Identification of active compounds from lindur root plants (Bruguiera gymnorrhiza) as α-glucosidase inhibitors

S P S Dia*, Nurjanah and A M Jacob

Aquatic Product Technology Department, Faculty of Marine Sciences and Fisheries, Bogor Agricultural University (IPB University), Bogor, Indonesia

*E-mail: putudia15@gmail.com

Abstract. Lindur (Bruguiera gymnorrhiza) is a plant that contains bioactive sources for antioxidants and α-glucosidase inhibitors. The α-glucosidase inhibitors are inhibitors of the α-glucosidase enzyme involved in digestion in the intestine. This study aimed to determine and select the potential inhibitory activity of the α-glucosidase enzyme from the crude extract of lindur by in vitro. This research was carried out through several studies that involved multilevel extraction, testing α-glucosidase inhibitors from crude extracts of lindur root. Fractionated from active extracts of lindur used thin layer chromatography (TLC) & preparative thin layer chromatography (PTLC), and combined compositions bioactive from the most active fraction. The highest yield was obtained from root ethanol extract which was 4.68. Ethanol extract of stem and root bark had an activity of α-glucosidase enzyme inhibitors calculated by IC50 values 171.31 ppm and 153.07 ppm. The fractionation results used preparative (PTLC) showed 5 fractions in crude ethanol root extract. Fraction 1 with Rf 0.15 had the most potential activity of α-glucosidase enzyme inhibitors (161.05 ppm). Identification chemical composition used by Gas Chromatography Spectrometry (GC-MS) showed the composition contained in the fraction 1 of the root ethanol extract mostly (similar to > 90%) was hexadecanoic acid or palmitic acid and phenol.

Keywords: α-glucosidase inhibitors, Bruguiera gymnorrhiza, mangroves, roots

1. Introduction

Changes in lifestyle affect eating habits. People consumed fast food and sweet foods/drinks which are high in sugar. Consumption patterns and not healthy lifestyles caused various degenerative diseases one of them is diabetes. Diabetes mellitus (DM) is a metabolic disease characterized by hyperglycemia, which is an increase in blood sugar levels exceeds from normal levels (80-120 mg/dL), generally caused by inadequate insulin action (Lee 2007). DM has been categorized as a global disease by World Health Organization (WHO). 347 million people have problem with DM in 2012. Amount people with DM in Indonesia was the seventh rank after China. India, The United States, Brazil, Russia, and Mexico. Indonesia first rank in Southeast Asia with a prevalence of 8.4 million people in 2000 and is projected to increase 2.5-fold to 21.3 million by 2030 (WHO 2013).

Diabetes mellitus can be divided into two main types namely type 1 DM and type 2. DM type 1 disease occurs because pancreatic beta cells whose main job is producing insulin can no longer meet the body's insulin needs and even products can be stopped altogether. Whereas type 2 DM occurs due to lack of
insulin receptors found on the surface of intestinal cells (Pulungan and Herqutanto 2009). Handling and effective treatment methods are needed and expected to eliminate major side effects. One alternative approach that can be taken is to prevent hydrolysis of carbohydrates into simple sugars (glucose) in the intestine by inhibiting the performance of the α-glucosidase enzyme to reduce amount of blood sugar. Increase in the number of diabetics encourages development of various ethnobotany research. Most plants have contained bioactive compounds such as glycosides, alkaloids, terpenoids, and flavonoids. These compounds have antioxidant activity and α-glucosidase inhibitors (Suarsana et al 2008). The bioactive component is related to the antioxidant activity of a substance. According results of research by Dia et al (2015) antioxidant activity of ethanol extracts of leaves, bark, and roots of lindur plants are classified as very strong with IC$_{50}$ values 34.272 ppm, 19.6193 ppm, and 42.04 ppm, respectively. Similarly, the antioxidant activity of the ethyl acetate extract of leaves, bark is classified as a strong antioxidant category with IC$_{50}$ values 30.3964 and 14.2140 ppm. The difference in the value of antioxidant activity is influenced by the type of solvent, extraction method, and testing method (Shalaby and Sanaa 2012).

Phytochemical compounds found in plants e.g. alkaloids, flavonoids, quinones, tannins, polyphenols, saponins, steroids, and triterpenoids (Juniarti et al 2009). Mangrove plants are a new alternative as a source of bioactive components for the development of medicines and functional food. Indonesia’s coastal areas have potential mangroves sources. Several studies mention that mangrove plants as a source of bioactive compounds are very effective and potential in inhibiting the enzyme α-glucosidase (Wang et al 2013, Srivastava et al 2014, Hardoko et al 2015, Chan et al 2015). One of the plants has potential source of bioactive compounds α-glucosidase enzyme inhibitors derived from aquatic resources is mangrove plant (B. gymnorrhiza).

Rahman et al (2011) showed that methanol extract of the roots has bioactive compounds e.g. diterpene, triterpenes, steroids, flavonoids, glycosides, saponins, and tannins. Fruit of the lindur plant also has antiviral activity and can fight tumors of Sarcoma 180 and Lewis lung carcinoma and contain high carbohydrates so that it has potential as an alternative food source (Allen and Duke 2006). Its bark is used to treat empirical burns (Solomon Islands), diarrhea drugs and malaria (Indonesia and Cambodia) (Rahman et al 2011). According research conducted by Karimulla and Kumar (2011) ethanol extracts of bark and roots of the plants can reduce total cholesterol. Low-Density Lipoprotein (LDL) and triglycerides. Singh et al (2010) in his study stated that with the addition of 250 mg/kg of ethanol extract of the root of the lindur plant was able to reduce blood glucose levels to 85.81 mg/dL for 90 minutes in rats tested. Hardoko et al (2015) state that agaropectin fraction from Gracilaria gigas extract can inhibit the α-glucosidase enzyme with an IC$_{50}$ value of 158.34 ppm. Cahyani et al (2015) in their study stated that methanolic extract of the type of mangrove gastropod Cerithidea obtuse had antidiabetes activity with IC$_{50}$ value of 400 ppm. Research on the use of mangroves in Indonesia as a source of bioactive compounds in inhibiting the α-glucosidase enzyme is still not widely done. The compounds that act as inhibitors of the α-glucosidase enzymes from the lemon tree have not been studied comprehensively. Information about bioactive compounds that function as inhibitors of α-glucosidase enzymes is still lacking so scientific research and evidence are needed. This study was aimed to determine and select the inhibitor activity of the α-glucosidase enzyme from the crude extract of lindur (B. gymnorrhiza) in vitro.

2. Materials and methods

The first step was extraction using n-hexane, ethyl acetate and methanol solvent, calculation of extract yield, phytochemical test, and α-glucosidase enzyme inhibitor testing from crude extracts of crude plants. The second stage was carried out fractionation from the active extract of lindur plants using TLC & TLC. and testing the best fractionation results against the α-glucosidase enzyme. The third stage was
carried out by identifying the bioactive compounds from the fraction which had the best \( \alpha \)-glucosidase enzyme inhibitor activity using GC-MS.

2.1. Materials

Materials used leaves, bark, roots of lindur plants. Samples of leaf, bark, and roots from the mangrove ecotourism area of Sedyatmo Angke Kapuk Toll, Pantai Indah Kapuk, Penjaringan, North Jakarta, Indonesia at a coordinate location of -6° 7' 17.69'' LS and 106° 45' 21.61'' East. Leaf is taken from the first segment of the branch to before the youngest part of the plant branch. the bark is taken from the part after the first segment of the branch to the branch. The root part is taken from the surface to underground. and is used as a whole. The preparation process includes cleaning with a dry brush and drying sun for leaves. bark and roots of the lindur plants. Clean plants that have been reduced in size and dried in the sun for \( \pm 12 \) hours. Dry plants crush with a blender to obtain leave powder. Bark and dry root with a size 100 mesh and stored in plastic at freeze for further research.

The tools used including orbital shaker (WiseShake Japan), rotary evaporator (Buchi Rot. R-205 Singapore), UV-Vis spectrophotometer (Hitachi U-2800 Japan), ELISA reader (Epoch USA), thin-layer chromatography (gel silica 60 F254 Germany), GC-MS (Agilent Technologies USA).

2.2. Methods

2.2.1. Bioactive Compound Extraction (Modification of Rahman et al 2011). Leaf powder. bark and roots of dried lindur were extracted using three types of solvents based on the level of polarity, n-hexane (non-polar), ethyl acetate (semi-polar) and ethanol (polar). The stages of the extraction process of leaves, bark, and roots of lindur plants include the destruction of samples by blender, maceration, partitioning and evaporation. Samples were weighed as much as 75 g then macerated with n-hexane as much as 225 mL. 1:3 ratio at room temperature and shaken with the help of a shaker for 3x24 hours. Samples in n-hexane solution were filtered every 1x24 hours. Then the resulting filtrate was combined and concentrated using a rotary evaporator at a temperature of 40°C. The concentrated extract is then transferred to the vial bottle and the rendement is calculated. Residues extract from n-Hexane extract was repeated using ethyl acetate and ethanol. The yield is a comparison of the percentage of the weight of the extract produced with the initial weight of the lindur plant (leaf, bark, and roots) used.

2.2.2. Standard Dilution. Standard weighed as much as 5 mg dissolved in methanol 10 mL until a standard solution 500 ppm. 10 \( \mu \)L of a standard comparative solution was bottled on GF254 silica gel TLC plate.

2.2.3. Test Solution. The sample was weighed as 100 mg and dissolved in ethanol (10,000 ppm). The sample fluid is then analyzed by thin-layer chromatography. Each sample liquid was bottled 5-10 \( \mu \)L with a concentration of 10,000 ppm on the GF254 silica gel plate, followed by bottling each standard. Each sample was eluted with the appropriate eluent. Determination of flavonoid levels using ethyl acetate eluent: methanol (7:3). tannin levels using ethyl acetate eluent: methanol: acetic acid (2: 17:1). Saponins using chloroform eluent: methanol (3.5:1.5). Steroids using ethyl eluent acetate: methanol (8.5:1.5) and triterpenoids using ethyl acetate: methanol (8.5:1.5) eluent. Spots were observed under UV lamps with wavelengths of 254 and 366 nm. If the expected spots are not detected by UV. Then the spots are marked on the edge of the plate in accordance with the TLC samples that have been done previously and detected by reagents based on the standard used. The reaction used for example to detected flavonoids sprayed with AlCl\(_3\). Tannins sprayed with FeCl\(_3\). Saponins sprayed with Leiberman Buchard solution. Steroids sprayed with Leiberman Buchard solution. While triterpenoids were sprayed with H\(_2\)SO\(_4\) and heated on hot plates until clear spots appeared. Samples on the TLC plates that have been eluted are then analyzed with a densitometer at wavelengths of 254 and 366 nm. The extracted content was calculated by the method of comparing the test area to the comparator area in\%.
2.2.4. A-Glucosidase Enzyme Inhibitor Activity Test (Sancheti et al. 2009). The activity of α-glucosidase enzyme inhibitors was carried out in vitro using the α-glucosidase inhibition method. The samples tested were dissolved in dimethyl sulfoxide (DMSO) solvents with a concentration of 50, 100, 250, 500, 1,000 ppm. 1 mg/mL of α-glucosidase enzyme was dissolved in 100 mM phosphate buffer pH 7.0 (concentration 0.2 unit/mL) then add 200 mg bovine serum albumin (BSA) which was dissolved in 100 mM phosphate buffer (pH 7.0). The enzyme solution (1 unit/mL) was previously diluted 25 times with a 100 mM phosphate buffer (pH 7.0). The mixture of reagents used in this test contained 50 μL 0.1M phosphate buffer (pH 7.0), 25 μL 4-nitrophenyl α-D-glucopyranoside 0.5 mM (dissolved in 0.1 M phosphate buffer pH 7.0), 10 μL test samples with various concentrations (50, 100, 250, 500, 1000 ppm) and 25 μL α-glucosidase solutions (0.04 unit/mL). The reaction was started by adding 25 μL of the enzyme solution and 25 μL of phosphate buffer followed by incubation for 30 minutes at 37°C. The reaction was stopped by adding 100 µL Na₂CO₃ (0.2M). Acarbose is used as a positive control with a concentration of 0.1, 0.5, 1, 5, 10 ppm. The absorption of the solution was measured using an Elisa reader at a wavelength of 410 nm. The percentage of α-glucosidase inhibition is determined using the equation (1):

\[
\text{Inhibition (\%) = \frac{K - S}{K} \times 100\%}
\]

K = absorbance without a sample (control)
S = S1 - S0 (S1 sample absorbance with enzyme S0 Sabsoarnace sample without enzyme)

IC₅₀ values indicate the sample concentration needed to inhibit α-glucosidase activity by 50% and the value is determined by the regression equation.

2.2.5. Separation of Active Compounds. Process of separating active compounds from selected extracts of lindur (which exhibits the highest α-glucosidase inhibitor activity) involves two stages. The first step is finding the best eluent using a combination of eluents with different polarity using the thin layer chromatography (TLC) method. TLC aims to separate the active compound from the crude extract using the appropriate eluent. This effluent functions as the mobile phase in the separation of PTLC. The second step is separation using KLTP to obtain, isolate or collect separated factions.

2.2.6. Fractionation Using Thin Layer Chromatography (TLC) (Modified Gritter et al. 1991). Crude ethanol extract of the lindur plant was dissolved in the appropriate solvent of origin and as much as 0.5 mg extract is bottled on the TLC plate. After drying, it was eluted directly in a vessel which has been saturated by the steam eluent of the developer. Elution begins using a single solvent (n-hexane, ethylacetate, chloroform, acetic acid, ethanol, methanol), followed by searching eluents in combination with some of the best single eluents using a methanol: DCM eluent in a ratio of 1: 1; 1: 2; 1: 3; 1: 4; 2: 1; 2: 2; 2: 3; 2: 4; 3: 1; 3: 2; 3: 3; 3: 4. Elution stains or spots were observed under UV lamps at wavelengths of 254 and 366 nm (Gritter et al. 1991). TLC plates with selected eluents produce patches which were then observed and their Rf values were calculated (Kusumaningtyas et al. 2010).

2.2.7. Fractionation Using Preparative Thin Layer Chromatography (KLTP) (Gritter et al 1991). Preparative thin-layer chromatography (KLTP) using a glass plate measuring 20 x 20 cm² with the stationary phase of silica gel GF254. The active extract was dripped in length to form a ribbon on the glass plate and eluted with selected eluents from the results of the previous TLC. The glass plate was dried and observed with UV light with wavelengths of 254 nm and 366 nm. Taking preparative TLC compounds by careful scraping and the results were dissolved with the initial solvent ethanol extract then the fraction obtained is evaporated.

2.2.8. Identification of Active Compounds Using Gas Chromatography-Mass Spectrometry (GC-MS). Identification of active compounds lindur plants conducted with best activity values against the α-
glucosidase inhibitors (lowest IC\textsubscript{50}) samples. Next step sample injected in GC-MS test to determine active compounds contained. 300 mg of sample was dissolved with 3 mL of 99.9% ethanol and injected until 1 µL. HP-5 capillary columns (Agilent 19091J-433: 0.25 mm x 30 mm x 0.25 µm containing 5% diphenyl 95% dimethylpolysiloxane) was used in GC MS with flow rate 1.0 mL / minute with temperature 270⁰C splitless mode. and pressure was 18.21 psi. He (helium) used as carrier gas. GC MS parameter. To detecting compounds with mass of 40-800. MS conditions were define as MS quad temperature 150⁰C-200⁰C and MS source temperature 250⁰C-300⁰C. Results of chromatogram were analyzed in database to determine component compounds contained in extract.

3. Results and discussion

3.1. Identification and characterization of samples
Identification result was carried out by LIPI Biological Research Center, Bogor used the key of determination method (Blackwelder 1967). Result show samples of stem bark and plant roots used in this study were included in the type of \textit{B. gymnorrhiza}. Classification of lindur plants is kingdom: Plantae, division: Magnoliophyta, class: Magnoliopsida. ordo: Myrtales, family: Rhizophoraceae, genus: \textit{Bruguiera}, and species: \textit{Bruguiera gymnorrhiza}. These plants grow in tropical regions from South Africa, East. Madagascar. South Asia and Southeast Asia (including Indonesia and Malaysia) to northeast Australia, Micronesia, Polynesia and the Ryukyu islands (Allen and Duke 2006). Samples taken was 5 years old. Lindur leaves used average length of 17.51±1.74 cm and a width of 5.0±1.03 cm. stem bark used on average had a diameter of 3.51±0.47 cm and root had an average diameter of 3.28±0.60 cm.

Characteristics of the lindur leaves used are dark green when fresh. After drying and mashed into a green powder. Fresh lemon-colored stems are pale gray, after being dried and mashed into a greenish-brown powder. Root was dark brown color when fresh after being dried and mashed into a brown powder.

3.2. Quantitative bioactive components of extracts of leaves, bark and roots of lindur plants
Active chemical compounds found in plants was phytochemicals. Phytochemicals consist of primary and secondary metabolites. Secondary metabolites are bioactive components such as phenols. flavonoids, saponins, terpenoids, steroids, tannins, and alkaloids. Sample with highest yield extract based on (Sudirman \textit{et al} 2014, Khasanah \textit{et al} 2014) and most varied qualitative phytochemical content (Hardiana \textit{et al} 2012) was used for quantitative bioactive. According results of research by Dia \textit{et al} (2015) qualitative phytochemical components contained flavonoids and tannins ethanol extract of bark, phenols from ethyl acetate extracts of leaves, steroids from leave ethanol extracts. saponins and triterpenoids from root ethanol extracts.

Samples tested e.g bark ethanol extract, leave ethyl acetate extract, leave ethanol extract and root ethanol extract. Results of quantitative bioactive component content of the study sample are presented in table 1.

Research conducted by Sari (2010) states that the alkaloid and flavonoid compounds in fruit extracts Macassar (\textit{B. javanica} (L.) Merr) plays a role into inhibiting of the α- enzyme glucosidase. Various research results have suggested that the compound phytochemicals have the ability to inhibit the action of the α-glucosidase enzyme for example alkaloids from \textit{Tinospora cordifolia} plant with activity 9.8 ppm (Patel dan Mishra 2012), triterpenes from extracts of leaves and bark \textit{Fagus hayatae} can inhibit the action of the enzyme α-glucosidase up to 96% (Lai \textit{et al} 2012) and flavonoids from \textit{Psidium guajava} leaves with their activities 1,500 ppm (Wang \textit{et al} 2010). Research conducted by Lee \textit{et al} (2008) reported that phenolic specifically kaempferol type of flavonol from leaf extracts \textit{Machilus philippinense} has an α-glucosidase inhibitory activity with IC\textsubscript{50} values 6.10 µM (Lee \textit{et al} 2008).
Detection of flavonoid compounds in ethanol from the bark is related function of flavonoids on regulating growth, photosynthesis, antimicrobial and antiviral in plants (Robinson 1995). Flavonoids and their derivatives are a group of polyphenols that are abundant in plants (Markham 1988).

Table 1. Quantitative bioactive component of rough extracts of leaves, bark, and root lindur plant

| Sample                  | Bioactive compound | Active Component Concentration (%) |
|-------------------------|-------------------|------------------------------------|
| Bark Etanol extract     | Flavonoid         | 0.42                               |
| Bark Etanol extract     | Tannin            | 4.10                               |
| Leaves Ethyl Acetate extract | Phenol | 3.48                               |
| Root Etanol extract     | Saponin           | 0.32                               |
| Leaf Etanol extract     | Steroid           | 3.99                               |
| Root etanol extract     | Triterpenoid      | 0.10                               |

This Research (wb) Reference

Bark Etanol extract: 4.50% a
Bark Etanol extract: 34.65% a
Leaves Ethyl Acetate extract: 0.33% b
Leaves Ethyl Acetate extract: 17.26% c
Root Etanol extract: 26.10% d
Leaves Etanol extract: 4.17% e
Root etanol extract: 3.12% g

Source : aOsman dan Abkar (2015). bEstiasih dan Kurniawan (2006). cRahmawati et al (2013). dSani et al (2014). eAnaduaka et al (2013). fNweze dan Nwafor (2014). gBasyuni et al (2013)

Tannins was group of phenol polyhydroxy can be different due of their ability to precipitate proteins. Detection of tannin in stem bark ethanol extract is related to its function as self-defense from bacterial, fungal, viral, herbivorous and vertebrate herbivorous insects (Leinmuller et al 1991). This compound is also important to prevent excessive degradation of nutrients in the soil. This process is very important especially in maintaining nutrient deposits in the soil for the next vegetation period from plants (Leinmuller et al 1991).

Phenol compounds include a variety of plant-derived compounds which have the same characteristics, namely aromatic rings containing one or two hydroxyls. Phenol compounds tend to be soluble in water because they often bind to sugar as a glycoside and are usually found in cell vacuoles (Koche et al 2010). Detection of steroids in leave ethanol extract is thought to be related to its protective function. This compound not only works to reject some insects but also attracts several other insects (Robinson 1995).

The saponin compound detected in the ethanol extract of the root is thought to be related to the plant's efforts to protect itself from intruders. Saponins are amphipathic (hydrophilic and hydrophobic) compounds which generally have a triterpene or steroid or aglycone backbone. Saponins have a bitter and hard taste or are poisonous to animals commonly referred to as sapotoxins. The content of triterpenoids in the ethanol extract of the root of the lindur plant is 0.1%. Riyanto et al (2013) states that triterpenoid compounds in plants function as protectors to resist insects and microbial invasion. The presence of secondary metabolites in plants is influenced by various factors such as differences in climate, habitat soil nutrient conditions, and time to take plants. The effect of stress from the environment experienced by plants such as contamination and climate change can affect the production of these secondary metabolites (Koche et al 2010).

3.3. The activity of α-Glucosidase enzymes from coarse leave extracts. stem and lindur roots

The selection of extracts used in the α-glucosidase enzyme inhibitor test was based on quantitative phytochemical activity. Considering the extract yield value. The representative of each extract tested and the nature of each solvent used. In accordance with these results ethyl acetate leave extract. ethanol extract of bark and ethanol extract of the roots of lindur plants were selected. Testing of α-glucosidase enzyme inhibitor activity was carried out to determine the inhibitory activity of the α-glucosidase
enzyme from various extract concentrations by looking at the percent inhibition value and IC$_{50}$ value of each extract tested. The results of testing on the inhibitory activity of the α-glucosidase enzyme can be seen in Table 2.

The test results of the extract showed inhibition of enzyme activity. The percentage of inhibition above 50% is ethanol extract of bark and root ethanol extract. The percentage inhibition of acarbose was 91.73% at a concentration of 5 ppm. While the inhibitory percentage value of bark ethanol extract and root roots were 61.93% and 69.26% respectively at 250 ppm. This shows that acarbose has better inhibitory ability compared to the ethanol extract of the sample. The inhibition of root ethanol extracts from the results of the study was better compared to the inhibition of the results of research by Yin et al. (2008) on Cirsium japonicum root water extract which was 32.56% with a concentration of 1000 ppm. Acarbose has a better ability because with a smaller concentration, acarbose has greater inhibitory power. Ethanol extracts of the samples have less inhibitory power than acarbose and also require greater concentration.

Acarbose is an oligosaccharide compound derived from fermentation of microorganisms from the Japanese Actinoplanes. This complex oligosaccharide compound is a potential competitive inhibitor of the α-glucosidase enzyme that breaks down starch, dextrin, maltose, sucrose to produce digestible monosaccharides. Acarbose is one of the oral antidiabetic agents for type 2 diabetes mellitus patients. The use that exceeds the dose continuously for a long time can cause side effects including flatulence (flatulence), diarrhea, and abdominal pain (Hollander et al. 1997).

| Sample          | Concentration (ppm) | Inhibition (%) | IC$_{50}$ Value (ppm) |
|-----------------|---------------------|---------------|-----------------------|
| Ethyl acetate leaves | 50                  | -15.17        |                       |
|                  | 100                 | -8.90         |                       |
|                  | 250                 | -6.83         |                       |
|                  | 500                 | 6.678         |                       |
|                  | 1000                | 26.19         |                       |
|                  | 50                  | 19.25         |                       |
|                  | 100                 | 32.45         |                       |
| Etanol bark      | 250                 | 61.93         | 171.31±3.93           |
|                  | 500                 | 81.26         |                       |
|                  | 1000                | 92.38         |                       |
|                  | 50                  | 17.53         |                       |
|                  | 100                 | 35.46         |                       |
| Etanol root      | 250                 | 69.26         | 153.067±0.93          |
|                  | 500                 | 88.41         |                       |
|                  | 1000                | 94.42         |                       |
| Glukobay/acarbose| 0.1                 | 24.97         | 0.313±0.06            |
|                 | 0.5                 | 61.32         |                       |
|                 | 1                   | 77.80         |                       |
|                 | 5                   | 91.73         |                       |
|                 | 10                  | 95.25         |                       |

Table 2 also shows that the ethanol extract of bark and root has an α-glucosidase enzyme inhibitor activity indicated by IC$_{50}$ values of 171.31 ppm and 153.07 ppm, respectively. The existence of these activities shows that the crude ethanol extracts of bark and roots of molasses contain several compounds
that are able to inhibit the activity of the α-glucosidase enzyme so that it produces a synergistic effect. While the ethyl acetate extract leaves the inactive group because it has an IC50 of more than 1,000 ppm. Different inhibitors of α-glucosidase enzyme activity on leave ethyl acetate extract tested ethanol extract of stem bark and roots were thought to be due to differences in the distribution of active compounds contained in each part of the lindur plant (Markham 1988, Sani et al 2014). The research conducted by Sani et al (2014) reported that the differences in the distribution of active compounds would affect their content, this can be seen from the presence of flavonoids in the roots of the Eucalyptus camaldulensis plant which is 0.30 g/100g, while the leaves and bark of the stem do not contain flavonoids.

The enzyme inhibitor activity of the α-glucosidase samples tested was still less effective compared to the IC50 value of glucobay which was 0.313 ppm. The IC50 value of stem bark ethanol extract from the research results is still greater when compared to IC50 value of ethanol extract of orange parasite which was 70.71 ppm (Ramadhan 2013). The IC50 value of the ethanol extract of the root of the study results was lower than Dayak onion tubers in ethanol extract which was 241 ppm (Febrinda 2014). The difference in the value of this activity is influenced by the type of sample used, distribution and location of sampling, testing methods and others. Based on the results of testing the sample activity indeed far compares positive control. It may be necessary to use other methods of extraction or testing to compare α-glucoside activity. According to Kardono (2003), differences in the amount of inhibitory power of an extract can be caused by differences in the content and variations in each medicinal plant, the presence of confounding compounds, differences in extraction methods and differences in the use of types of solvents.

The ability of the inhibitor activity possessed by the ethanol extract of stem and root bark is inseparable from the synergistic effect of the phytochemical compounds contained in it. The more diverse the number of phytochemical compounds in an extract that forms a synergistic effect in inhibiting the performance of the α-glucosidase enzyme, the inhibitor activity is also higher. According to Kim et al (2008) stated that most α-glucosidase inhibitors work by imitating the transition position of the piroanosidic unit from natural glucosidase substrates so that this inhibiting mechanism is called competitive inhibition. This study has not yet conducted a test to determine the inhibitory mechanism so that further testing is needed to determine the inhibitory mechanism of bioactive compounds extracted samples competitively or not. The inhibitor testing activity of the α-glucosidase enzyme was measured based on the absorbance of p-nitrophenol at a wavelength of 410 nm. Harborne (1987) states that the wavelength range of 230-560 nm is the maximum absorption for flavonoids. This is the basis for determining the right wavelength in measuring the percentage of inhibition of the α-glucosidase enzyme.

3.4. Fractionation with thin layer chromatography (TLC) and preparative thin layer chromatography (PTLC)
Separation or fractionation was used to separate the compounds found in the crude ethanol extract of the roots of the lindur plant with the best α-glucosidase enzyme inhibitor activity. Hancu et al (2011) suggested that thin layer chromatography (TLC) is a relatively simple, fast and commonly used chromatographic method for identifying pharmaceutical substances. Based on the TLC analysis with single eluent, chloroform, dichloromethane (DCM), and methanol showed good separation, this was based on separability and number of spots formed.

The results of the analysis obtained using KLT GF254, showed that methanol and dichloromethane (DCM) with a ratio (2:3) gave good results among the other ratios with the most number of spots, but the resulting spots had poor separation so nonpolar solvents needed to separate the distance.

The combination of the use of non-polar solvents in the form of n-hexane with a ratio of methanol: dichloromethane: n-hexane was (2:3:0.5); (2:3:1); (2:3:1,5). Fractionation using TLC and observation with UV 254 nm light produced 5 fraction stains presented in figure 3. These results showed that the
methanol: dichloromethane: n-hexane (2: 3: 1) combination eluent provided 5 spots with good separation. The selected eluent was then used in fractionation with PTLC.

![Figure 1. Profile spot with the best eluent used methanol DCM: hexane(a) 2:3:0.5, (b) 2:3:1.5, (c) 2:3:1.](image)

The fractionation results using preparative thin layer chromatography obtained 5 fractions in the crude ethanol root extract, with eluent methanol: dichloromethane: n-hexane (2:3:1). The results of fractionation by preparative thin-layer chromatography and checking of each fraction can be seen in figure 2.

![Figure 2. Fractionation used by TLCP (A), (B) checking by TLC.](image)

Markham (1988) Rf value is used as a relative comparison value between samples. The Rf value also states the degree of retention of a component in the stationary phase so the Rf value is often also called the retention factor. The greater the Rf value of the sample, the greater the distance of movement of a compound on the TLC plate (Gritter et al. 1991).

The results of the fractionation stage using TLC and observations using UV lamps with a wavelength of 254 nm resulted in 5 spots of compounds with different Retardation factor (Rf) values. The Rf value of the ethanol fraction of the lindur plant roots is presented in table 3. The colors produced by UV light are brown, yellowish-brown, and purplish brown. Rf numbers are defined as the distance traveled by a compound divided by the distance traveled by the developer frontline (measured from the starting line).
The value of the Rf number is always smaller than 1.0 (Markham 1988). Rf value is used as the value of the relative comparison between samples. Rf value also states the degree of retention of a component in the stationary phase so that the value of Rf is often also called the retention factor. The greater the Rf value of the sample, the greater the moving distance of a compound on the TLC plate (Gritter et al 1991).

### Table 3. Rf value used eluen metanol : DCM : hexane.

| Compound | Solvent mileage (cm) | Component mileage (cm) | Rf value |
|----------|----------------------|------------------------|----------|
| Fraction 1 | 8.00 | 1.2 | 0.15 |
| Fraction 2 | 8.00 | 2.2 | 0.275 |
| Fraction 3 | 8.00 | 4.0 | 0.50 |
| Fraction 4 | 8.00 | 4.8 | 0.60 |
| Fraction 5 | 8.00 | 6.6 | 0.825 |

Table 3 shows that the Rf value of the study results is different from the results of the study by Sudirman et al (2014) on methanol fraction of B. gymnorrhiza fruit using methanol: water (4:1) eluent having 3 spots with Rf values of 0.31; 0.34; and 0.45. The difference is influenced by the polarity of each sample used so that the resulting Rf value is also different. An eluent solution that is non-polar will slow down the component chromatography process. The Rf value is also influenced by differences in solubility and the nature of the eluent mixture used in the test (Gritter et al 1991). The greater Rf value (close to 1) indicates that the compound is nonpolar, and vice versa (Markham 1988). Rf value can be used as a basis in identifying a compound. A compound which has the same Rf value as the comparison, the compound can be said to have the same characteristics, and vice versa.

3.5. The activity of α-glucosidase enzyme inhibitors of ethanol extract of lindur root

In accordance with the tests that have been done showed that fraction 1 with Rf 0.15 has the most potential α-glucosidase inhibitory activity (161.06), followed by fraction 2 activity which has an IC₅₀ value of 362,471 ppm. Fraction 3, fraction 4 and fraction 5 did not show any inhibitory properties as alpha-glucosidase enzyme inhibitors, this can be seen from the IC₅₀ value>1000 ppm (table 4).

In general, the IC₅₀ value of crude extracts of the root of the lindur plant roots was greater than the positive control, diatomaceous or glucobay. Positive control activity was still better compared to crude extract and ethanol fraction of lindur plant roots. The IC₅₀ value of fraction 1 from chromatographic results (161.06 ppm) was greater than the inhibition value of the α-glucosidase enzyme from crude ethanol root extract (153.067 ppm). This is thought to be due to the synergistic effect between the compounds in the extract and the fraction concentration obtained by chromatography is too small (Mangunwardoyo et al 2009). The reduced diversity of active compounds that form a synergistic effect on the fraction and the small concentration of fraction yields from TLC causes the compounds contained in the extract cannot inhibit the performance of alpha-glucosidase enzymes.

The activity of the α-glucosidase enzyme inhibitor of the research fraction was still smaller than that of Gultom (2011) on the methanol fraction of andaliman fruit (Zanthoxylum acanthopodium DC) with an IC₅₀ value of 117 ppm. The activity of the α-glucosidase inhibitor was higher than that of Priyono (2008) on the fraction of red fruit ethanol extract (Pandanus conoideus Lam) with 0.5% inhibition (5000 ppm); and 0.25% (25000 ppm).
Table 4. Result α-glucosidase enzyme inhibitor activity from root ethanol extract fraction.

| Sample       | Value IC\textsubscript{50} (ppm) | Compare with reference IC\textsubscript{50} (ppm) |
|--------------|----------------------------------|-----------------------------------------------|
| Fraction 1\textsuperscript{[p]} | 161.052                          | 218.79\textsuperscript{a}                     |
| Fraction 2\textsuperscript{[p]} | 362.471                          | 117\textsuperscript{b}                        |
| Fraction 3\textsuperscript{[p]} | >1000                            | 5000\textsuperscript{c}                       |
| Fraction 4\textsuperscript{[p]} | >1000                            | 2446.18\textsuperscript{d}                    |
| Fraction 5\textsuperscript{[p]} | >1000                            |                                               |
| Acarbose\textsuperscript{[q]}      | 0.247                            |                                               |

Note:
- \textsuperscript{[p]}: concentration 50,100,250,500,1000,
- \textsuperscript{[q]}: concentration 0.1; 0.5; 1; 5; 10
- a: fraction 1 ethyl asetat pandan wangi leave (Sukandar \textit{et al} 2012)
- b: fraction metanol andaliman fruit (Gultom 2011)
- c: fraction ethanol red fruit (Priyono 2008)
- d: fraction root plat \textit{Tephrosia tinctoria} (Rajaram and Suresh 2011)

The inhibitory activity of the α-glucosidase enzyme results of this study is greater than the activity of the ethanol fraction of the plant roots of \textit{Tephrosia tinctoria} as a result of research by Rajaram and Suresh (2011) with IC\textsubscript{50} values of 2446.18 ppm. Toma \textit{et al} (2015) stated that the ethanol extract of \textit{Moringa stenopatela} leaves had antidiabetic activity at a concentration of 500 ppm which was induced in rats thereby reducing postprandial glucose levels. Research conducted by Sukandar \textit{et al} (2009) on the fraction of 1 ethyl acetate fragrant pandan leaves as an inhibitor of the α-glucosidase enzyme showed activity with IC\textsubscript{50} value 218.79 ppm. The α-glucosidase enzyme is an enzyme that catalyzes the cutting of glycosidic bonds in oligosaccharides into mono-saccharide units, which can be absorbed and used by organisms (Melo \textit{et al} 2006). The inhibition of the action of the α-glucosidase enzyme can help overcome the condition of hyperglycemia in diabetics because the amount of monosaccharide absorbed by the intestine is reduced.

3.6. Identification of fractionated compounds

The identification of chemical compounds found in the fraction with the best α-glucosidase enzyme inhibitor activity, namely fraction 1 with IC\textsubscript{50} of 161.06 ppm was carried out using GC-MS. The results of compound identification in the root ethanol fraction with GC-MS by looking at the class of lindur root ethanol extract compounds produced by reading the highest and widest peak graph based on the catalog contained in GC-MS. GC-MS analysis with MS parameters used in this study to detect compounds with a mass of 40-800 da in the root ethanol fraction compound. The compounds detected with this instrument are compounds that can turn into gas or are volatile because the principle of this instrument is to evaporate compounds with high temperatures.

The results of the analysis showed that the components of the root ethanol extract fraction consisted of fatty acid and phenol groups. The compound detected has a low peak, this is presumably because the sample used for GC-MS testing has a very small concentration (0.1 ppm) and does not do the coupling (forming conjugate). Coupling aims to bind compounds that have large molecular weight by using methyl, permethyl, trimethylsilyl to be evaporated so that peak readings can be optimized separately (Abian 1999). Factors that influence the results of identification with GC-MS are optimum conditions of tool operation, type and diameter of columns used, gas flow velocity, speed of temperature rise and column cleanliness of other compounds.
Table 5 shows that the compounds contained in the fraction 1 of the ethanol extract of the root mostly (have a similarity >90%) are hexadecanoic acid or acid palmitate and phenol. Palmitic acid compounds contained in ethanol extract of root fraction 1 have the molecular formula $C_{17}H_{34}O_2$ with a molecular weight of 270.26 g/mol. Phenol or 2,2 methylenebis compounds found in the sample have the molecular formula $C_{23}H_{32}O_2$ with a molecular weight of 340 g/mol. The compound detected by GC-MS and estimated to have antidiabetogenic activity in this study was phenol. Phenol compounds are secondary metabolites found in plants and have antioxidant activity (Hans and Heldt 2005) and can also be an α-glucosidase inhibitor. Smith (1962) states that the value of Rf 0.17 is the value of Rf phenol isolated from pure phenol compounds that have been commercialized. This value is not much different from the Rf value of the research results in fraction 1.

Table 5. Grouping compound on root etanol fraction extract from GC-MS.

| No | Run time | Compound Name         | Similarity (%) | Content (%) | Chemical structure       |
|----|----------|-----------------------|----------------|-------------|--------------------------|
| 1  | 14.62    | Hexadecanoic acid,    | 95             | 18.52       | $C_{17}H_{34}O_2$ BM 270.26 g/mol |
| 2  | 20.42    | Phenol, 2,2 methylenebis | 96             | 81.48       | $C_{23}H_{32}O_2$ BM 340 g/mol |

Phenol compounds, 2,2 methylenebis has the name IUPAC 2-tert-butyl-6 - [(3-tert-butyl-2-hydrxy-5-methylphenyl) methyl] -4-methylphenol. This compound also has another name, namely bis (2-hydroxy-3-tert-butyl-5-methylphenyl) methane; 2,2'-methylenbis (6-tert-butyl-4-cresol); and GERI-BP002-A. Amorati et al (2003) stated that 2,2'-methylenbis (6-tert-butyl-4-methylphenol) compounds had higher antioxidant activity than 4,4'-methylenbis (2,6-di-tert-butylphenol). Takashi and Hiraga (1981) stated that administration of 2,2'-methylenbis compounds in Sprague-Dawley female rats for one week was able to control cholesterol levels at the level of 1,135 mmol/L. Büyükbalci and Nehir (2008) stated that the presence of phenol and antioxidants in the diet of type 2 diabetics can maintain plasma antioxidant levels and support the development of the vascular system. This is supported by research conducted by Saleem (2010) that the high phenol content associated with antioxidant activity and there is a high correlation (r = 0.86) between inhibition of α-glucosidase with phenol content in Apiaceae plant water extract.

Plants have phenol components which can interact with proteins and can inhibit enzymatic activity (Dawra et al 1988). Phenol compounds can stimulate the activation of adenosine monophospat and activation of protein kinase which can reduce the process of increasing glucose transport in the plasma membrane. The presence of phenolic bioactive components can regulate tissue in utilizing glucose,
which of course depends on insulin. This is supported by research conducted by Mahalingam and Kannabiran (2009) that phenol compounds with 2-Hydroxy 4-methoxy benzoic acid isolated from the roots of Hemidemus indicus (Linn.) have antidiabetic activity in STZ-induced mice with a mechanism to increase insulin secretion and reactivation of glucogen synthesis. Phenol also affects glucose absorption in gastrointestinal tissues (Mi et al 2009). The phenol component Moracin M, steppogenin-4’-O-β-D-glucosiade and mullberroside A isolated from the root bark of the Morus alba (Linn.) plant have hypoglycemic activity in alloxan-induced diabetic rats. The presence of hypoglycemic conditions will increase peripheral glucose consumption (Mi et al 2009). Nwosu et al (2011) state that polyphenols affect the response of diabetes due to the regulation of glucose absorption and control of oxidative stress. The presence of polyphenols can also inhibit the activity of starch-breaking enzymes. Phenol extract produced from Ascophyllum with a concentration of 10 mg/mL has an α-glucosidase inhibitory activity of 80% so that it is potentially applied to treatments using rats (Nwosu et al 2011). Phenol can inhibit α-amylase activity in rat plasma and control the increase in postprandial blood glucose levels. Research conducted by You et al (2012) showed that phenol compounds contained in the ethyl acetate fraction of Muscadine seeds had inhibitory activity against α-glucosidase and lipase enzymes with IC50 values of 1.92 and 34.41 ppm.

Palmitic acid compounds have the name IUPAC 2-methyl hexadecanoic acid. This compound also has another name, 2-methyl hexadecanoic acid; and 2-methyl palmitic acid. The results of research conducted by Warsinah et al (2011) showed that the methanol extract fraction of Sandoricum koetjape bark contained palmitic acid detected using GC-MS with Rt 22.651 minutes and abundance (area) 4.06. Marliyana et al (2012) stated in his research that the active fraction of red fruit n-hexane extract (Pandanus Conoideus Lamk.) Contained palmitic acid with Rt 31.828 minutes with an abundance of 11.21%. Hexadecanoic acid (as palmitate) includes fatty acids that have antifungal properties by damaging the structure of cell walls and membranes that are synergistically associated with various active compounds such as terpenoids, so as to increase antifungal activity (Padmini et al 2010). Palmitic acid detected in the ethanol fraction of the leaves of Dregea volubilis has a retention time of 10.03 (Natarajan and Dash 2013). Palmitic acid can increase the replacement of lipid profiles which contribute to hypolipidemic activity by inactivating the hepatic HMG-Coa reductase enzyme during cholesterol synthesis (Jing et al 2011). This is supported by research conducted by Natarajan and Dash (2013) that by giving palmitic acid contained in the ethanol extract of leaves D. volubilis can improve lipid profiles (reduce TGL, cholesterol and increase HDL) in diabetic rats, reduce blood glucose levels and prevent complications of diabetes.

4. Conclusion

The ethanol extract of the root contains bioactive components of saponins and triterpenoids which are 0.32% and 0.10%. Potential α-glucosidase inhibitors were ethyl acetate extracts of bark and root ethanol extract with IC50 171.306 and 153.067 ppm. The eluent was selected based on KLT GF254 chromatography, indicating that the methanol: dichloromethane: n-hexane (2:3:1) combination eluent provided 5 spots with good separation. The fractionation results using preparative thin layer chromatography showed 5 fractions in the crude ethanol root extract, with eluent methanol: dichloromethane: n-hexane (2:3:1). The colours produced from observations under UV light were brown, yellowish-brown, and purplish brown. Fraction 1 from root ethanol extract has the potential as an inhibitor of α-glucosidase enzyme with IC50 value of 161.05 ppm. Compounds found in fraction 1 with similarities> 90% are hexadecanoic acid and phenol, 2,2 methylene bis.

References
Abian J 1999 The Coupling of gas and liquid chromatography with mass spectrometry J. Mass. 34 157-168
Allen J A and Duke N C 2006 Bruguiera gymnorrhiza (large-leaveed mangrove). Species Profiles for
Pacific Island Agroforestry Apr; Ver 2.1.

Anaduaka E G , Ouguua V N, Egba S I and Apeh V O 2013 Investigation of some important phytochemical, nutritional properties and toxicological potentials of ethanol extracts of *Newbouldia laevis* leave and stem *AJST.* 12 5941-5949

Basyuni M Putri L A P and Oku H 2013 Phytomedicinal Investigation from Six Mangrove Species, North Sumatra, Indonesia *IJMS.* 18 157-164.

Blackwelder R E 1967 *Taxonomy A text and reference book* (New York: John Wiley & Sons, Inc)

Büyükbalci A and Nehir S 2008 Determination of in vitro antidiabetic effects, antioxidant activities and phenol contents of some herbal teas *Plant Food Hum. Nutr.* 63 27-33

Cahyani R T, Purwaningsih S and Azrifitria 2015 Antidiabetic potential and secondary metabolites screening of mangrove gastropod *Cerithidea obtuse* *J. Coast. Life Med.* 3 356-360

Chan E W C, Tangah J, Kezuka M, Hoan H D and Binh C H 2015 Botany, uses, chemistry and bioactivities of mangrove plants II: *Ceriops tagal* *ISME.* 13 39-43

Dawra R K, Makkar H P and Singh B 1988 Protein-binding capacity of microquantities of tannins *J. Anal. Biochem.* 170 50-53

Dia S P S, Nurjanah and Jacoeb A M 2015 Komposisi kimia dan aktivitas antioksidan akar, kulit batang dan daun lindur *JPHPI.* 8 204-218

Estiasih S and Kurniawan D A 2006 Aktivitas antioksidan ekstrak umbi akar ginseng jawa (*Talinum triangulare* *Wild*) *JTIP.* 17 166-175

Febrinda A E, Astawan M, Wresdiyati T and Yuliana N D 2014 Ka pasitas antioksidan dan inhibitor α-Glucosidase inhi bitory activities of *Rhizophora mucronata* fruit powder. *Int. J. Chem. Tech. Research* 8 211-215

Harborne J B 1987 *Metode Fitokimia* Edisi ke-2. Padmawinata K, Soediro I (Bandung: Institut Teknologi Bandung) Terjemahan dari: *Phytochemical Methods* p 102-117; 140-141

Hardiana R, Rudiyansyah and Zaharah T A 2012 Aktivitas antioksidan senyawa golongan fenol dari beberapa jenis tumbuhan famili *Malvaceae* *JKK.* 1 8-13

Jing P, Gong P and Linghai L 2011 Palmitic acid acutely stimulates glucose uptake via activation of Akt and ERK1/2 in skeletal muscle cells *J. Lipid Res.* 52 1319-1327

Junianti, Osmeli D and Uhermita 2009 Kandungan senyawa kimia, uji toksisitas (*Brine Shrimp Lethality Test*) dan antioksidan (*1,1-diphenyl-2-picrilhydrazyl*) dari ekstrak daun saga (*Abrusprecatorius L.*) *Makara J. Sci.* 13 50-54

Kardono L B S 2003 Kajian kandungan kimia mahkota dewa (*Phaleria marcocarpa*). Di dalam: *Prosiding Pameran Produk Obat Tradisional dan Seminar Sehari Mahkota Dewa* (Jakarta: Pusat Penelitian dan Pengembangan Farmasi dan Obat Tradisional Departemen Kesehatan) pp 72-76

Kim K Y, Nam K A, Kurihara H, Kim S M 2008 Potent α-glucosidase inhibitors purified from the red alga *Grateloupia elliptica* *Phytochemistry* 69 2820-2825

Koche D, Shirsat R, Imran S and Bhadange D G 2010 Phytochemical screening of eight traditionally
used ethnomedicinal plants from Akola district (MS) India JIPBS. 1 253-256
Kusumaningtyas E, Natasia M and Darmono 2010 Potensi metabolit kapang endofit rimpang lengkuas merah dalam menghambat pertumbuhan Escherichia coli dan Staphylococcus aureus dengan media fermentasi PDB dan PDY Prosiding Seminar Nasional Teknologi Peternakan dan Veteriner (Jakarta: Pancasila University) p 819-824
Lai Y C, Chen C K, Tsai S F and Lee SS 2012 Triterpenes as α-glucosidase inhibitors from Fagus hayate. J. Phytochem 74 206-211
Lee S K J 2007 Inhibitory activity Eusonimus salatus against α-glukosidase in vitro and invivo. J. Nutr. Res. Pract. 1184-188
Lee S S, Lin H C and Chen C K 2008 Acylated flavonol monorhamnosides, α-glukosidase inhibitor, from Machilus Filipinensis J. Phytochem. 69 2347-2353.
Leimuller E, Steingass H and Menke K H 1991 Tannins in ruminant feedstuffs. Anim Res Develop. 33 9-62
Markham K R 1988 Cara Mengidentifikasi Flavanoid. Terjemahan Padmawinata K, Penerjemah, Niksohirin S, editor (Bandung: Insitut Teknologi Bandung). Terjemahan dari: Techniques of Flavanoid Identification pp 76-82
Marliyana S D, Fajar R W, Nestri H and Rakhmawati R 2012 Uji toksisitas secara brine shrimp lethality ekstrak buah merah (Pandanus Conoideus Lamk.) Jurnal penelitian kimia. 8 24-33
Mahalingam G and Kannabirana K 2009 Hemidesmus indicus root extract ameliorates diabetes-mediated metabolic changes in rats Intern. J. Green Pharm. 3 314-318
Mangunwardoyo W, Cahyaningsih E and Usia T 2009 Ekstraksi dan identifikasi senyawa antimikroba herba meniran (Phyllanthus niruri L.) JKI. 7 57-63
Melo E B, Gomes AS and Carvalho I 2006 α- and β-glucosidase inhibitors: chemical structure and biological activity Tetrahedron 62 10277-10302
Mi Z, Man C, Qing Z H, Shi S, Bing X and Hua W F 2009 Invivo hypoglycemic effects of phenolics from the root bark of Morus alba, Fitoterapia. J. Nat. Prod. Rad. 80 475-477
Natarajan V and Dhas A S A G 2013 Effect of active fraction isolated from the leave extract of Dregea volubilis [Linn.] Benth. on plasma glucose concentration and lipid profile in streptozotocin-induced diabetic rats Journal Springerplus 2 2-6
Nweze N O and Nwafor F I 2014 Phytochemical, proximate and mineral composition of leave extracts of Moringa oleifera lam. from nsukka, south-eastern nigeria JIPBS. 9 99-103
Nwosu F, Morris J, Lund V A, Stewart D, Ross H A and McDougall G J 2011 Anti-proliferative and potential anti-diabetic effects of phenolic-rich extracts from edible marine algae. J. Food Chem. 126 1006-1012
Osman N A and Abkar F A 2015 Comparative evaluation of some selected bioactive constituents in the leaves and bark of Avicennia marina (Forsk.) Veirh from the Sudanese Red Sea Coast JFPI. 4 5-11
Padmini E A, Valarmathi A and Rani M U 2010 Comparative analysis of chemical composition and antibacterial activities of Mentha spicata and Camellia sinensis. Asian J. Exp. Biol. Sci. 1 772 - 781
Patel M B and Mishra S M 2012 Magnoflorine from Tinospora cordifolia stem inhibits α glucosidase and is anticyclic in rats. Journal Funcit. Foods. 4 79-86
Priyono S H 2008 Kajian konservasi buah merah melalui kultur jaringan tanaman; ekstrasi, fraksinasi buah, uji antioksidan, dan uji antidiabetik. JTL. 9 227-234.
Pulungan A and Herquanto 2009 Diabetes melitus tipe 1: penyakit baru yang akan makin akrab dengan kita MKI. 3 455-459
Rahman M A, Arif A and Sahid I Z 2011 Phytochemical and pharmacological properties of Bruguiera gymnorrhiza roots extract Int. J. Pharm. Res. 3 63-67
Rajaram K and Suresh K P 2011 In-vitro antioxidant and antiabietic activity of Tephrosia tinctoria PERS.: an endemic medicinal plant of South India J. Pharm. Res. 4 891-893
Ramadhan H 2013 Analisis inhibisi enzim α-glucosidase dan sitotoksisisitas ekstrak air-etanol benalu
jeruk (Loranthus spp.) [Undergraduate Thesis] (Bogor: IPB University)  
Robinson T 1995 Kandungan Organik Tumbuhan Tinggi (Bandung: Institut Teknologi Bandung) pp 281-282  
Riyanto E I, Widowati I and Sabdono A 2013 Skrining aktivitas antibakteri pada ekstrak Sargassum polycystum terhadap bakteri Vibrio harveyi dan Micrococcus luteus di Pulau Panjang Jepara. J. Mar. Res. 1 115-121  
Saleem F 2010 Anti-diabetic potentials of phenolic enriched chilean potato and select herbs of Apiaceae and Lamiaceae Families [Thesis] (Amherst: University of Massachusetts)  
Sancheti S, Sandesh S and Seo S Y 2009 Chaenomeles Sinensis: A Potent α-and β-Glucosidase Inhibitor Am. J. of Pharmacol. Toxicol. 4 8-11  
Sani I, Abdulhamid A, and Bello F 2014 Eucalyptus camaldulensis: Phytochemical composition of ethanolic and aqueous extracts of the leaves, stem bark, root, fruits and seeds. J. Sci.Innov. Res. 3 523-526  
Sari N 2010 Potensi Buah Makasar (Brucea javanica (L.) Merr) sebagai Inhibitor Enzim α Glukosidase [Undergraduate Thesis] (Bogor: IPB University)  
Shalaby E A and Sanaa M M S 2012. Comparison of DPPH and ABTS assays for determining antioxidant potential of water and methanol extracts of Spirulina platensis IJMS. 42 556-564  
Singh N, Patel A V, Alok S, Kannoja P, Garud N, Mehta S C 2010 Anti-diabetic activity of Bruguiera gymnorrhiza root Intern. J. Pharm. Sci. Res. 1 115-119  
Smith B 1962 A Simple Adsorption Chromatographic Method for the Investigation of Phenol Structure (Sweden: Acta Chemica Scandinavica) P 843-848  
Srivastava A K et al 2014 Antihyperglycaemic and antidysslipidemic activities in ethyl acetate fraction of fruits of marine mangrove Xylocarpus moluccensis J. Pharm. Pharm. Sci. 6 809-826  
Suarsana N I, Priosoeryanto B P, Bintang M, and Wresdiyati T 2008 Aktivitas hipoglikemik dan antiossidatif ekstrak metanol tempe pada tikus diabetes J. Veteriner 9 122-127  
Sudirman S, Nurjanah, and Jacoeb A M 2014 Proximate compositions, bioactive compounds and antioxidant activity from large-leaved mangrove (Bruguiera gymnorrhiza) fruit Intern. Food Res. J. 21 2387-2391  
Sukandar D, Hermanto S, and Mabrud I A. 2009. Aktivitas Senyawa Antidiabetes Ektrak Etil Asetat Daun Pandan Wangi (Pandanus Amaryllifolius Roxb.) Jurnal Sains dan Teknologi UIN Syarif Hidayatullah 3 269-273  
Toma A, Makonnen E, Mekonnen Y, Debella A, and Adisakwattana S 2015 Antidiabetic activities of aqueous ethanol and n-butanol fraction of Moringa stenopetala leaves in streptozotocin-induced diabetic rats BMC Complement. Altern. Med. 15 242-250  
Wang Z, Wang J, and Chan P 2013 Treating type 2 diabetes mellitus with traditional chinese and indian medicinal herbs Zhijun J. Hindawi Publishing Corporation 1 1-7  
Wang H, Du Y J, and Song H C 2010 α-Glucosidase and α-amylase inhibitory activities of guava leaves. J. Food Chem. 123 6-13  
Warsinah, Kusumawati E, and Sunarto 2011 Identifikasi senyawa antifungi dari kulit batang kecapi (Sandoricum koetjape) dan aktivitasnya terhadap Candida albicans identification of compound antifungi of S. koetjape stem and activity to Candida albicans. J M Obat Tradisional 16 16-173  
WHO 2013 Prevalence of diabetes worldwide (Geneva: World Health Organization)  
Yin J, Heo S I, Wang M H 2008 Antioxidant and antidiabetic activities of extracts from Cirsium japonicum roots Nutr. Res. Prac. 2 247-251  
You Q, Chen F, Wang X, Jiang Y, and Lin S 2012 Anti-diabetic activities of phenolic compounds in muscadine against alpha-glucosidase and pancreatic lipase Food Sci. Technol. 4 164-168