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Isolation and identification structure antioxidant active compounds of ethyl acetate fraction hypokotil *Bruguiera gymnorhiza* (L) Lamk.

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Abstract: *Bruguiera gymnorhiza* (L) Lamk is one of mangrove plants that commonly used as traditional food and medicine. As traditional medicine, it needs information on contained compound in the hypocotyls of *B. gymnorhiza*. In general, compound insulation only applied to the flowers, barks and leaves of *B. gymnorhiza*, while isolation from hypocotyls was not much practiced. The existence of antioxidant compounds in hypocotyls of *B. gymnorhiza* was essential information for the development of functional food and pharmaceuticals. This study aimed to isolate and identify the structure of the active compounds of the most active antioxidant in ethyl acetate fraction hypocotyls of *B. gymnorhiza*. Methods for isolation and identification of active antioxidant compounds are: maceration, evaporation, partitioning, and the structure determination of the compound. The results showed that the fraction of F4.80 to F4.120 produces a single spot stains and high antioxidant activity. Structure identification of the active antioxidant compounds was performed using ^{13}C-NMR spectrum and ^{1}H-NMR 2D. Based on the results of the reading on 135^{0} DEPT, ^{1}H-^{1}H-COSY, HMQC, and HMBC, the compound target has 29 carbon atoms, 50 hydrogen atoms and two oxygen atoms with molecular formula $C_{29}H_{50}O_2$. This compound is a derivative phenolic compound with molecular structure resembles the structure of the compound of α-tocopherol.
Keywords: active antioxidant compounds, *Bruguiera gymorhiza* (L) Lamk., ethyl acetate fraction.

Introduction

Bruguiera gymnorhiza (L) Lamk is an important mangrove plants, which it hypocotyls can be used as an alternative source of food, functional food[1,2], pharmaceutical and adhesive materials[3]. Similar to other mangrove plants, many compounds of *B. gymnorhiza* (L) Lamk contain steroids, saponins, tannins (condensed and hydrolysable tannins), flavonoids (flavan and polymeric flavan), carotenoids, anthocyanins, terpenoids (diterpene and triterpene), gibberelin, fatty acids, carbohydrates, salts, minerals and proteins[3-13]. Isolation results of bioactive compounds from *B. gymnorhiza* (L) Lamk includes ***one neolignans*** compound (dioctadecyl (7R*, 8S *, E) -3-hydroxy-5, 5'-Dimethoxy-8-3 ', 7-O-4'- neolign-7'-ene-9, 9'-dioate), ***4 diterpenes compounds***: (a) ent-13, 16β, 17-trihydroxykaur-9 (11) -en-19-oate; (b) beyeran-19-al-16 –one; (c) steviol, and (d) methyl ent-16β, 17-dihydroxykaur-9 (11) -en-19-oate), ***one glycosides*** (daucosterol), ***one long chain fatty alcohols*** (triacontanol), ***one long chain fatty acid*** (septadecanoic acid), ***two sterols*** (cholestol, and stigmasterol), ***one***

aromatic (methyl 4-hydroxybenzoate) and ***one triterpenes*** (oleanolic acid). The majority of these bioactive compounds have activity as natural antioxidants.[14].

Natural antioxidants generally have a hydroxyl group (OH) in the molecular structure, thus some components of antioxidant in plants can be identified based on its free radical scavenger[15,16.] Bioactive compounds in the hypocotyls of *B. gymnorhiza* have high antioxidant activity with IC_{50} values below 20 ppm [(17)], other sources mentioned at 9.42 ppm[18].

Antioxidants are essential compounds that give a positive effect on cardiac arteries (cardiovascular), blockage of blood vessels (atherosclerosis), cancer and aging prevention [19]. The human body does not have a reserve of antioxidants in excessive amounts, thus in case of excessive exposure to free radicals, the body requires exogenous antioxidants. There are worries of possible side effects and carcinogenic properties of synthetic antioxidants[20] causes a natural antioxidant become an alternative requirement [1,22].

Isolation and identification of active antioxidant compounds of *B. gymnorhiza* (L) Lamk generally assessed from the flowers, barks and leaves. Meanwhile the insulation on the fruit (hypocotyls) has not been conducted. Yet the information is required for the development of functional food and pharmaceutical ingredients. Therefore, this study aimed to isolate and identify the structure of the active antioxidant compounds in the hypocotyls of *B. gymnorhiza* which most active in the ethyl acetate fraction.

Materials and Methods

The Main Ingredient

The purple hypocotyls of *B. gymnorhiza* (L) Lamk were taken in the period July-September 2013 from the coastal location of mangrove forests Sawohan Village, Sidoarjo, East Java, Indonesia.

Chemical material:

We used chemicals that categorized as PA (Pro Analysis), i.e. methanol, ethanol, ethyl acetate, distilled water, n-hexane, chloroform, deuterium, silica gel F-254, the TLC plate. Chemicals for the isolation and identification were obtained from Chemical Research Laboratory of Padjadjaran University at Bandung and Indonesian Institute of Sciences (LIPI) Laboratory, Chemical Research Center, Serpong Jakarta.

Preparation of raw materials:

Hypocotyls of *B. gymnorhiza* from study sites were selective on purple color. It washed by water and put in a dark plastic bag to be taken to the laboratory. Samples in the form of dry powder were obtained by following steps. Hypocotyls was sliced crosswise and thinly using a slicer into chips. The chip was then dried using a vacuum drying (temperature: 50°C, pressure: -70 mmHg) for 8 hours, downsizing using attrition mill. The water content of the dry powder was 7.73 + 0.09% (dry weight).

Isolation and identification of active compounds antioxidant

Maceration [23] (modified):

Hypocotyls powder of *B. gymnorhiza* was immersed in the liquid methanol (1:5) for 3x24 hours in the dark and closed containers. Samples were stored in a room protected from light with maintained room temperature. During the maceration, solvent was replaced every 1x24 hours. Maceration results were filtered by vacuum filter to obtain filtrate and residue. The filtrate of maceration from first, second and third day were collected to be evaporated.

Evaporation[23] (modified):

The filtrate was put in vacuum rotary evaporator (Buchi 144 type) at temperature of 45°C and pressure of 360 mbar. Evaporation process was not terminated until the distillate produced methanol again. Extract was stored in dark bottles and stored at temperatures 4°C.

Partition n-hexane fraction, ethyl acetate fraction and the fraction of H_2O [24] modified:

Ethyl acetate fraction obtained from the methanol extract of *B. gymnorhiza* (L) Lamk through 2 phases. Phase 1 was making the fraction of n-hexane and Phase 2 was ethyl acetate fraction. **Phase 1**: methanol extract of *B. gymnorhiza*'s hypocotyls dissolved in n-hexane: water=1: 1 (v / v). It was shook in a flask separator for 15 minutes, and kept for 24 hours. The yellow n-hexane fraction will be at the top of a flask separator. Then, n-hexane fraction was taken and stored in vials. Remaining H_2O fraction in the flask separator re-dissolved with n-hexane:H_2O 1:1 (v/v) to obtain a fraction of n-hexane again. This partition was done repeatedly until we obtained a clear n-hexane fraction. Fraction of n-Hexane results in a partition vials were collected and evaporated until all n-hexane evaporates. **Phase 2**: The left water fraction at the bottom of the separation flask partitioned using ethyl acetate: H_2O 1:1 (v/v). By the same procedure as the partition with n-hexane, it will obtain the ethyl acetate fraction. The results as whole partition are the fraction of n-hexane, ethyl acetate fraction and water fraction.

Isolation of active antioxidant compounds ethyl acetate fraction [24] (modified):

Ethyl acetate fraction further elucidated to obtain pure compounds that have antioxidant activity. Ethyl acetate fraction was separated and column chromatography using a gradient of 10% (v/v) n-hexane-ethyl acetate for the target compound fraction F1, gradient of 5% (v / v) n-hexane-ethyl acetate for the target compound fraction F2, gradient of 1% (v/v) H2O-methanol for the target compound fraction F3. Fraction F3 further elucidated with isocratic chromatography column with eluent n-hexane-ethyl acetate 95:5 (v/v). Spot stain pattern observed with UV light λ 254 nm, λ 365 nm, and the spraying of 10% H_2SO_4. The antioxidant activity was observed by TLC plate in the DPPH immersion of 0.05%. Eluent resulted in fractions with a separate stain spots, had a high antioxidant activity and the number of large masses chosen as the target compound. When the results of the TLC identification provided a single spot, the continued determination of the structure of the target compound with a ^{13}C-NMR spectra and ^{1}H-NMR 2D.

Determining the structure of the compound [25,26] (modified):

Pure compounds isolated released from organic solvents. Then diluted with solvent deuterium compounds and measured shift. The results of ^{13}C-NMR, ^{1}H-NMR 2D spectrum subsequently determined the structure of compounds by methods 135^0 DEPT, ^{1}H-^{1}H-COSY, HMQC, and HMBC [24].

Results and Discussion

Isolation and identification of active compounds antioxidant

Isolation and identification of active compounds antioxidants can be done by using column chromatography and thin layer chromatography (TLC). Column chromatography fractions produce better results based on the level of the polarity of the eluent. While TLC used for the identification of pure compounds by elucidating fractions partition column chromatography results. TLC spot stain pattern can be seen with UV light irradiation λ 254 nm, λ 365 nm, and spraying with 10% H_2SO_4. The activity of antioxidant compounds can be viewed by dipping the TLC plate in 0.05% DPPH in ethanol.

F1 fraction of target compounds present in fractions F1.2 with a mass of 2.3 g. Single spot of F1.2 was evident at λ 365 UV irradiation and spraying H_2SO_4 10%, while the high antioxidant activity was evident with immersion DPPH a 0.01% (Fig. 1). Elucidation of fraction F1.2 produce stain spots with high antioxidant activity in fractions F2.12, F2.14 fractions and fractions F2.15. Among these fractions F2.14 with a mass fraction of 1.4 g was chosen as the target compound F2, because the fraction of the F2.14 provides more separate spot stain and the amount is less than the other fractions (Fig. 2).

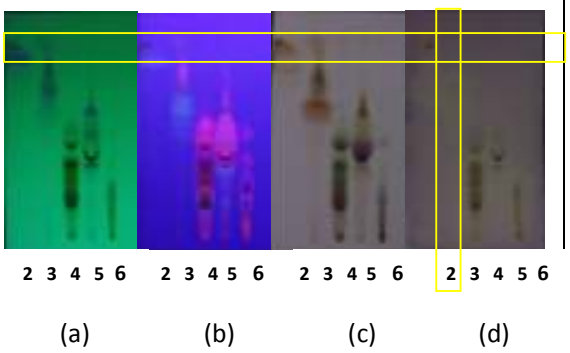


Fig. 1. TLC fractions F1.2 - F1.6 with n-hexane: ethyl acetate solvent (4: 1)

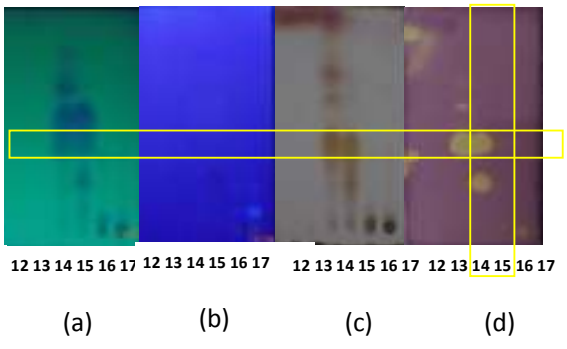


Fig. 2. TLC F2.12 - F2.17 with n-hexane: ethyl acetate solvent (9.5: 0.5)

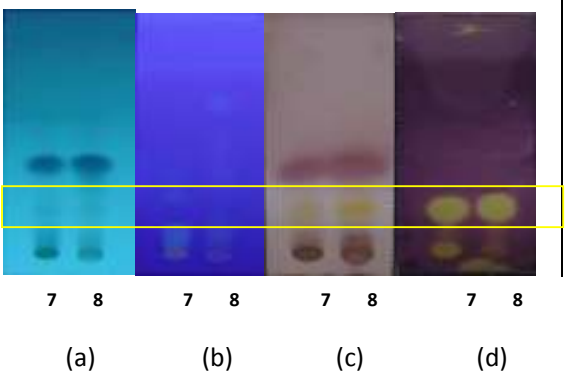


Fig. 3. TLC fractions F3.7 - F3.8 with methanol solvent

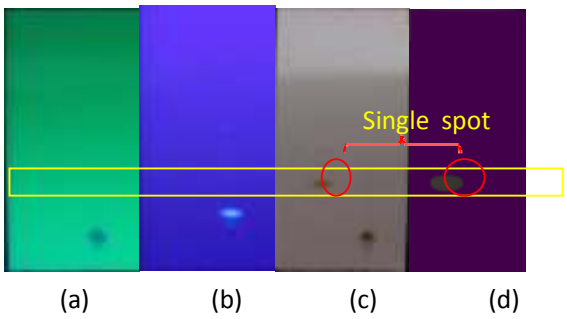


Fig. 4. TLC F4.80 and F4.120 with n-hexane: ethyl acetate solvent (95: 5)

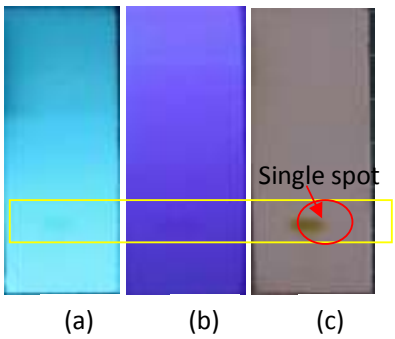


Fig. 5. TLC reversed-phase fraction F4.80-F4.120 with methanol

Fig. 1-5. Isolation and identification of active antioxidant compounds fraction of ethyl acetate (a) Viewed under UV 254 nm, (b) Viewed under UV 365 nm, (c) Sprayed with 10% H_2SO_4 in ethanol, and (d) Dipped in a solution of 0.05% DPPH in methanol

Results elucidation fraction F2.14 produce stain spots on the active antioxidant fractions F3.7 and F3.8 with the distance and the same pattern of staining (Fig. 3). Both fractions were selected as target compounds F3. The combined mass fraction of F3.7 and F3.8 is 150 mg. For further elucidation of F3.7 and F3.8 fraction, we conducted isocratic column chromatography to obtain the target compound F4. Single spot stains resulting in

fractions of up F4.120 F4.80 with a mass of 96.4 mg (Fig.4). To prove that the fraction of the F4.80 - F4.120 only have 1 spot stains, it is necessary to reverse TLC phase with eluent methanol. Results reversed phase TLC (Fig. 5) gives a single spot stain on UV radiation λ 254, uv λ 365 and spraying 10% H_2SO_4. Subsequently reversed phase TLC said as a pure compound.

Structure determination of antioxidant active compounds

Based on data from ^{13}C-NMR spectrum and ^{1}H-NMR, targeted antioxidant active compounds have 29 carbon atoms, 50 hydrogen atoms and two oxygen atoms with molecular formula $C_{29}H_{50}O_2$.

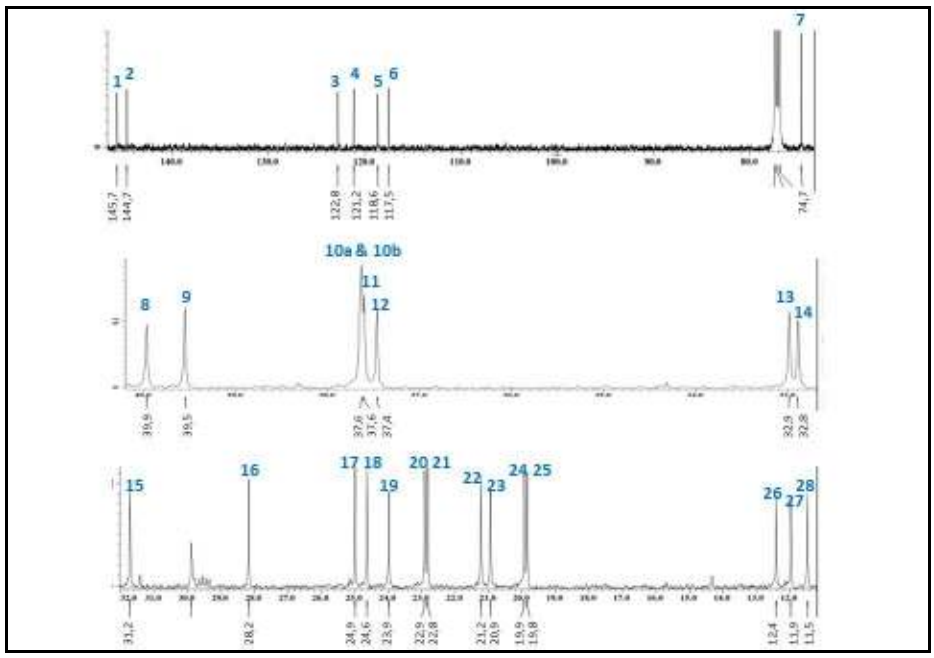


Figure 6. ^{13}C-NMR spectrum of the target compound (a) above 100 ppm; b) and c) below 100 ppm.

Fig.6 (a,b,c) showing signs of carbon. C1-C6 is a double carbon bond because the signal is above 100 ppm (Fig. 6a.). Sixth carbon is thought to form benzene ring which gives the value of DBE 4. While one another DBE value is derived from the cyclization carbon single bonds. Other carbon signals (C7-C28) is a single bond with the carbon signal values below 100 ppm[24,26] (Fig. 6b and Fig 6c). By comparing the ^{13}C-NMR spectral data with DEPT spectral data and HMQC spectrum, known target compound having seven carbon quarterner (-C-), three carbon metin (-CH), eleven carbon methylene (-CH_2) and eight carbon methyl (-CH_3).

DEPT data provide information C1-C7 carbon is quaternary carbon (-C-), carbon C13, C14 and C16 are metin carbon (-CH). Then eleven carbon methylene (-CH_2) is C8, C9, C10a, C10b, C11, C12, C15, C17, C18, C22 and C23. Furthermore, there are eight-carbon methyl (-CH_3) is C19, C24, C25, C26, C27 and C28.

HMQC spectrum of interpretation of the data will be obtained correlation or relationship between proton and carbon as a bond. This spectrum to determine the specific allegations of a carbon bonded to protons and how many protons are bonded to the carbon [24,26,27]. HMQC spectrum data reinforce and ensure data DEPT spectrum.

^{1}H-NMR spectrum of informing the number, type and environment of each proton contained in a compound. ^{1}H-NMR spectral data of target compounds in Fig. 9 shows eight methyl hydrogen signal that the chemical shift of 2.16 (3H, s), 2.11 (6H, s), 1.23 (3H, s) and 0.83 -0.87 (12H) ppm. Signal hydrogen hydroxy (-OH) is available at 4.18 (1H, s). Proton signal at 2.60 (2H; t) indicates the presence of methylene (-CH2) which has two neighboring hydrogen. Other hydrogen signal is a signal of hydrogen to the aliphatic chain.

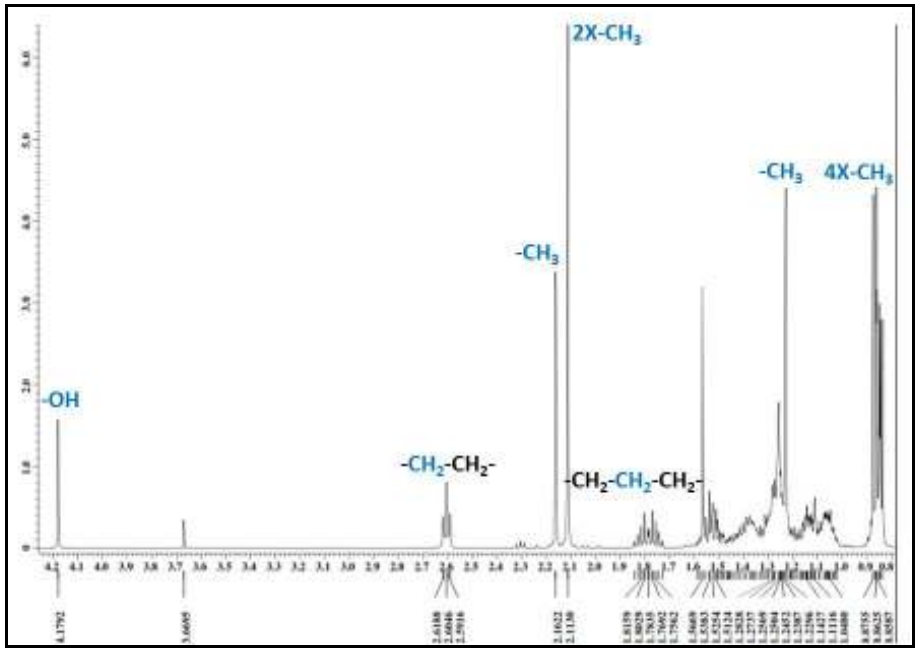


Fig. 7. ^{1}H-NMR spectrum of target compounds (500 MHz in chloroform-D)

Spectrum HMBC (Heteronuclear Multiple Bond Connectivity) can be used to determine the position of functional groups in a compound with the correlation between the proton and carbon a distance of two to three bonds (2J and 3J), but sometimes capable of measuring up to four bonds (4J) [24.26,27]. HMBC spectral data of target compounds show that there is a correlation between protons of hydroxyl (-OH) with C5, proton correlation methyl (-CH_3) to C28 (H28) for C1 and C5, proton correlation methyl (-CH_3) to C27 (H27) with C1, C3 and C4, and correlation proton methyl (-CH_3) to C26 (H26) with C2, C3 and C4. Third correlation methyl protons (-CH_3) is (H26, H27 and H28) with some carbon shows that the position of the OH group bonded to C1, where C1 is the neighboring carbon C3 and C5. This group is at benzene ring (aromatic system) because the carbon C1-C5 which is correlated with one another is a sp2 carbon.

Methylene (-CH_2) of carbon C23 correlated with C6. Correlation C16 to C15 C6 reinforces that are in addition to C23. Substituents at C2 allegedly are O or N atoms because the value of the shift is similar to the C1 binding OH group. C15 fragment containing further established by HMBC spectrum interpretation. Correlation C23 and C15 are suspected of mutual neighbors, this was confirmed by the correlation of C15 to C23 Hydrogen. Correlation atoms C2, C6, C23, C15 and C7 form a cyclic furan, the oxygen atom is located between C2 and C7. Illustration alleged ring structure formation / cyclic furan, shown in Figure 8.

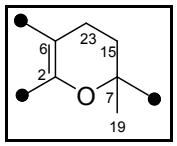


Fig 8. Alleged cyclic / furan ring formed by C2, C6, C7, C15 and C23

Fragments HMBC spectrum interpretation results, showing that C19 (methyl) have the relationship with the C7, C8 and C15. C7 atom which is oxygenated quaternary carbon binds the three more carbon C8 (methylene), C15 (methylene) and C19 (methyl).

1H-1H COSY provides information regarding the correlation of H-23 to H-15. Correlations of H-16 with geminal methyl hydrogen (H-20 and H-21), the correlation of hydrogen methyl C24 and C25 respectively to carbon metin (-CH) at C13 and C14. Correlation of Hydrogen methylene (-CH2) on C12 with hydrogen methylene (-CH2) at C10a, C10b and C11. All correlations were obtained from the interpretation of 1H-1H COSY spectrum is the formation of aliphatic chains. Based on the NMR spectrum of the fragments, we obtained as following Fig 9.

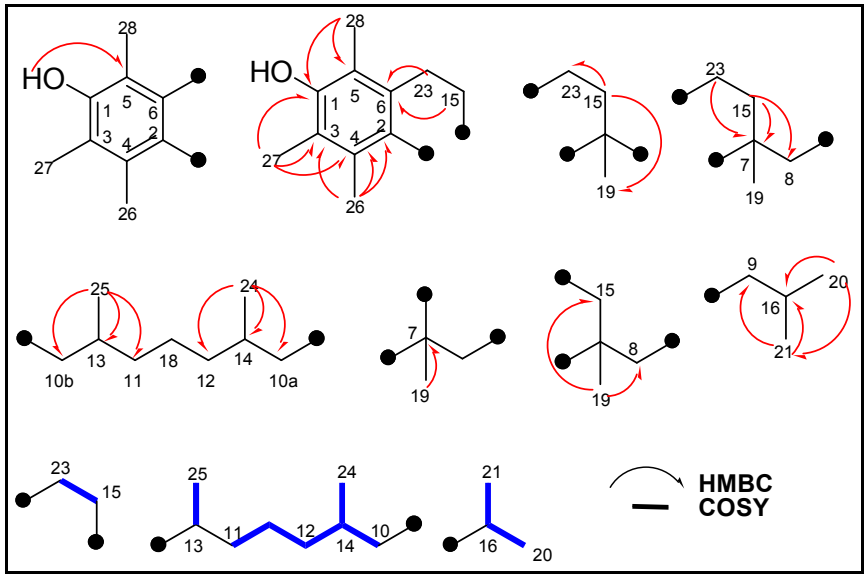


Fig. 9. Fragments of the target compound

Table 1. Chemical shift data of 1H-, 13C- and 2D-NMR target compound.

No	d_C (ppm)	d_H (ppm)	DEPT	HMBC	COSY
1	145,7	-	C	-	-
2	144,7	-	C	-	-
3	122,8	-	C	-	-
4	121,2	-	C	-	-
5	118,6	-	C	-	-
6	117,5	-	C	-	-
7	74,7	-	C	-	-
8	39,9	1,56 (2H; *m*)	CH_2	-	-
9	39,5	1,14 (2H; *m*)	CH_2	-	-
10-a	37,6	1,26 (2H; *m*)	CH_2	-	H-12
10-b	37,6	1,26 (2H; *m*)	CH_2	-	H-12
11	37,6	1,26 (2H; *m*)	CH_2	-	H-12
12	37,4	1,05 (2H; *m*)	CH_2	-	H-10, H-11
13	32,9	1,40 (2H; *m*)	CH	-	-
14	32,8	1,40 (2H; *m*)	CH	-	-
15	31,2	1,78 (2H; *m*)	CH_2	C-6, C-7, C-8, C-23	H-23
16	28,2	1,52 (1H; *m*)	CH	-	-
17	24,9	1,26 (2H; *m*)	CH_2	-	-
18	24,6	1,26 (2H; *m*)	CH_2	-	-
19	23,9	1,23 (3H; s)	CH_3	C-7, C-8, C-15	-
20	22,9	0,87 (3H; s)	CH_3	C-16, C-20, C-21, C-9	-
21	22,8	0,86 (3H; d;)	CH_3	-	-
22	21,2	1,35 (2H; *m*)	CH_2	-	-
23	20,9	2,60 (2H; *t*)	CH_2	C-6, C-7	H-15
24	19,9	0,84 (3H; *d*)	CH_3	C-10, C-12, C-14	H-14
25	19,8	0,83 (3H; *s*)	CH_3	C-10, C-11, C-13	H-13
26	12,4	2,16 (3H; *s*)	CH_3	C-2, C-3, C-4	-
27	11,9	2,11 (3H; *s*)	CH_3	C-1, C-3, C-4	-
28	11,5	2,11 (3H; *s*)	CH_3	C-1, C-5	-

Based on data from the NMR spectrum, the structure notion of the active compounds is in the form of targeted antioxidant phenolic derivatives. To support the notion of structure, NMR data is compared with the target compound α-tocopherol compounds [28,29] which is derived phenolic compounds (Table 2 and Fig.10).

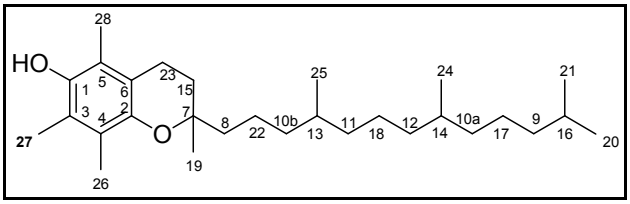


Fig.10. Alleged target compound structure

Comparative data value 1H- and 13C chemical shift NMR-active compounds targeted antioxidant α-tocopherol compounds [28.29] can be seen in Table 2. The structure of comparative compounds can be seen in Fig.11.

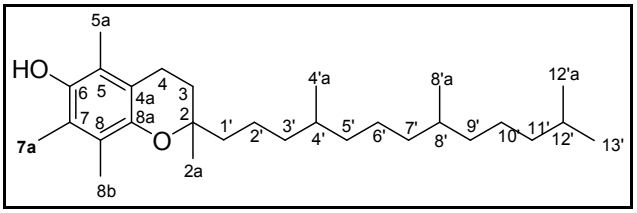


Fig.11. Structure of α-tocopherol

Chemical shift data similar to the data target compound α-tocopherol chemical shift. The chemical shift difference is caused by differences in the solvent used at the time of measuring the NMR and power tools used NMR spectroscopy.

Table 2. Data 1H- and 13C-NMR target compounds and α-tocopherol.

No	Position	d_C (ppm)		d_H (ppm)	
		Target compound	α-tocopherol	Target compound	α-tocopherol
7	2	74,7	74,6	-	-
15	3	31,2	31,6	1,78 (2H; *m*)	1,78 (2H; *m*)
23	4	20,9	20,8	2,60 (2H; *t*)	2,62 (2H; *t*)
5	5	118,6	118,5	-	-
1	6	145,7	145,6	-	-
3	7	122,8	122,6	-	-
4	8	121,2	121,1	-	-
19	2a	23,9	23,8	1,23 (3H; *s*)	1,25 (3H; *s*)
6	4a	117,5	116,4	-	-
28	5a	11,5	11,2	2,11 (3H; *s*)	2,13 (3H; *s*)
27	7a	11,9	11,8	2,11 (3H; *s*)	2,13 (3H; *s*)
2	8a	144,7	144,6	-	-
26	8b	12,4	12,2	2,16 (3H; *s*)	2,14 (3H; *s*)
8	1'	39,9	39,9	1,56 (2H; *m*)	-
22	2'	21,2	21,1	1,35 (2H; *m*)	-
10	3'	37,6	37,3	1,26 (2H; *m*)	-
13	4'	32,9	32,8	1,40 (2H; *m*)	-
11	5'	37,6	37,5	1,26 (2H; *m*)	-
18	6'	24,6	24,5	1,26 (2H; *m*)	-

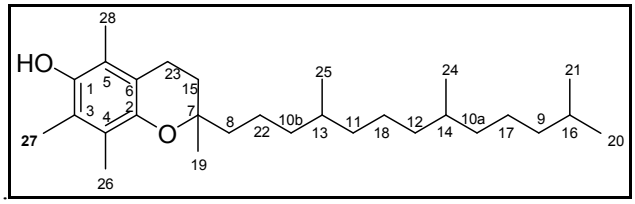
a) Varian Mercury plus 400 NMR spectrometer

b) Spectrometer JEOL

Conclusion

The results of column chromatography isolation and identification of TLC showed that the fraction of F4.80 to F4.120 have high antioxidant activity with a single spot stain. Pure compounds were obtained at 96.4 mg of 22.2 g of ethyl acetate extracts hypokotil *B. gymnorhiza.*

Based on the analysis 135^0 DEPT, ^{1}H-^{1}H-COSY, HMQC, and HMBC, the structure of the active compounds antioxidants fraction F4.80 - F4.120 has 29 carbon atoms, 50 hydrogen atoms and two oxygen atoms ($C_{29}H_{50}O_2$). This compound is a derivative of phenolic compounds with molecular structure resembles the structure of α-tocopherol compounds



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References

1. Purnobasuki, H. 2004. The potential of mangroves as a medicinal plant. Department of Biology, Faculty of Mathematics and Natural Sciences, Airlangga University, Surabaya. http: www.irwantoshut.com.
2. James A, Allen JA, Duke NC. *Bruguiera gymnorrhiza* (large-leafed mangrove), Rhizophoraceae (mangrove family). Species Profiles for Pacific Island Agroforestry. www.traditionaltree.org, 2006.
3. Bandaranayake WM. Bioactivities, bioactive compounds and chemical constituens of mangrove plants. AIMS Research. Australia Institute of Marine Science, http: www.aims.go.au., 2002.
4. Achmadi S, Syahbirin G, Choong ET, Hemingway RW. 1994. Catechin-3-O rhamnoside chain extender unitsin polymeric procyanidins from mangrove bark. J. Phytochemistry, 35: 217–219.
5. Bandaranayake WM, 1994. Phyto-chemical constituents and pigments in mangrove species and mangal associates of Northern Australia. Australian Institut of Marine Science, Townsville.
6. Balasooriya SJ, Sotheeswaran S, Balasubramanium S. Economically useful plants of Sri Lanka. Part IV.Screening of Sri Lanka plants for tannins. J. Nat. Sci. Counc. Sri Lanka, 1982, 10, 213–219.
7. Ganguly SN, Sircar SM. Gibberellins from mangrove plants. J. Phytochemistry, 1974, 13: 1911–1913.
8. Ghosh A, Misra S, Dutta AK, Choudhury A. Pentacyclic triterpenoids and sterols from seven species of mangrove. J. Phytochemistry, 1985, 24: 1725–1727.
9. Hogg RW, Gillan FT. Fatty acids, sterols and hydrocarbons in the leaves from eleven species of mangrove. J. Phytochemistry, 1984, 23: 93–97.
10. Ravi AV, Kathiresan K. Seasonal variation in gallotannin from mangroves. Indian J. Mar. Sci., 1990, 25: 142–144.
11. Rollet B. Bibliography on mangrove research. 1600–1975. UNESCO Paris. Pub. Information Retrieval Ltd., London, 1981, 479 pp.
12. Seshadri TR, Venkataramani B. Leucocyanidins from mangroves. J. Sci. Ind. Res., 1959, 18B: 261–262.
13. Shinoda Y, Ogisu M, Iwata S, Tajima T. Chemical composition of mangroves. 11. Gifu Daigaku Nogakubu Kenkyu Hokoku, 1985, 50: 155–165.
14. China paper (*China's Outstanding Master's Theses Part C)*. Chemical constituents from Mangrove *Bruguiera Gymnorrhiza* and their bioactivities, 2010.

15. Duh PD. Antioxidant activity of burdock (*Arctium lappa* Linne): its scavenging effect on free radical and active *oxygen*. J. Am. Oil chemist' Soc., 1998, 75: 455-465.
16. Duh PD, Tuh YY, Yen GC. Antioxidant activity of water extract of *Harng Jyur (Chrysanthemum morifolium* Ramat*)*. Lebne-smittel-Wissenschaft and Technologie, 1999, 32: 269-277.
17. Bunyapraphatsara N, Jutiviboonsuk A, Somlek P, Therathanathorn W, Ksormkaew S, Fong HHS, Pezzuto JM, Kosmeder J. Pharmacological studies of plants in the mangrove forest. J. Phytopharm., 2003, 10 (2).
18. Jacoeb AM, Suptijah P, Zahidah. Chemical composition, bioactive component and antioxidant activity of large-leafed Mangrove (*Bruguiera gymnorhiza*) Fruit. Aquatic Product Technology Department, Faculty of Fisheries and Marine Science. Bogor Agricultural Institute. Indonesia, 2013.
19. Packer L. The antioxidant miracle – your complete plan for total health and healing. New York: Wiley, 1999.
20. Ito N, Fukushima S, Hasegawa A, Shibata M, Ogiso T. Carcinogenecity of butylated hydroxylanisole in F 344 rats. J. National Cancer Institute, 1983, 70: 343-347.
21. Sunarni T. Free radical scavenger antioxidant some sprouts from seeds fam. Papilionaceae. J. Pharm. Indonesia, 2005, 2 (2): 53-61.
22. Rohdiana D. Free radical scavenger of polyphenols in tea leaves. Magazines of Journal Indonesia, 2001, 1: 53-58.
23. Harbone JB. Chemical Method: how to analyze modern plants. Publisher ITB. Bandung, 1987.
24. Supratman U. Eludation structure of organic compounds: spectroscopy methods for determining the structure of organic compounds. Widya Padjadjaran, Bandung, Indonesia, 2010.
25. Jeol Ltd. Global Solution Provider for Advanced Technology. Tokyo, 196 8558, 2005.
26. Kosela S. Determination of molecular structure based data spectra (NMR, MASS, IR, UV). Publisher Institute Faculty of Economics, University of Indonesia. Jakarta, 2010.
27. Jane UA, Kardiono LBS, Hanafi M, Rumampuk RJ, Darmawan A. modern techniques of NMR Spectroscopy: Theory and applications in structure elucidation of organic molecules and biomolecules. Indonesian Institute of Sciences. Jakarta. 2006.
28. Chen CR, Chao LH, Pan MH, Liau YW, Chang CI. 2007. Tocopherols and Triterpenoid from Side acuts. J. Chinese Chem. Soc., 54: 41-45
29. Odinokov VN, Spivak AY, Emelyanova GA, Mallybaeva MI, Nazarova OV, Dzhemilev UM. Synthesis of α-tokopherol (vitamin E), vitamin K_1-chromanol, and their analog in presence of aluminosilicate catalysts Tseokar-10 and Pentasil. ARKIVOC, 2003, XII: 101-118.

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