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The Isolation and Characterization of Phenolic Compound of Euphorbia Plant/Patikan Cina (*Euphorbia thymifolia* Linn)

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Abstract. Euphorbia plant, *Euphorbia thymifolia* Linn, is one the Euphorbiaceae families that still need to be developed due to its benefits. An effort to be able to use this plant maximumly is by studying the active component in the plant. This study aims to isolate and characterize phenolic compound in euphorbia plant extract using ethyl acetate. 500 gram of euphorbia plant (Patikan Cina) powder was macerated using methanol. Then, the macerated extract was evaporated to eliminate excess solvent and as a result, a solvent-free extract was obtained. Next, the result was partitioned with a solution of methanol : ethyl acetate (1:1) in order to get methanol and ethyl acetate extracts. The ethyl acetate extract of the partition was evaporated to get concentrated ethyl acetate extract. Next, it was isolated in a vacuum liquid and gravitational column chromatography to get pure isolate. Silica gel 60 (0.040 – 0.063 mm) and n-hexane motion phase were used in the separation of concentrated ethyl acetate in vacuum liquid chromatography. There were 16 fractions produced from the yield. Then the fractions were put in TLC. With silica gel 60 (0.2 – 0.5 mm) mesh and n-hexane mobile phase, ethyl acetate gave 5 fractions in gravitational column chromatography and fraction 3 produced a single spot. UV and IR spectroscopy were used to determine the constituents in the isolate. From UV spectrum, λmax is 268.97 nm. While IR data shows the presence O-H group in 3521.38 cm^{-1}, C – H (alkane) presents at wave number 2926.45 cm^{-1} reinforced by the appearance of 5 other alkanes absorption at 2857.00 cm^{-1}, 1447.31 cm^{-1}, 1370.18 cm^{-1}, 868.774 cm^{-1} and 757.887 cm^{-1} wavelengths. There is also C = O (carbonyl) groups in wave number 1693.19 cm^{-1}. The identification of structures based on UV and IR spectra data has shown that the isolate is a phenolic compound.

Keywords:*Euphorbia thymifolia* Linn, isolation and characterization, phenolic

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1 Introduction

Medicinal plants can be used as traditional medicine to cure various diseases. Since a long time ago, medicinal plants have been used as an alternative to chemical drugs that are relatively expensive by the people in Indonesia, both in the city and countryside, to treat the diseases they are suffering from.

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Indonesia has a tropical climate and has an abundant supply of medicinal plant. There is a lot of plants that can be used as medicine to cure various of diseases, one of them is euphorbia plant, Patikan Cina (*Euphorbia thymifolia* Linn).

The existence of the plant in the nature seems to not receive enough attention from the people. Although it is a wild plant, Patikan Cina has the potential to be a medicinal plant. People who live in the countryside usually use all the parts of Patikan Cina plant for bacillary dysentery, typhus, herpes zoster, enteritis, diarrhea, bleeding hemorrhoids, eczema and dermatitis (Utami, 2008).

Based on the background described, the author meant to do a research for "The Isolation and Characterization of Phenolic Compound of Euphorbia Plant/Patikan Cina (*Euphorbia thymifolia* Linn)". The objectives of this study are:

1. To isolate phenolic compound from Patikan Cina (*Euphorbia thymifolia* Linn) plant using methanol extraction

2. To characterize phenolic compound from Patikan Cina (*Euphorbia thymifolia* Linn) plant

Patikan Cina (*Euphorbia thymifolia* Linn) is still in one family with Patikan Kerbau, which is in Euphorbiaceae family. Patikan Cina is a small herbaceous plant that creeps or sometimes half upright. The stems and leaves are reddish and secretes sap when broken. The leaves are ovoid, with even fins, located face to face and have good smells. The flower is light pink (Utami, 2008; Supandiman,2000). Patikan Cina is used to cure dysentery, hemorrhoids, anemia, wound, swollen and dirty blood. All parts of the plant can be used to cure diarrhea and wormy because of roundworms (Handayani, 2003; Permadi, 2006).

2 Materials and Methods

Sample used was all parts of Patikan Cina (*Euphorbia thymifolia* Linn) plant. The equipment used in this study was organic chemical equipment, distillation set, Thin Layer Chromatography set, evaporator, Liquid Column Chromatography set, Gravity Column Chromatography set, hotplate, UV light, laboratories glassware, filter paper and camera. In vacuum liquid chromatography, silica gel used was Merck 60 (0.040 0 0.63 mm) while in gravity chromatography column silica gel used was Merck 60 (0.2 – 0.5 mm). Next TLC analysis used 60 F254 (0.25 mm, Merck). Spots were observed under the UV light (254 mm – 365 mm) and the reagent to show the spot was added. While the material used in this study is all part of Patikan Cina plant, methanol, ethyl acetate, n-hexane, chloroform, aquadest and cerium sulfate to show the spots.

The method used in this study: 400 gram powder of all parts of Patikan Cina plant macerated in 2.5 L of methanol for more or less 3 days. After that, it was filtered with filter paper to get

extract methanol sample (filtrate). The filtrate was evaporated in a rotary evaporated and partitioned in n-hexane with a ratio of 1:1 for 3 times. Before the separation was conducted, sample had been analyzed using thin layer chromatography (TLC). This approach is to know the right eluent to be used in vacuum liquid chromatography and gravity chromatography column in order to get pure isolate.

The pure isolate obtained was then analyzed with UV spectroscopy to confirm the existence of conjugated double bonds and to determine the chromophore in the compound. Then IR spectroscopy was used to determine the type of functional groups in bioactive compound isolated.

3 Results and Discussion

Maceration extraction method was used because this method does not have heating process involved, therefore the plant components are not destroyed. The solvent used in this research was methanol as methanol is a universal solvent that is able to dissolve both polar and non-polar compounds. Moreover, methanol has a low boiling points hence evaporates easily. Besides that, methanol is economical. 1.5L of methanol extract was evaporated to remove excess solvent and to obtain concentrated extract. The result obtained is 27.93 gram. Methanol extract was partitioned with a ratio of 1:1 methanol and ethyl acetate. After that ethyl acetate partitioned was evaporated and 10.02 gram concentrated ethyl acetate extract was obtained. The analysis of the isolate with UV spectroscopy can be seen in the picture below:

The UV spectrum of ethyl acetate fraction shows absorbance peak at 268.97 nm wavelength. It can be suspected that there is a transition from n - σ*. Based on the energy needed from electrical transition, absorbance bigger than 185 nm automatically means that energy is smaller than 150 kcal with an electron transition happens from n - σ* (Fessenden dan Fessenden, 1982). Isolate analysis using infrared spectroscopy is shown in the picture below:

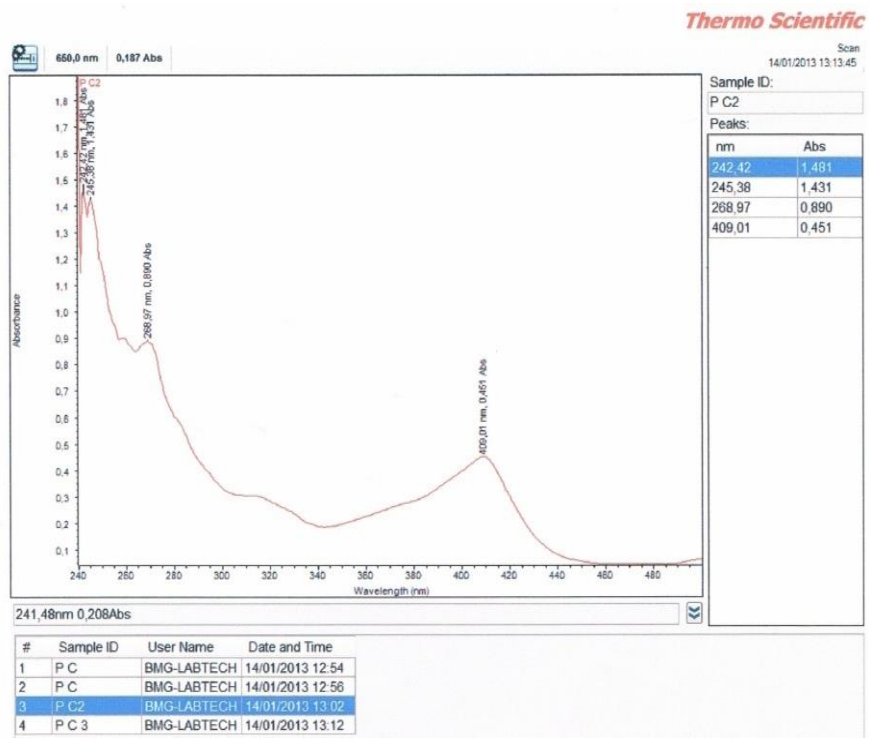
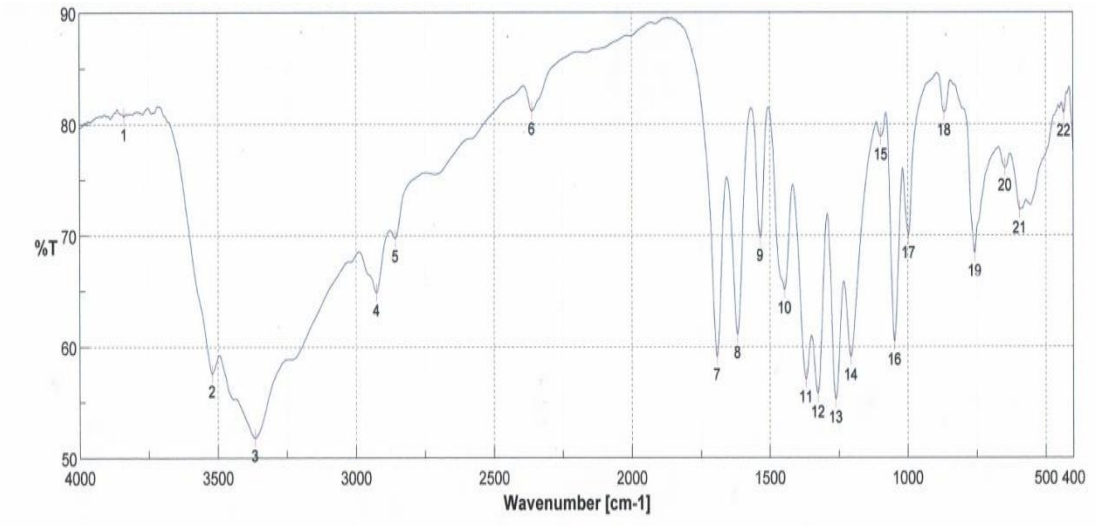


Figure 4.1 UV Spectrum



Accumulation	16	Resolution	4 cm-1
Zero Filling	ON	Apodization	Cosine
Gain	Auto (4)	Scanning Speed	Auto (2 mm/sec)
Date/Time	11/22/2012 4:43PM	Update	11/22/2012 4:45PM
Operator	rina		
File Name	Unja 3		
Sample Name	PC 3		
Comment			

No.	cm-1	%T	No.	cm-1	%T	No.	cm-1	%T	No.	cm-1	%T	No.	cm-1	%T
1	3838.61	80.7025	2	3521.38	57.6096	3	3367.1	51.8372	4	2926.45	64.888	5	2857.99	69.7763
6	2362.37	81.203	7	1693.19	59.1386	8	1617.98	61.0999	9	1535.06	69.8331	10	1447.31	65.1187
11	1370.18	57.1045	12	1326.79	55.7532	13	1262.18	55.2977	14	1207.22	59.1152	15	1098.26	78.8982
16	1049.09	60.4801	17	997.982	70.0776	18	868.774	81.0839	19	757.887	68.44	20	647.965	76.0968
21	594.932	72.3022	22	433.905	81.0124									

Figure 4.2 Infrared spectrum

Infrared spectrum of ethyl acetate fraction above shows that there is a probability of several functional groups contained in the compound, such as O-H (carboxylic acid) in wavelength 3521.38 cm^{-1}, also there is O-H (alcohol, phenol (H bond)) in 2362.37 cm-1 wavelength. Whereas C-H (alkanes) is shown in 2926.45 cm-1 wavelength, reinforced by the existence of 5 absorbance areas of C – H in 2857.99 cm-1, 1447.31 cm-1, 1370.18 cm-1. Also C - H (alkenes) are shown in 868.774 cm-1 and 757.887 cm-1. There is also C=O (carbonyl) functional group shown in 1693.19 cm-1 area and C – O (alcohol, ether, carboxylic acid and ester in 1098 cm-1 wavelentgh (Bresnick, 2003)

4 Conclusion

Based on the research and discussion elaborated, it can be concluded that phytochemistry test of Patikan Cina (*Euphorbia thymifolia* Linn) isolates positively contains phenolic compound with black color appearance after the addition of $FeCl_3$. Based on the data of UV and IR spectra, the compound isolated is a phenolic compound with the characterization of O – H, C – H, C = O and C – O functional groups.

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