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PHYTOCHEMICAL STUDY OF KETAPANG BARK (*TERMINALIA CATAPPA* L.)

Ade Zuhrotun[1]*, Asep Gana Suganda[2], As'ari Nawawi[2]
[1]*Faculty of Pharmacy, Universitas Padjadjaran*
[2]*School of Pharmacy, Institut Teknologi Bandung*

**Author for Correspondence: Jl. Raya Bandung-Sumedang KM 21, Jatinangor, West Java, Postal Code 45363, Phone/Fax 022-7796200, Mobile Phone 081322517899, email address: airkusaja@yahoo.com*

Abstract: *Terminalia catappa* L. is a tree which is known in Indonesia as ketapang or in other countries like England as tropical/indian almond. All part of ketapang used as traditional medicine and its pharmacological activity had been reported by many researchers. In this present work, the phytochemical study was covered material preparation, extraction, fractionation, characteristic determination of crude drug and extract, separation, purification and identification of isolated compound. The bark was extracted by reflux with 95% ethanol. An amount of extract was dissolved in hot water, then fractionated by liquid-liquid extraction (LLE) using n-hexane, chloroform, and ethyl acetate respectively. The extract and fractions analyzed by thin layer chromatography (TLC) and showed that n-hexane fraction had an interested compound. Further separation and purification of n-hexane fraction by combination of classical column chromatography and preparative TLC, have got isolated compound BK. Characterization of BK by ultraviolet-visible spectrophotometry, infrared spectrophotometry, liquid chromatography-mass spectrometry (LC-MS/ESI), and nuclear magnetic resonance spectroscopy (^{1}H, ^{13}C, HMQC and HMBC) identified that BK was steroidal compound with 390,20 (m/z) of molecular weight and $C_{27}H_{34}O_2$ of molecular formula, and predicted have aromatic ring in A and B position and acetoxy group in C-3 position.

Keywords: Ketapang, *Terminalia catappa* L., phytochemical study, steroids

1. INTRODUCTION

Terminalia catappa L. are naturally widespread in subtropical and tropical zones of Indian and Pacific Oceans and planted extensively throughout the tropics. In Indonesian archipelago, ketapang grown wild at low altitude, seashore or near by littoral area of Java Island until 800 m above the sea level and cultivated usually to take the fruits [Heyne, 1950; Thomson and Evans, 2006].

The leaves of this Combretaceous plant were widely used as a folk medicine in Southeast Asia for dermatosis and hepatitis. A lot of pharmacological studies have reported that the extract of leaves and fruits have anticancer, antioxidant, anti-HIV reverse transcriptase, anti-inflammatory, antidiabetic and hepatoprotective activities. This plant was popularly known as 'deshibadam' in Ayurvedic medicine. Juice of young leaves are employed externally in ointment for leprosy and scabies and internally for colic and headache. In addition, the seed also have aphrodisiac activity [Jing *et.al,* 2004; Ratnasooriya and Dharmasiri, 2000; Tenpe et al., 2007].

In Indonesia, ketapang bark was empirically used on medication of dysentery. The kernel seed used for laxantia, and the leaves for treatment of rheumatism, headache, colic, tanning and dye black agent on fabrican cloth [Heyne, 1950; Kasahara and Hemmi, 1995].

All ethanol extract of 12 species of Terminalia genera (Combretaceae) leaves have shown an antimicrobial activity minimal against three of *Escherichia coli, Staphylococcus aureus, Candida albicans* and *Microsporum gipseum*. Further research showed that ethanol and water extract of fresh fallen leaves gave the largest inhibition diameter against the same

microbe and *Pseudomonas aeroginosa, Trichophyton mentagrophytes, Pityrosporum ovale* and *Epidermophyton flocosum*. Furthermore, antifungal testing showed that ointment with 10% of this extract healing up on rabbit's skin infected by *Epidermophyton flocosum* and *Candida albicans* [Suganda, A.G. et al., 2004, 2006, Sukandar, E. Y. et al., 2007].

Ketapang contains several chemicals that distributed on every parts of this plant. The kernel seed consist of fatty acids and tannins like punicalin, punicalagin, terflavins A ,B, and tercatein. The dried fallen leaves was present of flavone glycosides like apigenin 6-*C*-(2²-*O*-galloyl)-b-*D*-glucopyranoside, apigenin-8-*C*-(2²-*O-g*alloyl)-b-*D*-glucopyranoside,isovitexin, vitexin, isoorientin, and rutin. [Lin Hsu and Ta-Chen, 1999; Heyne, 1950; Lin et. al., 2000]. Phenolic compounds were found in root, fruit shell and bark. The fruit had also cyanidin-3-glucoside and corilagin. The bark possessed of gallic acid, ellagic acid, 2,3-(*S*)-HHDP-D-glucose, casuarinin, castalagin, grandinin, castalin; 3-methoxy-4-hydroxy phenol-1-*O*-b-D-(6-*O*-galloyl)-gluco side; 3,5-dimethoxy-4-hydroxyphenol-1-*O*-b-D-(6-*O*-galloyl)glucose de;(-)-epicatechin-3-*O*-gallate; (-)-epigallocate chin-3-*O*-gallate, procyanidin B-1; 3-*O*-galloyl procyanidin B-2; acutissimin A and eugenigrandin A [Lin et. al., 2000; Thomson and Evans, 2006; Nagappa et. al., 2003].

Phytochemical study of ketapang bark original species from Indonesia has not been reported. Previous screening indicated only the presence of flavonoid, tannin, saponin, kuinon, and mono/sesquiterpene [Sumintir, 2008]. These research was done to provide basic chemicals reference of ketapang and hopefully an isolated compound would intended to be direction for further elaboration.

2. METHODS AND RESULTS

2.1 Materials and tools

Materials: ketapang bark, 95% ethanol, aquadest, n-hexane, chloroform, ethyl acetate, toluene, ether, acetone, methanol, amilalcohol, hydrochloric acid, sulfuric acid, nitric acid, boric acid, citric acid, acetic acid glacial, formic acid, formaldehyde, ammonia, bismuth-subnitric, mercury(II)chloride, alumunium (III) chloride, chloral hydrate, kalium hydroxide, sodium acetic, magnesium powder, alumunium foil, precoated silica gel GF254, and silica gel 60 (0,063-0,200 mm) for column.

Tools: grinder, reflux, rotavapor (BUCHI), electric dryer (MITSEDA HD 350), separatory funnel, freeze dryer (EYELA FD 81), microscope (OLYMPUS CX31), crucible porcelain, furnace (CARBOLITE), toluene-distillator, classical column chromatography, common glassware in laboratory, high performance liquid chromatoghraphy (HAWLETT PACKARD 1100), ultraviolet-visible spektrophotometer (DESAGA), infrared spectrophotometer (FT/IR JASCO 4200), Liquid Chromatography- Mass Spectrometry /Electron Spray Ionization (LC-MS/ESI), Mariner Biospectrometry), and nuclear magnetic resonantion spectrometry (JEOL JNM ECA 500).

2.2 Sample preparation

The bark from main wooden rod of ketapang, was collected on September 2008 from local area of Indonesian Institute of Science (LIPI) Bandung. The Material was sorted, cleaned, cuted in small quadrangle (2,5-3,0 cm), air-dried then heated in oven (40°C), and finally grinded.

The plant was identified in Herbarium Bandungense, School of Science and Biological Technology (SITH) ITB, that belonged to Combretaceae, species *Terminalia catappa* L, with sinonim name *Terminalia moluccana* Lamk., *Terminalia procera* Roxb., and *Terminalia mauritiana* Blanco.

2.3 Extractions

Powdered crude drug was Extracted by reflux using 95% ethanol (6 hour). Filtrat was condensed first using vacuum rotavaporator then electric dryer. A 973,04 g of extract was obtained from 4 kg of crude drug, equivalent with 24,32%(w/w) of extractive matters (rendemen).

2.4 Fractionation

Fractionation conducted by liquid-liquid extraction (LLE) using n-hexane, chloroform, and ethyl acetate respectively, where the extract was dissolved in aquadest previously. About 200g extract fractionated

resulted in 2,81 g n-hexane fraction (1,4%); 1,04 g chloroform fraction (0,52%); 1,50 g ethyl acetate fraction (0,75 %) and 102,85 g of water fraction (51,42%).

2.5 Determination of paramater quality

Crude drug and extract were determined by strandard procedure [Ditjen POM, 1987; 1979], parameters was covered water-soluble extractive matters, ethanol-soluble extractive matters, loss on drying, total ash content, water-soluble ash content, acid-insoluble ash content, and water content. Specifically to crude drug followed by macroscopical and microscopical analysis.

Macroscopical analysis of katapang bark showed the appearance of cuted bark in long fibrous layer, hard, thick (2-5 mm), visible like soft path in inner surface and outer surface was rugged, wrinkled, fractured, brown color and specific odor. Powdered crude drug have cream-pale brown color. By microscopical analysis fragments identified were shclerenchime, parenchime, fibrous bark, rocked cells, amylum, oil cells, and chrystal of rossette oxalate. The result of determinations are available in Figure 1 and Table 1.

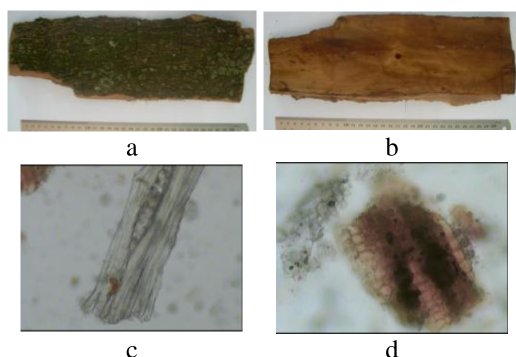


Figure 1 Characteristic of macroscopic and microscopic

Note :
(a) Outer-(b)inner-surface (c)fibrous bark(d)oil cells and chrystal of rossette oxalate in parenchime

Table 1 Characteristic of Quality

Parameter	Content % (w/v)	
	Crude Drug	Extract
Water-soluble extractive matters	14,50	49,45
Ethanol-soluble extractive matters	19,00	64,55
Loss on drying	10,54	21,63
Total ash content	15,07	1,67
Water-soluble ash content	4,50	0,38
Acid-insoluble ash content	11,09	1,27
Water content (% v/w)	8,88	17,85

2.6 Chemical content investigation

Chemical content investigation was done by phytochemical screening and thin layer chromatography (TLC).

a. Phytochemical screening

Phytochemical screening were determined from crude drug, extract and all fractions of LLE, that covered alkaloid, flavonoid, kuinon, saponin, tannin, steroid dan triterpenoid using standard procedures [Ditjen POM, 1979]. The results are available in Table 2.

Table 2 Result of Phytochemical Screening

Compounds	I	II	III	IV	V	VI
Alkaloid	-	-	-	-	-	-
Flavonoid	+	+	+	-	+	+
Kuinone	+	+	+	+	+	+
Saponin	+	+	+	-	-	-
Catechin	+	+	+	+	-	-
Gallotanin	+	+	+	+	-	+
Steroid/Triterpenoid	+	+	-	+	+	+

Note:
I=crude drug; II= ethanol extract ; III=water fraction ; IV = n-hexane fraction; V = chloroform fraction; VI= ethyl acetate fraction; + = present; - = absent

b. Thin layer chromatography (TLC)

Ethanol extract and LLE fractions were investigated by TLC using precoated silica gel GF254 and toluene:ether (1:1) solvent mixture. Chromatogram was observed under 254 nm, 366 nm and after 10% of sulfuric acid as spray reagent. Revealed the profile or numerous chemical contents of ketapang bark marked by every spots.

In Figure 2 showed that are two spots which have dominan in intensity. They're observed under UV 366 nm, as greenish-blue flourescence spot ('biru kehijauan'=BK) in n-hexane fraction (Rf 0,78-0,85) and yellow flourescence spot (Rf 0,45-0,54) in chloroform fraction. At this research, for further process initially focused on n-hexane fraction to isolate the greenish-blue flourescence spot ('biru kehijauan' = BK).

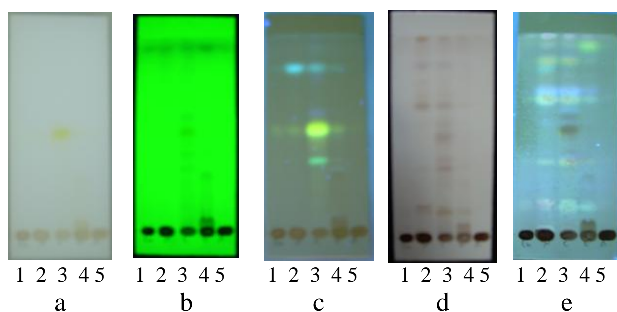


Figure 1 Chromatogram of extraction and fractionation result

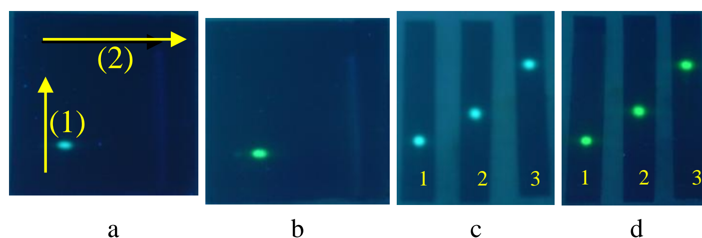
Note:
Sample: (1) extract, (2) n-hexane fraction, (3) chloroform fraction, (4) ethyl acetate fraction, (5) water fraction. **condition**: precoated silica gel GF254, eluen: toluene:eter (1:1) solvent mixture, **observed** under (a) visible (b) UV 254 nm (c) UV 366 nm (d) visible with 10% of sulfuric acid (e) UV 366 nm with 10% of sulfuric acid

2.7 Separation and purification of fractions

Classical column of chromatography (CC) and preparative TLC was chosen for further separation and purification of BK in n-hexane fraction. n-hexane:ethyl acetate (11:1) solvent mixture used as mobile phase, where BK identified in Rf 0,35. Classical column of chromatography was conventionally prepared as follow: about 450 ml of eluen; 30,14 g silica gel for column; size 2 cm of column diameter; 17,5 cm of column heigh after stable. Sample 1,03 g of n-hexane fraction putted down carefully on surface column to formed a flat layer. Elution process began slowly until interested compound fully separated, this process monitored by TLC.

From CC obtained 43 of sub fractions where BK was concentrated in 17-26 sub frations. Base on similarity pattern of chromatogram TLC then clasified as grouped 17-18 of sub fraction (4,2 mg), 19-22 sub fraction (8,2 mg) and 23-26 sub fraction (7,3 mg). Each groups then purified by preparative TLC under the same condition. BK on band shape then removed or scraped from the plate, redissolved with eter, liquid phase decanted, air-dried or placed in acid room to remove the solvent, residue was BK.

Purity of isolate BK was checked by two dimensional TLC, TLC with three diffrerent mobile phase, HPLC and LC (connected with MS in LC-MS/ESI). On each chromatogram appeared only one spot with different value of Rf or Rt during observation under their specific condition. These may conclude that isolate BK was pure. In chromatogram resulted by LC-MS/ESI, showed that isolate BK was dominan of peak although small peak of impurities stil exist. After direct calculation on data, area under curve was 77,45% of precentage. It's also figure out the purity level of isolate BK. Chromatogram are available in Figure 3.



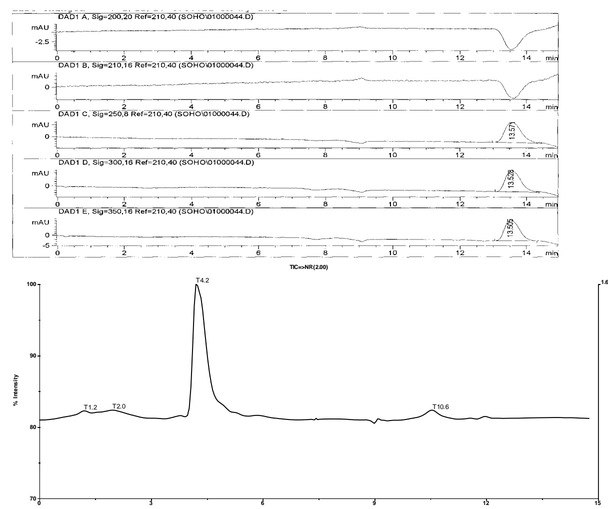


Figure 2 Chromatogram of purity tests

Note:
TLC: stationary phase: precoated silica gel GF 254, mobile phase (1) n-hexane:ethyl acetate (11:1) solvent mixture and (2) n-hexane:chloroform (3:7) solvent mixture (3) chloroform:ethyl acetate (9:1) solvent mixture, obseved under UV 366 nm (a and c), UV 366 nm with 10% sulfuric acid (b and d). **HPLC :** ODS hypersil column, mobile phase methanol (100%), flow rate 1 ml/minute, DAD detector, temperature 25°C, stop T 15 minute. **LC(LC-MS/ESI):** mobile phase: methanol:water (80:20) solvent mixture; Injection Volume 20 µl; flow rate 1 ml/minute; C18 (RP 18) Supelco column, size 150mm of lenght, size 5 µm of particle. System used: ESI (*electrospray ionization*), positive ion mode.

2.8 Characterization of Isolated compound

Isolate BK visually like a stiky mass, fair white and almost transparent when dissolved. It's soluble in ethanol, methanol and ethyl acetate but more soluble in chloroform, n-hexane, diethyl ether.

Monitored by TLC, BK appeared as greenish-blue flourescence spot ('biru kehijauan'=BK) under UV 366 nm, but under UV 254 nm this invisible. After sprayed with Liebermann-Burchard (LB) reagent, BK turned into green flourescence spot under 366nm, this also appearance BK after sprayed with 10% sulfuric acid, beside as light green spot visually when heated. Reaction BK with anisaldehyde reagent was appeared as yellow spot in orange/pink backgrounds under UV 366 nm and visible. Isolat BK it's not reacted with 5% aluminium (III) chloride solution and 5% sitroboric solution when used as spray reagent. Chromatogram are available in Figure 4.

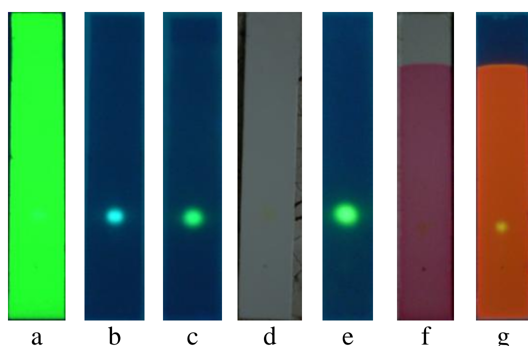


Figure 3 Chromatogram of BK with some spray reagent

Note:
stationary phase: precoated silica gel GF 254, mobile phase n-hexane:ethyl acetate (11:1) solvent mixture; obseved under (a) UV 254 nm (b) UV 366 nm (c) UV 366 nm with 10% sulfuric acid, (d) visible with LB reagent, (e) UV 366 nm with LB reagent, (f) visible with anisaldehyde, (g) UV 366 nm with anisaldehyde

Application of TLC also verified that BK was not an arthefak. It's done by spotting an amount of isolate BK and extract under the same condition. This co-chromatogram pattern showed that isolate BK contained in the extract in small portion, so that BK must not formed as arthefak.

Profile of BK by utraviolet-visible spectrum, described the presence of conjugative bond within it's structural compound. As the absorbance was detected at wavelenght of 208 nm dan 355 nm. Previously, 95% ethanol was measured as blank solvent to proof that spectrum only derived from sample.

Infrared spectrophotometry was measured with potasium bromida (KBr) pellet as sample handling. Base on spectrum, structure of BK possessed some fungsional groups as follow: hidroxy (O-H) at 3378,67 cm^{-1}, stretched C-H at 2931,27 cm^{-1} and 2857,99 cm^{-1}, group C=O at 1735,62 cm^{-1}, and bended C-H at 1461,78 cm^{-1} and 1376,93 cm^{-1}. Beside that, mass spectrum of LC-MS/ESI showed the ion peak at $[M+1]^+$ 391,20 (m/z), this implied that the molecular weight of BK was 390,20 (m/z).

The H-NMR spectrum of BK used $CDCl_3$ as solvent, have shown a strong and sharp signal at δ 0,8-1,0 ppm that indicated of methyl groups ($-CH_3$). signal also appear at existence area of group-CH_2 (δ 1,2-1,4 ppm), group C-H (δ 1,4-1,7), group CH_3-COO- (δ 2,0-2,2 ppm), group CH- (bound to esther group) (δ 3,3-3,9 ppm), group $R_2C{=}CH_2$ (δ 4,6-5,0 ppm), $R_2C{=}CHR$ (δ 5,2-5,7 ppm), and proton aromatic (ArH) (δ 6,0-9,0 ppm). Whereas spectrum C-NMR showed appearance of signals that indicated of alkyl groups (δ 5-60 ppm), group C–O (δ 45-90 ppm), carbon in aromatic ring (δ 90-60 ppm), carbon of alkena (δ 100-170 ppm), group –COO- (δ 150-185 ppm). Spectrum are available in Figure 5.

Measurenment by two dimensional NMR comprise of *Heteronuclear Multiple Quantum Coherence* (HMQC) and *Heteronuclear Multiple Bond Correlation* (HMBC). Data summaries in Table 3 and Table 4.

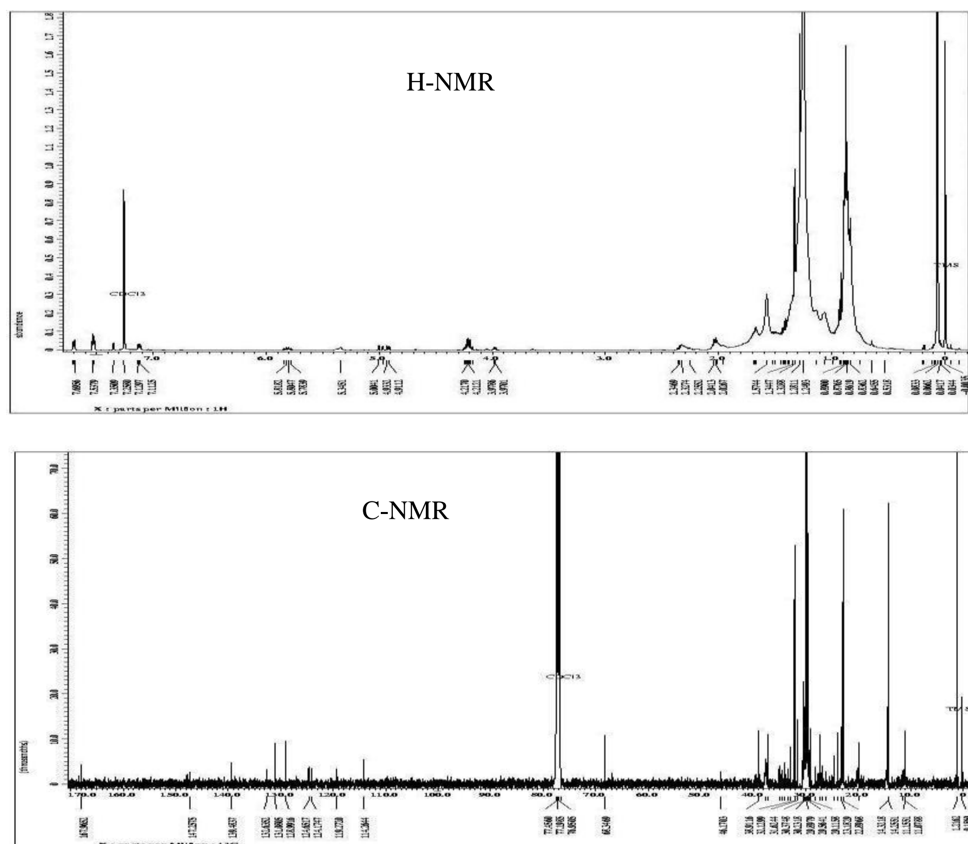


Figure 5 H- and C- NMR spectrum of BK

Table 3 Data Summaries from HMQC Spectrum

δ (ppm) C	δ (ppm) H	δ (ppm) C	δ (ppm) H
11,1551		46,1705	
14,3218	0,880	68,3469	4,21
19,9208	0,838	114,2644	4,9-5,0
22,8908	1,331	119,2720	7,53
23,1829	1,26	124,2747	7,12
23,9269		124,6517	7,35
27,2844		128,9916	7,7
29,5641	1,25	131,0805	7,52
29,8979		132,6352	
30,3748		139,4837	
31,6244		147,2575	
32,1299		147,8012	
37,2901		167,9652	
38,9116			

Table 4 Data Summaries From HMBC spectrum

δ (ppm) H	δ (ppm) C	δ (ppm) H	δ (ppm) C
0,838	37,2901	2,02	114,2644; 139,4837
0,877	32,1299	4,21	23,9269; 30,5465
0,880	22,8908	7,12	124,6517; 147,80
0,918	38,9116	7,35	124,1747; 147,80
1,253	29,8979	7,52	139,4837, 147,2575
1,281	14,3218; 147,2575	7,70	131,0805
1,331	30,3748		

3. DISCUSSION

Ketapang that used in this research was determined belonged to Combretaceae, species *Terminalia catappa* L, with sinonim name *Terminalia moluccana* Lamk., *Terminalia procera* Roxb., and *Terminalia mauritiana* Blanco. The bark was taken from main wooden rod of ketapang, then cleaned, air-dried then powdered in order to be extracted, fractionated, and characterization.

The result of macroscopical and microscopical analysis showed a similarly profile of ketapang with the bark of other medicinal plants. Interested fragments appeared was the presence or abundance of oil cells with specific odor and chrystal of rossette oxalate.

By determination of quality parameters and phytochemical screening, was figured out the quality and chemical component katapang bark. Hopefully this will provided basic reference to explore ketapang in herbal medicine.

Qualitative information from TLC after extraction and fractionation process, directed focused on the objective of further separation and purification i.e isolation of greenish-blue flourescence spot ('biru kehijauan'=BK) in n-hexane fraction. The result of previous condition on chromatogram TLC, BK sited at Rf 0,78-0,85. After re-optimize the condition resulted an appropriate mobile phase for further separation and purification,that was n-hexane:ethyl acetate (11:1) solvent mixture, where BK sited in Rf 0,35. By the combination methods of CC and preparative TLC, BK was fully isolated. Purity level checked by two dimensional TLC, TLC with three diffrerent mobile phase, HPLC, and LC-MS/ESI, under specific condition concluded that isolate BK was dominan with 77,45% of purity level.

In chromatogram by TLC, after sprayed by Liebermann-Burchard reagent, BK appear as green flourescence spot under 366nm, as light green color visually, supposed that steroidal compound. According to Goad and Akihisa (1997), steroidal compound reacted positively with LB if produced green or blue or pink color. From that reference also stated that in UV-Vis spectrum, double bond of steroidal compound will indicated at range wavelength of 190-220 nm. As result that the absorbance of BK indicated at wavelength of 208 nm dan 355 nm implied that BK owned conjugative double bond, had also clarified of it's green flouresence under UV 366nm. In addition from infrared spectrum showed that BK possessed fungtional group of O-H, C-H dan C=O within structural compound.

The H-NMR spectrum of isolat BK showed a similarly with specific pattern of stearyl acetate, that marked as strong and sharp signal at δ 0,8-1,0 ppm that indicated of methyl groups ($-CH_3$), signal at δ 4,2 ppm and δ 2,02 ppm indicated of acetoxy group, and signal at δ 4,9-5,0 ppm indicated of vinilic double bond. From C-NMR spectrum peak was appeared signals at δ 5-60 ppm indicated of alkyl groups, signal at δ 68,34 ppm indicated of carbon bound to esther group, and signal at δ 167,96 indicated of carbonyl group. Further analyze of compiled data from H- and C-NMR ensure the previously prediction that BK was steroidal compound. In general steroidal skeletone of cholestane, subtitution of acetoxy group possible in C-3 position (3β-hydroxyl).

Two dimensional NMR spectrum of HMQC and HMBC showed some kind position of carbon such as the presence of carbon connected with proton aromatic, carbon connected with proton vinilic, one esther carbon connected with their proton, carbon connected with proton of methyl, and carbon disconnected with any protons (carbon without proton). These conclude that isolate BK in their structure possibly have aromatic bonding, vinilic bonding, and or binding with methyl groups in side chain.

Mass spectra of LC-MS/ESI given clued that the molecular weight of BK was 390,20 (m/z). In general, steroidal compound have 388- 426 (m/z) of molecular weight which depend on parent skeletone and side chain of their structure. These information was supported the previously prediction that BK was steroid compound. And then, molecular formula possibly predicted as $C_{27}H_{34}O_2$. The value of Hydrogen Deficiency Index calculated based formula, that was 11. It's mean that within structural molecul, BK had 11 of double bonding. It can breaking down by decreased 4 from the value allocated for the skeleton (siklo-pentano-perhidro-fenantren ring system), and decreased 1 the value for double bond of one carbonil group, so that there still remain 6 double bond within structure of BK. In such a way that formerly prediction of the presence oromatic ring within structure of BK more supported.

Steroidal compounds that have been reported had aromatic ring in their structure, are very rare derive from plants. As a mean to identify and elucidate molecular structure of BK, so dealing with alternative approached. And then the resulted data of characterization were compared with reference resources. Herein some reference that reported of steroid with aromatic ring in their structural skeletone. Aromatic ring in A position can be found in steroidal hormone like estradiol, estrone,

Table 5 H-NMR Spectrum of BK, Equilenin and It's derivative

Isolate BK	Equilenin (AL)	Equilenin (CDU)		17α-dihidro-Equilin sulfate		Equilin sulfate (6-OH)	
δ (ppm)	δ (ppm)	δ (ppm)	H	δ (ppm)	H	δ (ppm)	H
0,838	1,0	1,30	18	0,52	18	0,70	18
0,877	1,3	1,68	12b	1,17	20	1,17	20
0,880	1,7-1,9	1,93	12a	1,52	15a	1,78	12a
0,918	1,9-2,02	2,01	16b	1,60	6a	1,88	15a
1,253	2,2-2,7	2,11	16a	1,73	12a	2,00	12b
1,281	3,0-3,2	2,19	15b	2,04	12b	2,32	16a
1,331	3,4	2,44	15a	2,16	15b	2,39	15b
2,02	7,1	2,80	11b	2,27	16b	2,62	16b
4,21	7,2	2,90	11a	2,99	11a	3,08	11a
4,9-5,0	7,5-7,6	3,05	14	3,09	19	3,10	19
7,12	7,8	6,93	7	3,10	14	3,12	11b
7,35	9,6	6,96	2	3,13	11b	3,14	14
7,52		7,18	4	3,76	17	6,69	7
7,53		7,72	6	4,50	17(OH)	7,33	2
7,70		8,10	1	6,62	7	7,77	1
		9,43	3(OH)	7,30	2	7,89	4
				7,75	1	9,83	6(OH)
				7,85	4		
				9,64	6(OH)		

Note:

(shaded) Showed a similarly of chemical shift value

AL = chemical shift value of equilenin from the Aldrich Library; CDU= chemical shift value of equilenin in NMR prediction using software (Chemdraw ultra); 17α-dihidro Equilin sulfate and Equilin sulfate (6-OH) used as standard on measured condition in natrium salt form

estriol and geodisterol in marine sponge [Gui-Yang-Sheng Wang and Crews, 1999; Ikan, 1991]. Aromatic ring in B position can be found in fungi of species *Phycomyces blakesleeanus*, such as *Phycomysterol A, Phycomysterol B* and *Neoergosterol* [Barrero et. al., 1998]. And Aromatic rings both in A and B position like equilenin and its derivative [Pouchert and Behnko, 1993; Hill et. al., 2008]. Structure of each compound are available in Figure 6.

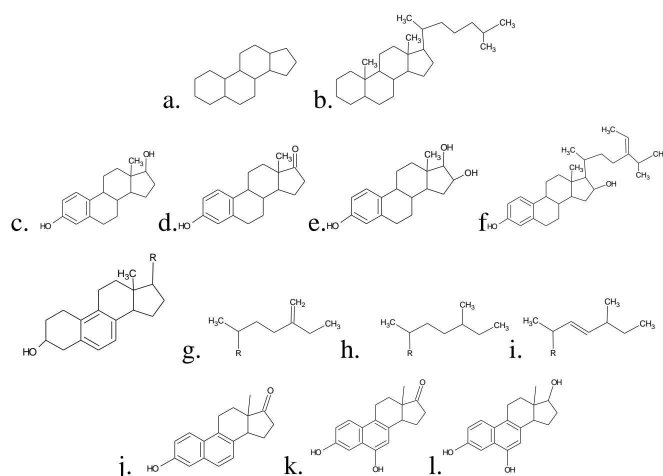


Figure 6 Structure of selected steroid

Note:
(a).Siclopentanoperhydrophenantren ring system; (b).Skeletone of cholestane;(c). Estradiol;(d). Estrone; (e).Estriol; (f).Geodysterol; (g). Phycomysterol A; (h). Phycomysterol B; (i). Neoergosterol; (j). Equilenin; (k).6-hidroksi Equilin; (l). 17α-dihidroequilin

Chemical shift value of proton aromatic in NMR spectrum at δ 6,0-9,5 ppm. Data on Table 5 showed a similarly of chemical shift value at proton aromatic at isolat BK with equilenin and its derivative. Its meant that skeletone of isolat BK have similarly in aromatic rings position with Equilenin and its derivative, that was in both A dan B rings. One of the difference was disappearance on chemical shift value proton of hydroxyl group from BK unlike in C-3, C-6 and C-17 position from equilenin and its derivative. Its may conclude that hydroxyl group of BK binding with another fungtional group. As predicted previously that BK might subtituted by acetoxy group in C-3 position. It also explained that chemical shift value of hydroxyl group from BK in C-3 position was disappeared.

Chemical shift value of carbon aromatic in NMR spectrum at δ 90-160 ppm. Data on Table 6 showed a similarly of chemical shift value at carbon aromatic in isolat BK with equilenin and its derivative . Its meant that skeletone of isolat BK have similarly in aromatic rings position with Equilenin and its derivative, that was in both A dan B rings. One of the difference was appearance on chemical shift value at δ 160-220 ppm indicated of carbonyl group. In this case, represented to acetoxy in C-3 position of isolate BK and represented to ketone group in C-17 position of Equilenin and its derivative.

The similarity on chemical shift value of H- and C-NMR spectrum at aromatic ring position from BK with Equilenin and it's derivative, implied that each compound have similar parent steroidal skeleton and location of

Table 6. C-NMR Spectrum of BK, Equilenin and It's derivative

Isolate BK	**Equilenin (AL)**	**Equilenin (CDU)**		**17α-dihidro-Equilin sulfate**		**Equilin sulfate (6-OH)**	
δ (ppm)	**δ (ppm)**	**δ (ppm)**	**C**	**δ (ppm)**	**C**	**δ (ppm)**	**C**
11,1551	11,00	18,0	18	8,6	20	8,6	20
14,3218	21,00	21,8	12	15,6	18	12,7	18
19,9208	23,00	26,4	11	23,4	11	21,4	15
22,8908	28,50	28,9	15	24,6	15	22,9	11
23,1829	36,00	36,8	16	29,2	12	28,8	12
23,9269	38,00-40,00	46,7	13	32,9	16	36,1	16
27,2844	45,00	47,5	14	44,4	13	45,7	19
29,5641	46,00	106,5	7	44,5	14	45,9	14
29,8979	110,00	118,0	6	45,7	19	46,9	13
30,3748	118,00	125,2	4	77,3	17(OH)	106,0	7
31,6244	124,00	125,7	2	107,5	7	111,7	4
32,1299	124,00	127,8	10	111,7	4	120,1	9
37,2901	126,00	128,4	1	119,9	9	121,8	2
38,9116	130,00	129,1	9	121,5	2	123,7	1
46,1705	133,00	129,6	8	123,6	1	124,2	5
68,3469	139,00	133,9	5	123,7	5	129,4	10
114,2644	154,00	153,4	3	129,4	10	133,3	8
119,2720	178,00	203,0	17	136,0	8	149,7	3
124,2747				149,4	3	151,2	6(OH)
124,6517				150,7	6(OH)	218,9	17
128,9916							
131,0805							
132,6352							
139,4837							
147,2575							
147,8012							
167,9652							

Note:
Showed a similarly of chemical shift value
AL = chemical shift value of equilenin from the Aldrich Library; CDU= chemical shift value of equilenin in NMR prediction using software (Chemdraw ultra); 17α-dihidro Equilin sulfate and Equilin sulfate (6-OH) used as standard on measured condition in natrium salt form

aromatic ring, that sited in A and B position. However, the configuration or structural complete of BK are still unclear.

4. CONCLUSION

Phytochemical study of katapang bark was conducted by analyze of specific and non specific parameter of plant, provided information of characteristic of its chemical compounds. By the process of extraction, fractionation, separation and purification, with monitored by TLC, have been fully isolated compound BK from n-hexane fraction. Characterization of BK by ultraviolet-visible spectrophotometry,Infrared spectrophotometry, liquid chromatography-mass spectrometry (LC-MS/ESI), and nuclear agnetic resonance spectroscopy (^{1}H, ^{13}C, HMQC and HMBC) identified that BK was steroidal compound with 390,20 (m/z) of molecular weight and $C_{27}H_{34}O_2$ of molecular formula, and predicted have aromatic ring in A and B position and acetoxy group in C-3 position.

Advanced research may directed to optimize condition of purification in order to isolation BK with higher of purity level, besides the other chemicals of ketapang.

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