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Isolation and Characterization of Scopoletin from The Bark of *fagraea ceilanica thumb* and Antioxidants Tests

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***Abstract*— Have done the isolation scopoletin compound from the bark of fagraea ceilanica Thunb by maceration method using n-hexane, ethyl acetate and methanol. Column chromatography with silica gel used for the isolation of the ethyl acetate extract fraction with a mixture of n-hexane and ethyl acetate as the mobile phase in the SGP (step Gradient Polarity). This way produces a dark yellow crystals (26 mg) with a melting point = 205-207 ° C. TLC (Thin Layer Chromatography) were eluted using a mixture of n-hexane: ethyl acetate (7: 3) gives (Rf 0.42). Based on the UV spectrum, shift reagents, IR spectrum and melting point of this compound compared with standard scopoletin showed that the isolated compounds are the same compound or isolated compound is scopoletin. The results of antioxidant test with DPPH of isolated compounds as weak antioxidants with IC 50 = 358.71 mg / L.**

***Keywords*— Fagraea ceilanica Thunb, coumarin, DPPH, antioxidant.**

I. INTRODUCTION

Fagraea is a plant that has been used traditionally as a medicine, perfume, as well as ornamental plants. This plant is found widely in some parts of the world, such as in India, Southeast Asia, southern China, northern Australia and in the islands of the Pacific [1].

Several studies on the *fagraea* genus been reported to have a variety of chemical compounds contained in these plants, among chemical compounds that have been reported are lariciresinol, isolariciresinol, 7,8-dihydro-7-oxy-koniferil alcohol, methyl p-kumarat, methyl caffeate, syrinate methyl, methyl sinapate, and sweroside glucoside [2].

Coumarin compounds from the bark of *fagraea ceilanica Thunb*. has been carried out and proved that the bark of this plant positive for coumarin compounds. Therefore, further studies with coumarin compound isolated from the bark of *fagraea ceilanica Thunb*., And testing its for antioxidant activity.

II. MATERIAL AND METHODS

A. Chemicals, equipment and instrumentation

The chemicals used include hexane , ethyl acetate , methanol , silica gel 60 , TLC plate (silica gel 60 F 254), Whatman filter paper No. 1, NaOH 5%, $FeCl_3$ 5%, and DPPH (1,1-diphenyl-2-pikrihidrazil), shift reagents = NaOAc, NaOH, $AlCl_3$, $AlCl_3$ + HCl and NaOAc + H_3BO_3.

The equipment used are a set of distillation apparatus, rotary evaporator (Heidolph Laborota 4000), Melting Point (Stuart SMP10), ultraviolet visible spectrophotometer (Shimadzu UV-1700 PharmaSpec), infrared spectrophotometer (Thermo Scientific Nicolet is10), column chromatography and UV light (254 nm and 365 nm).

B. Research procedure

1) Sample Preparation, Samples of ceilanica Fagraea bark as many as 2500 g was taken in the campus of the University of Andalas, Limau Manis, Padang. Samples cut , dried, grindered until smooth, and weighed [3].

2) *Extraction*, Fine powder of bark fagraea ceilanica = 600 g macerated with hexane solvent for 3-4 days repeatedly. Results of maceration then filtered and concentrated in vacuo with a rotary evaporator to obtain a concentrated hexane extract. Subsequently, the sample was macerated with ethyl acetate and then methanol solvent in the same way and obtained concentrated ethyl acetate extract and the concentrated methanol extract.

3) *Purification*, Before further isolated, for each test extract TLC and coumarin content test with 5% NaOH on the TLC plate under UV light. Ethyl acetate extract is used for further purification using column chromatography with SGP system (Step Gradient Polarity) and preparative paper chromatography [4]. Furthermore eluted with different polarity solvent, starting from hexane, hexane - ethyl acetate, ethyl acetate solvent. TLC test of column chromatography

obtained 13 fractions are A-M fractions. Preparative paper chromatography with 15% acetic acid showed a coumarin positive of H Fraction. From the results of purification, obtained isolated compounds that provide a single stain bright purple (Rf = 0.42) with eluent hexane: ethyl acetate (7: 3) and *Fluorescence* even brighter after sprayed with NaOH 5% under 365 nm UV light.

4) The purity test of isolated compounds, The isolated compounds was measured melting point, then TLC test with a variety polarity of the eluent and revealed with 5% NaOH reagent.

5) Characterization, Isolated compounds were characterized using UV, IR spectrophotometer and melting point.

6) *Antioxidant Activity Test Methods*, 2,2-diphenyl-1-picrylhydrazyl (DPPH) was weighed as much as 3.94 mg, diluted to 100 mL with methanol. As many as 3 mL pipette solution, put in vials and added 1 mL of methanol. The solution was allowed to stand for 30 minutes in dark place, then the absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 517 nm and used as control absorbance. Each hexane extract, ethyl acetate extract and methanol extract weighed 10 mg, diluted to 10 mL with methanol and obtained a concentration of 1000 mg / L and made various concentration. The hexane, ethyl acetate and methanol extract made into 50, 100, 150, 200 and 250 mg / L. Also isolated compounds was measured antioxidant activity in solution concentration 20, 40, 60, 80 and 100 mg / L. Do the same way, the absorption solution is measured. Antioxidant activity is determined by the amount of free radicals barriers [5].

III. Result and Discussion

A. Analysis of isolated compounds

Table I shows the data obtained from the melting point determination of standard Scopoletin [6] & isolated compound . Melting Point of Isolated Compound and Standard Scopoletin are same.

TABLE I
MELTING POINT OF STANDARD SCOPOLETIN AND ISOLATED COMPOUND

Compound	Melting Point ^{0} C
Scopoletin (Standard)	200-207
Isolated Compound	205-207

From the results of the TLC test in different variations of the eluent polarity (Table II), compounds still provide single spot (Rf = 0.42) with eluent hexane: ethyl acetate (7: 3) and fluorisensi even brighter after sprayed NaOH 5% under 365 nm UV light.

TABLE III
RESULT OF TLC TEST OF ISOLATED COMPOUND WITH VARIETY OF ELUENT POLARITY

No.	Eluen	Rf
1.	heksana : etil asetat (7 : 3)	0,42
2.	heksana : etil asetat (5 : 5)	0,62
3.	heksana : etil asetat (3 : 7)	0,82

The results obtained by UV spectroscopy of isolated compound & standard Scopoletin, in methanol and after addition of NaOAc or NaOH indicate the λ value as given in Table III and that can be seen in Figures 1, 2 , 3 , 4 and 5.

TABLE IIIII
THE DATA FROM UV SPECTRUM OF STANDARD SCOPOLETIN [6], [7] AND ISOLATED COMPOUND

Sample	λ max (nm)		
	Me OH	After additional NaOAc	After additional NaOH
Standard Scopoletin	344.40 295 252 228	391 277	390.29
Isolated Compound	345 295.80 252 228.80	391.40 276.60	391.60

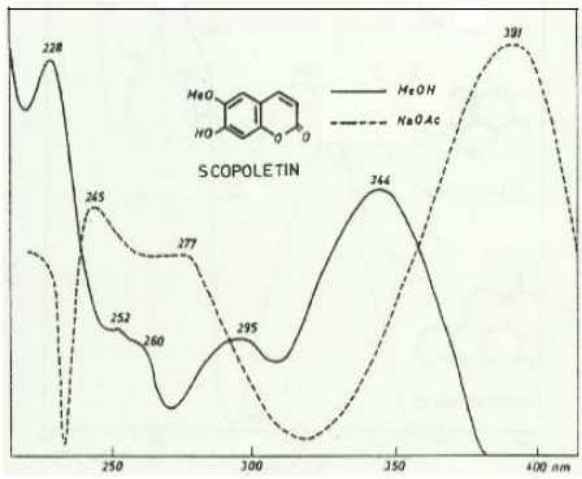

Fig.1. UV spectrum of standard scopoletin in MeOH and NaOAc,

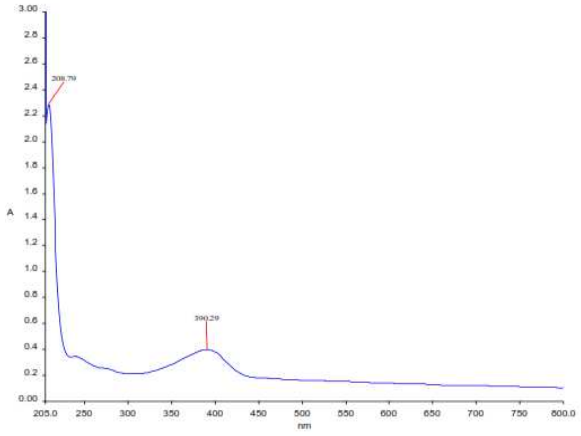

Fig.2. UV spectrum of Standard Scopoletin in Methanol after addition NaOH

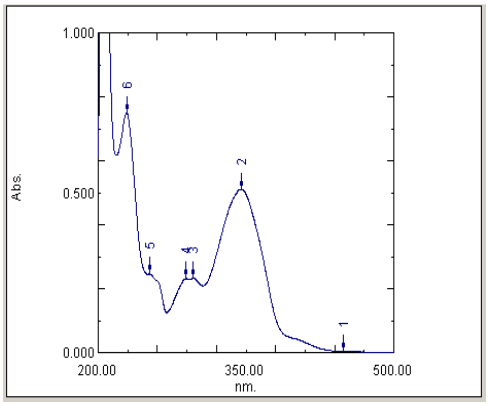

Fig. 3. UV spectrum of the isolated compound in MeOH

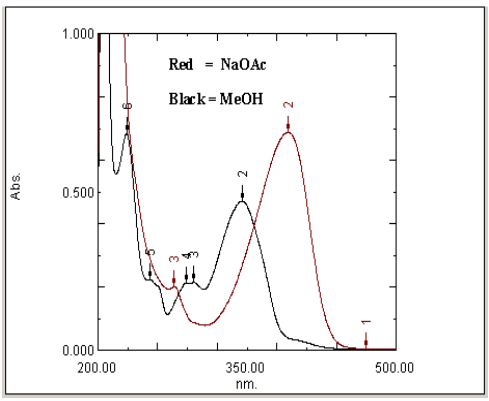

Fig. 4. UV spectrum of the isolated compound in MeOH and NaOAc

After addition of NaOAc in Methanolic solution and NaOH in Methanolic solution of Isolated Compound, bathochromic shift was observed which also confirms that Isolated Compound is Scopoletin, that can be seen in Figures 4 and 5.

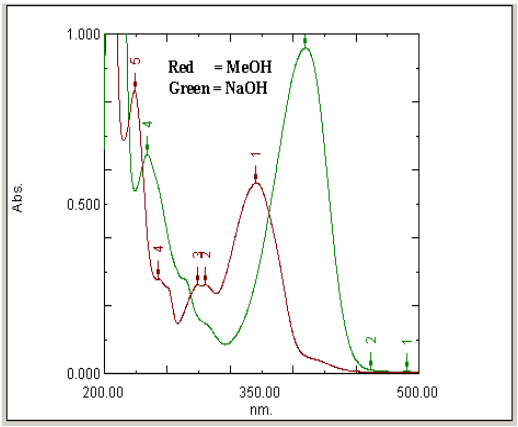

Fig. 5. UV spectrum of the isolated compound in MeOH and NaOH

The addition of reagents sihft of $AlCl_3$, $AlCl_3$ + HCl and NaOAc + H_3BO_3 not show bathochromic shift, this indicates the absence the ortho-di OH group in the compound, that can be seen in figure 6, 7 and 8.

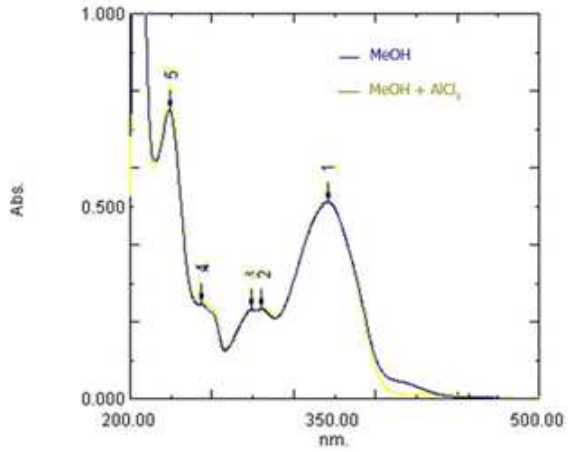

Fig. 6. UV spectrum of isolated compounds MeOH, MeOH + AlCl3

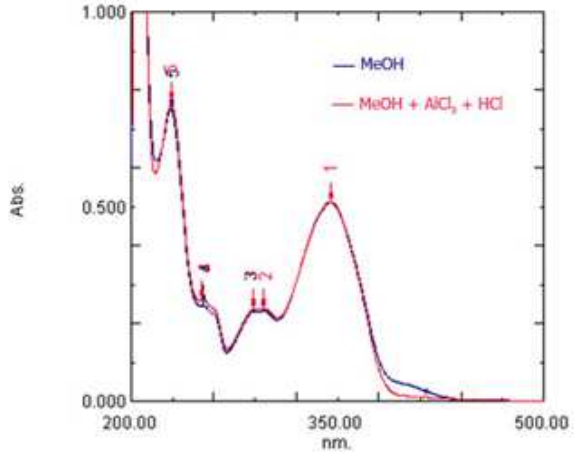

Fig. 7. UV spectrum of isolated compounds MeOH, MeOH + AlCl3 + HCl.

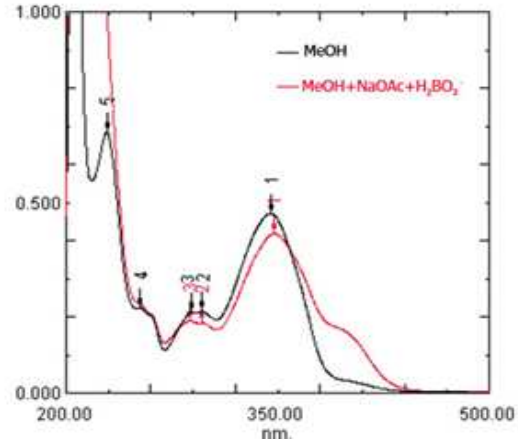

Fig. 8. UV spectrum of isolated compounds in MeOH + NaOAc + H_3BO_3.

Table IV shows the data obtained from FTIR Spectroscopy and possible functional groups present. In the IR spectral analysis, the Peak at 3337.44 & 3318 cm-1, a band is most probably the result of O-H stretching vibrations of phenol OH group. The peak at 2850.97 - 2990.45 & 2926 - 2990 cm^{-1} showed C-H Streching due to –CH. The peak at 1702.90 & 1698 cm^{-1} indicates the presence of -C=O, Carbonyl group. The peak at 1608.09, 1565.06, 1510.53 & 1602, 1567.68 , 1517.01 cm^{-1} indicates the presence of

benzene ring in both standard Scopoletin and isolated compound respectively and that can be seen in Figure 9 & 10. The above Comparision with standard confirms that isolated compound is Scopoletin.

TABLE IVV
THE DATA FROM FTIR SPECTRUM OF STANDARD SCOPOLETIN [6] AND ISOLATED COMPOUND

Peaks (cm^{-1})		**Functional group**
Standard Scopoletin	**Isolated Compound**	
3337.44	3318	O-H Alcohol group present
2850.97 - 2990.45	2926 - 2990	C-H group present
1702.90	1698	Carbonyl C=O group present
1608.09	1602	C=C *stretching* of aromatics
1565.06	1567.68	Benzene ring present
1510.53	1517.01	Benzene ring present

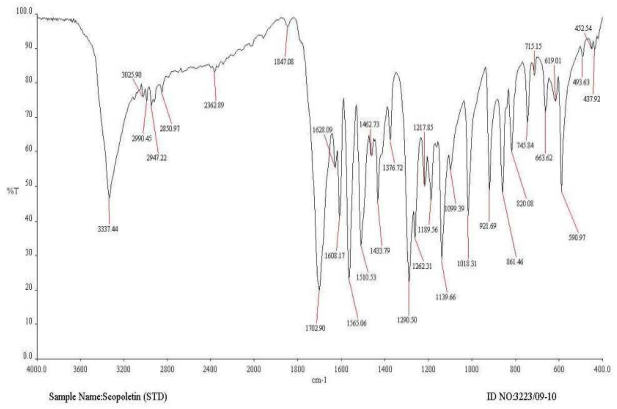

Fig. 9. IR spectrum of standard scopoletin

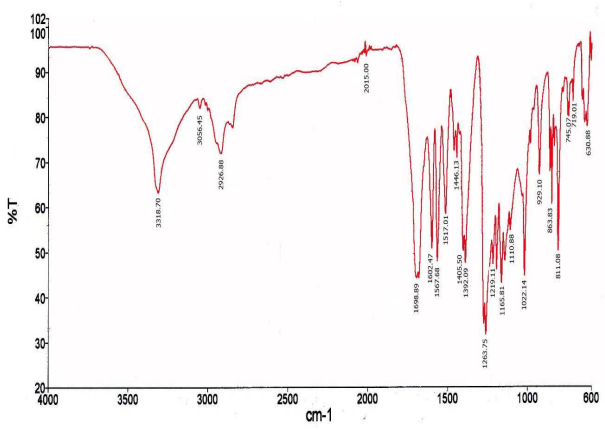

Fig. 10. IR spectrum of the isolated compound

Based on information obtained from the UV spectrum, sihft reagents, IR spectrum and Melting Point from isolated compounds compared to standard scopoletin showed that the isolated compounds are the same compound, namely scopoletin.

B. Antioxidant activity test

The antioxidant activity test of the extract of hexane, ethyl acetate extract, methanol extract and isolated compounds using DPPH method. The results of these tests obtained regression equation that can be seen in Figure 11 and 12.

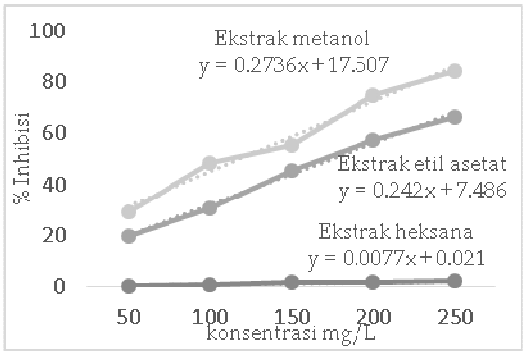

Fig. 11. Regression equation of antioxidant measurement by the DPPH method of hexane, ethyl acetate and methanol extract.

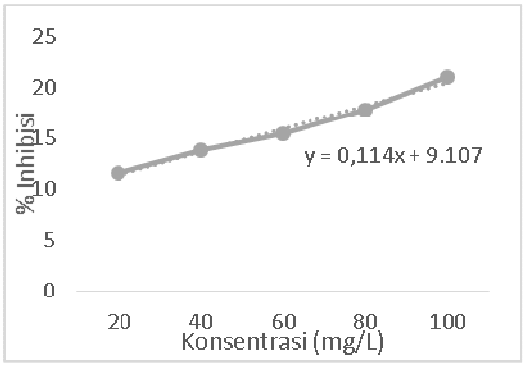

Fig. 12. Regression equation of antioxidant measurement by the DPPH method of isolated compounds.

The regression equation obtained the IC 50 values for methanol and ethyl acetate extracts had IC 50 respectively at 118.76 and 175.68 mg / L. As for the hexane extract is not active as an antioxidant with very large IC50 is 6490.78 Mg/L. For isolated compounds were classified as weak antioxidant activeness with IC 50 of 358.71 mg / L.

IV. CONCLUSIONS

From this study it can be concluded that the isolated compounds, from the ethyl acetate extract of *fagraea ceilanica thunb* is Scopoletin (Fig. 13), due to the measurement of UV spectroscopy, shift reagents, IR spectroscopy and melting point same as the standard scopoletin. These compounds are weak as an antioxidant activity with IC 50 values obtained at 358.71 mg / L.

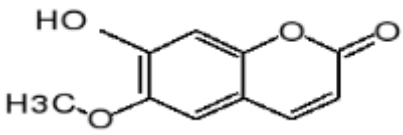

Fig. 13. Scopoletin

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