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International Journal of Chemistry; Vol. 6, No. 2; 2014 ISSN 1916-9698 E-ISSN 1916-9701 Published by Canadian Center of Science and Education 15 Isolation and Structure Elucidation of Bioactive Compounds Chemical as Inhibitors of the Enzyme ? -Glucosidase Raru Bark Ethanol Extract (Vatica pauciflora Blume) Ida Duma Riris1, Tonel Barus2, Partomuan Simanjuntak3 & Basuki Wirjosentono2 1 Faculty of Mathematics and Natural Sciences, State University of Medan, Indonesia 2 Faculty of Mathematics and Natural Sciences, North Sumatera University, Medan Indonesia 3 Research Centre for Biotechnology, Indonesian Institute of Sciences (LIPI), Cibinong Indonesia Correspondence: Ida Duma Riris, Facu lty of Mathematics and Natural Sciences, State University of Medan, Indonesia. E-mail: dumariris@gmail.com Received: January 5, 2014 Accepted: Februa ry 11, 2014 Online Published: March 6, 2014 doi:10.5539/ijc.v6n2p15 URL: http://dx.doi.org/10.5539/ijc.v6n2p15 Abstract Inhibitory compound of a -glucosidase bioactivity was isolated from ethanol extract of the stem bark of raru (Vatica pauciflora Blume) which, is a wild plant that grows in Tapanuli state in Indonesia.The ethanol extract of the stem bark of raru was partitioned and was chromatogr aphed on columns with stationare fase silica gel 60 mesh F254 (0.063 mm-0.200 mm).

The ethanol extract of isolates showed the enzyme a -glucosidase inhibitor IC50 of 93.46. The next step is the determination of the chemical structure of the Ultraviolet–visible spectroscopy (UV Vis); FT-IR (Fourier Transform Infrared spectroscopy), NMR (Nuclear Magnetic Resonance) namely COSY, Heteronuclear Multiple-Quantum Correlation (HMQC); and Heteronuclear Multiple-Bond Correlation (HMBC).

It found of compounds consist of two of methoxy, one of aromatic, and one of carbonil

compounds. The isolat is a 3,4,9,Trihidroxy-2-(hydroxymethyl)-8-10-dimethoxy-2-3-4atetrahydropyrano(3,2-c)isochromen-6(10bH)-one acording to International Union of Pure and Applied Chemistry (IUPAC). Keywords: raru (Vatica pauciflora Blume), antidiabetic, a -glucosidase inhibitor 1.

Introduction The use of traditional medicine is Indonesian culture that goes back thousands years ago. It is a cultural heritage of Indonesia that need to be explored, researched, and developed (Hedi, 2007). Consumed plant extracts for the treatment, which is made in the form of decoction, herbal remedies and herbal capsule form.

Things like this do to treat diabetic mellitus. Diabetic Mellitus (DM) is a co ndition of the concentration of glucose in the blood is chronically higher than normal value (hyperglycemia). This is caused by a deficiency of insulin or the insulin does not function effectively.

Diabetic can lead to variou s diseases such as hypertension, stroke, coronary heart disease, and kidney failure (Guyton & Hall, 2007). Measurement of glucose levels can be determined in vitro by enzymatic methods (Lucile, 1997). Spectrophotometer for color intensity is used hereinafter blood glucose levels can be determined.

According to World Health Organization (2005), more than 80% of the population in developing countries consume natural ingredients derived mainly from plants, either as a form of maintaining health and medicine. The use of natural medicine is intended to avoid the side effects of chemical drugs. And plant-based treatments currently has a market share of about 30%.

Adebayo (2008) found extracts of plants as antidiabetic impact activities such as: Vermonia amygdalina, Bidens pilosa, Carica papaya, Citrus aurantiifolia, Ocimumgratisimum, Momordica charantia and Morindalucida these plants have been consumed in Nigeria. Likewise Gunawan (2009) discovered a flavonoid in four raru plant species, namely (1) CotylelobiummelanoxylumPierre, (2) Shoreabolancarpoides Symington, (3) Cotylelobiumlanceolatumcraib, and (4) Pierre Cotylelobiummelanoxylon be expressed that these compounds can lower blood sugar levels in vitro.

There are three compounds that have activit y flafonoid as antidiabetic, namely (1) 3- ß -hydroxynaringenin or www.ccse n isoaroma d Compoun d ethanol e x COSY, H M extract fr o 8-geranyl - In the eth a b arrier tes t b ark raru stem bark raru type enzima -gl u elucidatio n chemical s HMBC), a 2.

Metho d Raru bark levels of p toxicity w of the co m smaller t h Artemiasa having L C Bioactivit y inhibition The enzy m spectroph o Extract th a extraction n et.org / ijc d endrin, (2) ta x d s 1 and 2 w e x tract of Salvi a M QC and Nu c o m the leaves - 4,5,7-flavone a nol extract o t conducted b y (Vatica pauci f extract is hig h s Vatica pa u u cosidase. To n pe r formed o s tructure dete r a nd HR MS.

d s and Mater i samples (Vat i p olarity, name l ith BSLT met h m pound that gi v h e value, the a lina Leach ha s C 50 > 1000 mg / y inhibition o of solving the m e activity w a o tometry at ? a t has the hig h scheme show n Figure 1. E x ifolin, and (3) e re found fro m a L. verbenac a c lear Overhau s of plants Art o trihidroksi.

T h f the stem ba r y Ida (2013) i n f lora blume), h er than ethyl a u ciflora Blu m xicity tests c o o n extracts th a r mined by spe c i als i ca p auciflora l y n-hexane, e t h od. With thi s v es as much a greater the t o s LC 50 < 30 m g / mL (Steven & o f the enzym e model substr a a s measured b 400 nm (M a h est bioactivi t n in Figure 1.

E xtraction sch e Internationa l) 5-hydroxy-3, m plant Eupho r a Flavonoids w s er effect spe c o carpus com m h is can inhibit r k raru contai n n vitro against obtained bio a a cetate, hexan e m e extracted, o nducted by B a t have inhibit o c troscopic spe blume) extra c t hyl acetate, 9 6 s method, t he t s 50% mortali o xicity LC 50 . g / mL. LC 50 h & Russell, 199 3 e a -glucosida s a te p-nitrophe n b ased on the a tsui, 2001).

P t y separated b y e me, fractiona t l Journal of Ch e 16 4', 7 trimetho x r bia cuneata V w ere identifie d c troscopy (NO m unis flavono i a -glucosidase n ed flavonoi d hexane, ethyl a ctivity of a -g l e and water b y and further B rine Shrimp o ry activity o f ctral data (U V c ted by solven t 6 % ethanol, a n t oxicity of a c ty rate. Activ e A sample sai h as declared t o 3).

s e carried ou t n ol- a -D-gluco p absorbance o f P hytochemica l y column chr o t ion of the ste m e mistry x iflavonone. F V ahl, and com p d by spectros c O ESY) (Bahar, i d found to h a enzyme IC 50 1 d s are phytoch acetate, ethan o l ucosidase in h y using acarbo testing of Lethality Te s f a -glucosidas V , FT IR1, N M t extraction m n d water.

Eac h ompound wit h e compounds w d to be high l o xic when 30- t in vitro by p yranosid to p f p-nitrophen o l test was co n o matography. m bark of raru F lavonoids ext r p ounds to the c opic data 1 H , 2005). Isolat i a ve activity a s 1 8.12 pg mL -1 emicals. Anti d o l, and water f h ibition of et h se as a contro l the inhibitor y s t (BSLT).

Fu r e enzyme of t M R1D, 2D N M m ethod, that st r h extract was c h LC 50 value i w ill provide a h l y toxic to s h 1000 mg/mL a the method o p -nitrophenol a o l right gener

a n ducted usin g The shape of (Vatica pauci f Vol. 6, No. 2; r acted with et h three found i n , 13 C NMR, D i on of ethyl a c s an antidiabe t (Puspa, 2008) d iabetic bioac t f rom the plant h anol extract o l .

In this stud y y activity a g r ther isolatio n t he most high . M R, COSY, H M r atified by dif f c oncentrated, t s the concent r h igh mortalit y h rimp larvae w a nd less toxic w o f Kawanishi , a nd glucose y e a ted by U V -v i g Harborn m e the stem bar k f lora) 2014 h anol. n the D EPT, c etate i c, ie) . t ivity stem o f the bark g ainst n and .

The M QC, f erent e sted r ation . The w hen w hen that e llow. i sible t ho d . k raru www.ccsenet.org/ijc International Journal of Chemistry Vol. 6, No. 2; 2014 17 2.1 Fractionation by Column Chromatography Method Extract ethanol fractionation by chromatography column I, performed by taking as much as 5.0 g of ethanol extract, and further fractionated by column chromatogra phy using silica gel stationary phase 60 mesh.

And the mobile phase used was chloroform-methanol (40:1~1:1). The compounds in botanicals, grouped by polarity in the form of fractions. To obtain a more simple fractions, done by combining fractions that have the same pattern of the chromatogram. Results fractions of column 1 are combined into 14 fractions, and then tested the inhibition of the enzyme a -glucosidase.

The test results showed inhibition of the enzyme a -glucosidase is the fraction 9. Extract ethanol fractionation by column chromatography II made to the fraction 9 that the same method as in column I. Results fractions were combined, and obtained six fractions, were then tested for inhibition against a -glucosidase enzyme.

Further separation of fraction VPEt-9 with chromatography columns III conducted in fractions 9-4 with the same method as in column I. Results fractions were co mbined and gained 5 fractions. Tests conducted on the inhibition of the enzyme a -glucosidase. Provided that fractions 9-4 VPEt-4 of the ethanol extract had a -glucosidase inhibitory activity of the most high, and further separated by column chromatography.

This was done to obtain a pure compound. Purity test using 2-dimensional TLC performed on fractio ns VPEt-9-4-4 obtained from test results antidiabetic. Analysis was performed using silica gel stationary phase GF 254 plates with a mobile phase of chloroform-methanol (2:1), and chloro form-acetonitrile (2:1). Chemical stru cture determination carried out by UV-Vis spectroscopy, FT-IR, NMR analysis (1H.13C-NMR and DEPT) and 2D NMR (COSY, HMQC, HMBC), and the number of isolates VPEt-9-4-4 reconstituted with CD3OD . 3.

Results The yield of extraction results shown in Table 1, where the highest obtained in the extraction of ethanol. Table 1. The results of the yield of the extract of the stem bark of raru (Vatica pauciflora Blume) Sample Weight (g) The yield of (%)* n-hexane 6,21 0,62 Ethylacetate 58,62 5,86 Ethanol 76,13 7,61 Distilled water 19,47 1,95 Description: * 1 kg calculated on the dry crude drug.

The result of extract toxity test of n-Hexane, ethylacetate, ethanol and water and water by BSLT method, each of them is n-hexane extract LD 50 = 368,51 ppm, ethylacetate = 19,45, etha nol = 5,76 ppm, dan air = 36,22 ppm. It shown that ethanol extract has the least toxity. The results of Antidiabetic test of each extract by a -glucosidase inhibitory method is shown in Table 2. Table 2.

Test results antidiabetic extract using an enzyme inhibition method a glucosidase Extract Inhibisi (%) n-hexane 28,98 Ethylasetate 60,83 Ethanol 91,08 Water 78,34 Phytochemical test results on ethanol extract of bark which has bioactivity antidiabetic raru highest, with Harborn method, indicates that there are flavonoids, sapon ins, quinones, tannins, triterpenoids, essential oil, and coumarin.

The results of the analysis of NMR (Nuclear Magnetic Resonance) 1-dimensional (1H and 13C-NMR) is based www.ccse n on Silvers deutereu m JEOL 500 1H- N MR indicate t h (OCH 3) i n Some me t characteri s (s), which 13C- N M R Enhance m extract ha d The carbo n n et.org / ijc tein (1991).

P m methanol (C spectrophoto m spectra of et h h e type and n u n the area of c h t in proton (C H s tic for the pr o does not reso n R spectra To m ent by Polari d inhibition a g n yl atom loca t P roton spectra D3OD) (0.5 m m eter (1 H- N M h anol extracts u mber of pro t h emical shift H) are the ch o tons that res o n ate with othe r Isolate 9-4-4 - zation Transf e g ainst a -gluco s Figur t ed very down Internationa l (1 H- N MR an d m L), respecti v R MHz and 13 to isolate 9-4 - t ons containe d (chemical shi f emical shifts o nate with the r protons. -1. 13C- N M R e r) shows the r s idase enzyme e 2.

DEPT 13 C field that is a t l Journal of Ch e 18 d carbon (13 C- v ely in the N M C- N MR at 12 5 - 4-1. Proton n d in t he ethan o f t) d H 3.87 a n d H 3.53; 3.5 4 oxygen atom, R spectra an d re are 15 car b highest as sh o C - N MR spect r t d C 166.15 (s) e mistry - N MR) was d o M R tube (5 m 5 MHz). n uclear magne t o l extract of i n d 3.97 (3H) i n 4 ; 3.77; 3.82; and one olef i d DEPT expe b on on the c h o wn in Figure 2 r um of isolate s) .

Methoxy pe a o ne by dissol v m m). Spectra w t ic resonance solates 9-4-4- n the singlet s 3.96; 3.99, a i nic proton at riments anal y h emical struc t 2 . s a ks are at d C 6 Vol. 6, No. 2; v ing the samp l w ere recorded spectra (1H- N 1. Proton me t plitting patter n a nd 4.80, whi d H metin that y sis (Distorti o t ure of the et h 6 1.43 (q) and 6 2014 l es in on a N MR) h oxy n (s). c h is 7,32 nless h anol 6 2.02 www.ccsenet.org/ijc International Journal of Chemistry Vol. 6, No. 2; 2014 19 (q). The presence of the chemical shift in the high field region is about d C 62.6 (t) and 71.71 (d), 73.26 (d), 76.05 (d), 81.91 (d), 82.70 (d), all of which resonate carbon atoms with oxygen atoms. And for the aromatic carbon present in d C 115.00 (d), 120.62 (s), 125.97 (s), 148.72 (s), 152.64 (s) and 152.72 (s).

The relationship between carbon and proton in the ethanol extract of the isolates showed no association between the signal and the carbon nucleus on the same proton. Spot spectrum indicates the two are directly related to the bond. Proton signal at d H 3.79 ppm seen (H1-13) and d H 3.99 (H2-13). C-12 at d C 73.26 with d H 4.80 (H-12), C-3 at d C 115.00 with d H 7.32 (H-3).

The relationship between protons and protons in the chemical structure of 9-4-4-1 isolates appear from analysis of COSY spectra. The correlation sugge sts that the protons of methylene (CH 2) d H 3.79 (H1-13) and 3.99 (H2-13) associated with the proton at d H 4.80 (H-12); d H 4.80 (H-12) with d H 3.96 (H-11). Likewise d H 3.53 (H-10) with d H 3.82 (H-9); d H 3.82 (H-9) with d H 3.54 (H-8).

O O OH OH OH O HO OCH 3 H3CO H H H H H H H H Figure 3. Cosy spectra analysis results for isolates chemical structure 9-4-4-1 The results of the analysis of HMBC spectra of carbon visible at the position C-1 (d C 166.15) C-2 (d C 125.97), C-4 (d C 152.64), C-5 (d C 152.72) and C-7 (d C 120.62). The existence of long-range HMBC Correlation of the spectra looks like Figure 3.

Chemical shifts for proton and carbon chemical structure isolates 9-4-4-1 shown in Figure 4. O O OH OH OH O HO OCH 3 H3CO H H H H H H H H Figure 4. Isolates chemical structure 9-4-4-1 HMBC analysis results www.ccsenet.org/ijc International Journal of Chemistry Vol. 6, No. 2; 2014 20 . O O OH OH OH O HO OCH 3 H3CO H H H H H H H H 12 7 8 9 12 13 3,79 3,99 62,66 (t) 4,80 73,26 (d) 3,99 81,91(d) 3,82 76,05(d) 3,53 71,71(d) 166,15 (s) 3,54 82,70 (d) 120,62 (s) 3,87 (s) 62,02 (q) 3,93 (s) 61,43 (q) 152,64 (s) 148,72 (s) 152,72 (s) 7,32 (s) 115,00 (d) 125.97 (s) Figure 5.

Proton and carbon chemical shifts for chemical structure isolates 9-4-4-1 Carbon chemical shift data for the proton and the chemical structure of 9-4-4-1 isolates presented in Table 3 below. Table 3. Carbon chemical shift correlation and H-13 C NMR

to isolate 9-4-4-1 based on 2D NMR HMQC No. d C (ppm)/DEPT d H (ppm) Number of Carbon according to Chemical Structure 1. 61,43 (q) 3,97 Ome 2. 62,02(q) 3,87 Ome 3. 62,66(t) 3,79;3,99 C-13 4.

71,71(d) 3,57 C-9 5. 73,26(26) 4,80 C-12 6. 76,05(d) 3,82 C-10 7. 81,91(d) 3,99 C-11 8. 82,70(d) 3,54 C-8 9. 115,00(d) 7,32 C-3 10. 120,62(s) - C-7 11. 125,97(s) - C-2 12. 148,72(s) - C-5 13. 152,64(s) - C-6 14. 152,72(s) - C-4 15. 166,15(s) - C-1 Analysis of mass spectroscopy (M S) is conducted by the HR-MS (H igh Resolution Mass Spectroscopy) instrument, showed a molecular ion at m/z 342 (M) +. This indicates isolates 9-4-4-1 has a Molecular Weight (MW = 342) for the molecular formula mass C 15H18O9.

Spektra isolate compounds 9-4-4-1 gave molecular ion peaks and fragment ions in the spectra of m/z: 32; 265; 165 and 121. 4. Conclusions Isolation and structure elucidation of chemical that has activity as an inhibitor of a -glucosidase enzyme from the stem bark raru (Vatica pauciflora Blume) concluded that, (1) Bark raru efficacious as antidiabetic. Inhibit the www.ccsenet.org/ijc International Journal of Chemistry Vol. 6, No.

2; 2014 21 activity of the enzyme a -vitro glucosidase by VpEt fraction 9-4-4-1, that is equal to IC50 93.46. (2) The chemical structure of spectral data based on UV-Vis spectrophotometer, FT-IR and NMR spectrometer and proton carbon Nuclear Magnetic Resonance (COSY, HMQC, and HMBC); HRMS isolates obtained VpEt 9-4-4-1 is bergenin dimethoxy compound. The isolate is a acording to International Union of Pure and Applied Chemistry (IUPAC).

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