Chemical Constituens of Ethyl Acetate Fraction From The Methanol Extract of *Typhonium flagelliforme* Leaves, Araceae.

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Abstract

Typonium flagelliforme (Lodd.) Blume), familia Araceae, is one of the plant for anti cancer until there's so much research is done to know the contents of active compounds of the plants. This study was isolated and identification of chemical compounds from ethyl acetate fraction from Typonium flagelliforme. This research is continued from previous research which is done by phytochemical screening, the ethyl acetate fraction was isolated using Vacuum Liquid Chromatography, then the fractions were fractionated with column chromatography and the pure compound was identified by UV-VIS, FTIR, LCMS-MS and 1H-NMR, 13C-NMR, 2D-NMR, including HMQC, HMBC, DEPT and COSY. The result from the isolation of the ethyl acetate fraction T.flagelliforme (Lodd) Blume obtained 2 compounds, namely adenosine and adenine.

Keywords : *chemical constituents, Typhonium flagelliforme, ethyl acetate fraction, adenosine and adenine*

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INTRODUCTION

Indonesia is a tropical country that has the second largest biodiversity in the world after Brazil, has the natural resources that have not been fully utilized. Therefore, research of natural resources as sources of medicines has developed especially discovery of active compound in order to find new drugs, one of them as anticancer.

Keladi Tikus (T.*flagelliforme* (Lodd) Blume), familia Araceae, commonly known as the 'rodent tuber', is often included as an essential ingredient in various herbal remedies recommended for cancer therapies in Malaysia (Choo et al., 2001). This plant is widely used in traditional medicine in Southeast Asia to treat various diseases. This plant is used to soothe swelling, coughing and more predominantly for the treatment of cancer (Lee and Wong, 2004). anti-inflammatory, analgesic and sedative (Zhong et al., 2001); as antibacterial and antioxidant cctivities (Mohan, et al., 2008).

A number of plant secondary metabolites including flavonoids, saponins, alkaloids and terpenoids have previously been reported from this plant.

Several chemical constituents had been identified from *T.flagelliforme*. The hexane extract was reported to contain saturated hydrocarbons and aliphatic acids (Choo et al., 2001a), while the ethyl acetate extract was found to contain aromatic fatty acids (Chen et al., 1997). The aim of this research is to determine the active compounds of *T.flagelliforme* leaves and their bioactivities.

MATERIALS AND METHODS

General procedures

The UV spectra were recorded with a Hitachi model U-2000, and FTIR spectra were recorded with a Shimadzu 8400 S FTIR spectrophotometer using KBr for solids.. The NMR spectra were recorded on a Jeol USA ECA-500 (500 MHz for ^{1}H and 125 MHz for ^{13}C NMR, 1D, 2D techniques) using CD_3OD as solvent and TMS as an internal standards. LCMS analysis was performed on Mariner Biospectrometry. Column chromatography were packed with silica gel 60 (0,015 – 0,040 mm) and Sephadex LH20 (Sigma USA), TLC analysis was performed using silica gel plates (Merck Kieselgel GF_{254}, 0,25 mm 20 x 20 cm), with visualization under UV (254 and 366 nm).

Plant material

The Leaves of *T.flagelliforme* were collected in Balittro, Bogor, Indonesia. A voucher specimens has been deposited at Herbarium Bogoriense, LIPI, Cibinong Bogor.

Extraction , isolation

The dried powdered leaves (3,4 kg) of *T.flagelliforme* was macerated with methanol at room temperature for 24 hours (three times), and the crude methanolic solution was subsequently concentrated using rotary evaporator. The methanol extract was partitioned by the following solvents with increasing polarity: *n*-hexane, ethyl acetate, and *n*-buthanol. The phases were concentrated to dryness by rotary evaporator. Each phase was analyzed for their biological activity using BSLT method, the potency as antioxidant using DPPH method, the antimicrobial activity using agar diffusion. The active phase (ethyl acetate) was fractionation using VLC with a step gradient of dichloromethane, isopropanol mixture, the fractions were tested using BSLT method. The active fraction were further column chromatography using Sephadex LH20 with methanol as mobile phase, and further identified using the preparative reversed phase HPLC analysis using gradient methanol in water as mobile phase, to yield compound 1 (7,5 mg), and compound 2 (5,9 mg).

RESULT AND DISCUSSION

The results for activity test of phase from methanol extract T.flagelliforme were shown in Figure 1. (biological activity using BSLT

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method), Figure 2 (antioxidant activity using DPPH method), Table 1 (antibacterial activity using diffusion method) and Table 2. (cytotoxic activity against breast cancer line T-47D using MTT method).

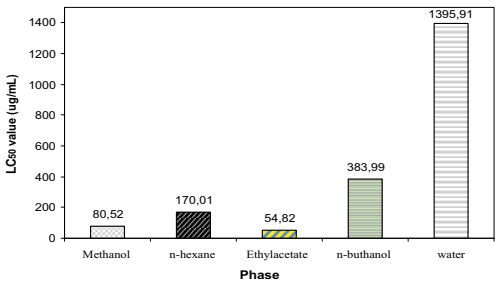

Figure 1. LC_{50} values (µg/mL) of the partition of methanol extract

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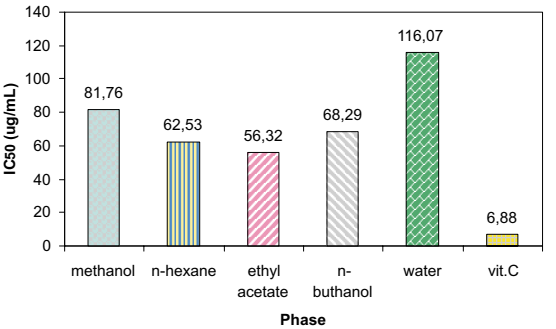

Figure 2. IC_{50} values (µg/mL) of the partition methanol extract

Table 1. Antimicrobial Activity of the Partition Methanol Extract

Concentration (mg/mL)	Zone of inhibition (diameter in mm)											
	n-hexane phase			Ethyl acetate phase			*n*-butanol phase			Water phase		
	PA	BS	CA	PA	BS	CA	PA	BS	CA	PA	BS	CA
50%	-	-	-	16,2	18,1	-	12,1	14,8	-	9	8,5	-
25%	-	-	-	13,1	15	-	10,2	13	-	8,5	7,5	-
12,5%	-	-	-	10,3	11,6	-	8,2	9,6	-	7,4	7,2	-
6,25%	-	-	-	9,1	9,2	-	7,1	7,3	-	7,1	7,1	-
Reference std (antibiotic)	27,5	24,1	24,4	27,9	24,1	-	28	25	-	28	23,4	-

Values are mean inhibition zone (mm) of three replicates
Notes: PA = *Pseudomonas aeruginosa* CA = *Candida albicans*
BS = *Bacillus subtilis*

Table 2. Cytotoxic Activity the Ethyl Acetate Phase against T-47D using MTT

Concentration (ppm)	Log Concentr	Absorban	Negative control	%PP	Probit	IC_{50} (µg/mL)
10	1	0,3256	0,4885	33,35	4,56	31,41
25	1,4	0,2870		41,25	4,77	
50	1,7	0,1970		59,67	5,25	
100	2	0,1150		76,46	5,71	
250	2,4	0,1270		74	5,64	
500	2,7	0,1230		74,82	5,67	
Positive control (Cisplatin)						3,07

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The results from the fractionation the ethyl acetate phase using VLC and column chromatography using Sephadex LH20 were obtained 2 compounds.

Compound (1), **adenosine**, white crystalline, UV ëmax (MeOH): 260, 213 nm: LCMS m/z 268,10 $[M+H]^+$; ^{1}H NMR (500 MHz, CD_3OD) ; ^{13}C NMR (125 MHz, CD_3OD):
^{1}H NMR (500 MHz, CD_3OD): 3,33 (1H,s), 3,35 (1H,s), 3,74 (1H,d,2,85Hz), 3,89 (1H,d,2,85Hz), 4,17 (1H,m,2,3Hz), 4,33 (1H,dd,5,15;2,3Hz), 4,73 (1H,dd,5,75Hz), 5,96 (1H,d,6,85Hz), 7,88 (2H,s), 8,18 (1H,s), 8,31 (1H,s).
^{13}C NMR (125 MHz, CD_3OD): 63,55 (C'5'); 72,75 (C-3'); 75,57 (C-2'); 88,27 (C-4'); 91,32 (C-1'); 142,11 (C-8); 153,59 (C-2); 157,67 (C-6).
FTIR (KBr): 3147 cm^{-1} (-OH, stretch vibr), 1679-1554 cm^{-1} (C=C, stretch vibr.), 1606-1579 cm^{-1} (C=C, aromatic), 1479 cm^{-1} (C-H), 1384 cm^{-1} (bending vibr), 1298-1205 cm^{-1} (C-O-C, eter), 1030 cm^{-1} (C-H out of plane). HMQC and HMBC Chemical shift (Table 3 and Figure 3.

Table 3. HMQC and HMBC Chemical shift of Compound (2)

Position of ^{1}H and ^{13}C	^{1}H - NMR (δ_H, ppm)	HMQC (δ_C, ppm)	HMBC (δ_C, ppm)
2	8,18	153,59	157,67
8	8,31	142,11	157,67
1'	5,96	91,32	75,57
2'	4,73	75,57	91,32
3'	4,33	72,75	88,27
4'	4,17	88,27	72,75
5'	3,89	63,55	72,75

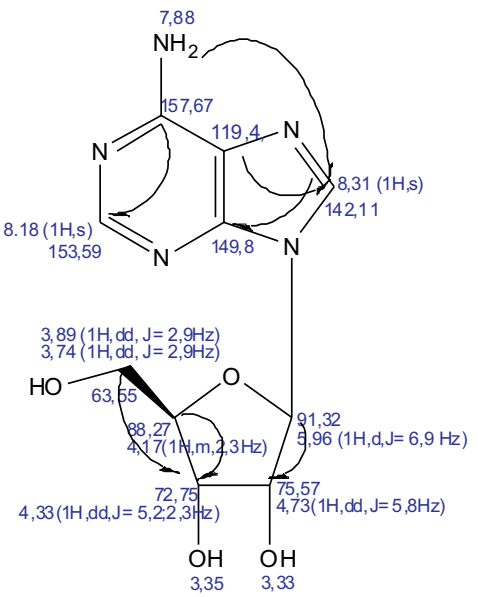

Figure 3. Analysis of HMQC and HMBC of Compound 2. (Adenosine)

Compound (2), Adenine , white powder, UV ëmax (MeOH):215, 261 nm, LCMS m/z 136,06 $[M+H]^+$; ^{1}H NMR (500 MHz, CD_3OD) ; ^{13}C NMR (125 MHz, CD_3OD):
^{1}H NMR (500 MHz, CD_3OD): 1,89 (1H,s), 7,90 (d), 8,11(1H,s), 8,18 (3H,s).
^{13}C NMR (125 MHz, CD_3OD): 119 (C-6); 141,64 (C-8); 152,61 (C-4); 153,67 (C-2); 156,78 (C-5).
FTIR (KBr): 3111 cm^{-1} (-NH, aromatic), 2796 cm^{-1}(C-H stretch vibr.), 690 cm^{-1} (C=C,stretch vibr, aromatic), 717-640 cm^{-1} (C-H bending vibr).

HMQC and HMBC Chemical shift (Table 3 and figure 4).

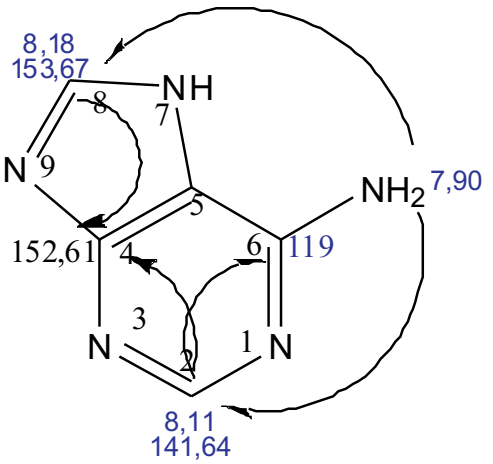

Figure 4. Analysis of HMQC and HMBC of Compound 3 (Adenine)

No biological activity against T 47D breast cancer cell line of compound (1) dan compound (2) with IC_{50} = 236,04 ppm and the compound (2) has weak/no cytotoxic activity against murine cell leukemia P-388 (IC_{50} = 71,36 ppm).

The chemical structure of two compounds of ethyl acetate fraction from the methanol extract of *Typhonium flagelliforme* leaves as seen in Figure 5

CONCLUSION

1. The ethyl acetate phase has biological activity against larvae *A.salina*, has potential antioxidant activity, has antibacterial activity against *B.Subtilis* and *P.aeruginosa*, has cytotoxic activity against breast cancer cells T-47D.
2. From the ethyl acetate fraction of methanol extract *T.flagelliforme* leaves was isolated two compounds, namely compound (1) adenosine and compound (2) adenine.

ACKNOWLEDGEMENTS

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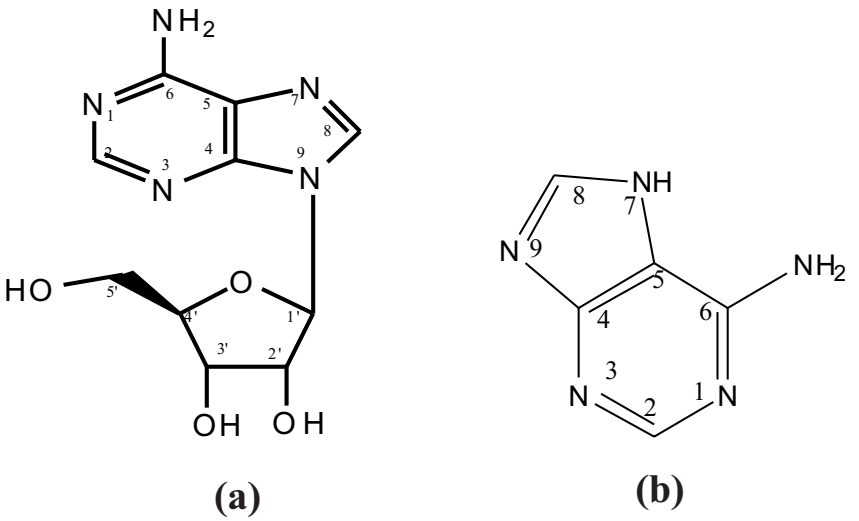

Figure 5. Chemical Structure of Compound 1 (a) and 2 (b)

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