain a concentrated methanol extract as much as 215 mL.

Separating the Chloroform Extract using Gravity Column Chromatography (GCC) Method

The separation process was conducted by using gravity column chromatography (GCC). Before processing, the column should be packed first, and the extract used should be as much as 1.00 grams, because the diameter of the column was just 2 cm wide. The solvent used in this process was selected by *trial and error* method, with two solvents mixed by TLC. The TLC result edinan appropriate eluent i.e. a mixture of n-hexane and ethyl acetate in a ratio of 6: 4. In 20 bottles of fraction were obtained from this process, each of which were then identified using the TLC, eluted using a mixture of n-hexane and ethyl acetate with the ratio 6: 4.

Furthermore, the concentrated methanol extract was partitioned using chloroform. The partitioning process aimed to separate compounds based on the polarity of the more-specific secondary metabolites. The concentrated methanol extract was partitioned using n-hexane and then the rest of the methanol in this partition was again partitioned using chloroform. The tools used in this partitioning process was separating funnel. The partitioning process was repeated three times, each with the ratio of 6:4 between n-hexane and ethyl acetate. The partitioning process resulted in as much as 215 mL ethyl acetate fraction. Then this fraction is evaporated to produce concentrated chloroform fraction as much as 110.6 mL.

The next stage was the separation process using chromatographic techniques with Vacuum Liquid Chromatograph, Gravity Column Chromatography (GCC), and Thin Layer Chromatography (TLC). Prior to the separation, the *ethyl acetate* fraction concentrated in advance using silica gel was impregnated intended that the compound in the fraction bound to the silica gel so that secondary metabolites can be eluted only with a suitable solvent in the separation process with GCC. Thin Layer Chromatography (TLC) function to determine the appropriate eluent to be used in the process of GCC, as well as to test the purity of the isolated compounds.

The TLC resulted in the appropriate eluent, i.e. a mixture of n-hexane and ethyl acetate with a ratio of 6:4. This process generated 20 bottles of fraction, which were then identified with TLC from each bottle of fraction using a mixture of n-hexane and ethyl acetate with the ratio of 6:4. The 20 bottles of fraction resulted from TLC were grouped based on the pattern of separation and the same value of R_f, so that there were 5 groups/fractions, namely I (1-2), II (3-4), III (5-8), IV (9-13) and V (14-20). Group/fraction V (14-20) was assumed to produce a single node so that this group of bottles was identified to be further examined.

From fraction V (14-20), the results were then gathered into one and dried, in order to obtain as much as 0.1 gram. This fraction showed a single node on the chromatogram, so that a further test for purity using TLC with three kinds of eluent i.e. n-hexane: ethyl acetate (6:4) (Figure A), acetone : ethyl acetate (4:1) (Figure B), and dichloromethane : acetone (9:1) (Figure C). The chromatogram as a result of purity test is shown in Figure 13.

Separation with Vacuum Liquid Chromatography clicking to use tools such as a vacuum pump to accelerate the rate of the eluent. Eluent used include 100% n-hexane, n-hexane: *ethyl acetate* with the ratio of 6:4; 4:1; 9:1, *ethyl acetate* 100%, and acetone 100%. Eluent-column eluent is passed on by the increase in polarity then vacuum and collected in bottles fractions. Thus obtained 20 bottles fraction, the fraction of each bottle is identification using Thin Layer Chromatography (TLC) with a mixture of n-hexane eluent: *ethyl acetate* in a ratio of 6:4. Based on the results of TLC R_f price similarity then 8 bottles fractions were grouped into two fractions, namely fraction A, fraction B and Fraction C. Based on the chromatogram, selected fractions A for further separation using column chromatography Gravity 1.

The separation process with CCG 1 aims to obtain more pure fractions with a single stain on the TLC plate. Eluent used in the separation process is a mixture of n-hexane: *ethyl acetate* in a ratio of 6:4. This process produces 20 bottles fraction, then be identified using TLC techniques. Based on the results 1 TLC chromatogram, taken one of the most dominant fraction group and have the same price of R_f (0.2) is the fraction F6A. These groups combined and concentrated fractions and tested for purity using TLC techniques. The purity of the test is known that has not produced a single stain on a TLC plate. Therefore, this fraction is separated group returned with techniques 2.

Separation process of CCG2 using the eluent a mixture of n-hexane: *ethyl acetate* in the ratio of 6:4. In this separation process produces 20 bottles of fractions, then identified using TLC techniques. Furthermore, the fractions were grouped by similarity price R_f and obtained two groups of fractions. Then the selected group of the most dominant fraction is the fraction of R_f 0.45. These groups combined and concentrated fractions and tested for purity using TLC techniques.

Test purity by TLC technique using the eluent mixture of chloroform with 3 kinds of comparisons among others (6:4), (4:1), (9:1). The value of R_f calculations on this test can be seen in Appendix 2. Relatively pure compounds in the yellow-green room temperature.

Analysis using UV-Vis, IR spectrometer, and GC-MS spectrometer

Analysis with UV-Vis spectrophotometer aimed to investigate the electronic transition and the chromophore group in the identified compounds. Chromophore group is a functional group that can absorb ultraviolet radiation near the visible region.

Solvent used in this analysis was methanol, since methanol can dissolve the sample well and do not absorb ultraviolet radiation. The results of the analysis using UV-Vis spectrophotometer showed the maximum wavelength (λ_{maks}) at 402.0 nm and 664.5 nm.

The Infrared (IR) spectrometer analysis aimed to determine the functional groups contained in the isolated compounds. Based on the IR spectra of isolated compounds showed an -OH group at 3429.59 cm^{-1} , CH aliphatic at 2951.62 cm^{-1} and 2839.31 cm^{-1} , group C=C in $1644,01\text{ cm}^{-1}$, -CH₃ in 1405.86 cm^{-1} , and C=O at 1018.12 cm^{-1} .

CONCLUSION

Based on the discussion above some conclusions can be drawn as follows:

1. Analysis compound in the leaves *Tithonia diversifolia* using a UV-Vis spectrophotometer showed the maximum wavelength (λ_{maks}) at 220.80 nm and 400.20 nm. Based on the analysis of IR spectrometer it is known that the compound contains -OH group, aliphatic CH, the C=O ester group, and the C=C double bond.
2. Mass spectrum of GC-MS shows the m/e value of 332 which is similar to 17 β -(acetyloxy)-2 α -methyl-5 α -estran-3-one with the abundance of 69.53%. The secondary metabolite compound which can be isolated from chloroform fraction of *Tithonia diversifolia* leaves is steroid.

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