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Bioactive Compound Impacting the Metabolism and Antibacterial Activity of *Gadung* Tuber (*Dioscorea hispida* Dennst)

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Abstract. Plant extracts continue to represent an untapped source of renewable therapeutic compounds for the prevention of illnesses including metabolic disorders such as antihyperglycemia. This study was to determine and identify the chemical compounds in leaves and *gadung* tuber (*Dioscorea hispida* Dennst), α -glucosidase inhibitory effects of leaves and to test antibacterial activity of tuber against *Staphylococcus aureus* ATCC 25923 (Gram positive) and *Escherichia coli* ATCC 25922 (Gram negative). Identification of the chemical compounds was conducted using GC-MS technique, α -glucosidase inhibitory effects test was measured with spectrophotometric and antibacterial activity test was performed in vitro using agar disc diffusion assay. The bioactive compound evaluation of leaves confirmed the presence of dl-chimyl alcohol (11.82%); 10 (E), 12(Z)-conjugated linoleic acid (10.84%); stigmasterol (9.55%); heptadecane (5.94%); campesterol (5.91%). The analysis of *gadung* tuber revealed the presence of 7-Azabicyclo [4.1.0] heptane, 1-methyl- (23.16%); n-Hexadecanoic acid (18.85%); 10E, 12(Z)-Conjugated linoleic acid (13.73%); 1, 4, 7, 10, 13, 16 - hexaoxacyclooctadecane (4.34%); 5-Hydroxymethylfurfural (4.07%). IC₅₀ values inhibition of α -glucosidase extract was >300 ppm and glucobay standard was 0.210 ppm. The antimicrobial activity was performed against bacteria, its inhibitory effect. The results concluded that *gadung* tuber has great potential to invade some human pathogenic bacteria as it showed zone inhibition. Results from this research show that the concentration of *gadung* tuber compound has a low inhibition of alpha glucosidase activity. Further research is required to fully elucidate the bioactive compounds in this plant using vigorous analytical methods to be potentially responsible for health benefits

1. Introduction



It is estimated that a large portion of the world's population depend on medical nutrition therapy and bioactive compounds of plants may improve physical health in people. Plants are used as a source of medicine and many programs will continue to develop for use in pharmaceuticals. The volunteers claim about the ability of foods and their constituents of secondary metabolites to modify health and/or the risk of disease. Phytochemical compound in plant such as whole grains, cereal, vegetables, fruits including phenolic acids, flavonoids, lignans are associated with lowered risk of disease [1].

Tuber of *gadung* (*Dioscorea hispida* Dennst) internationally known to exhibit nutritional biological activity when compared with other roots, can lower blood glucose levels [2], plays a role in reducing lipogenesis pathway with the benefit of reducing the risk of obesity, diabetes and prevention of cardiovascular disease [3], and antitumor activity [4]. The leaves and *gadung* tuber crude extract in 96% ethanol was investigated for inhibition of α -glucosidase enzyme. It is the key enzyme catalyzing the step in the process of carbohydrate and retard the liberation of d-glucose from dietary complex carbohydrate. This enzyme hydrolyses oligosaccharides, trisaccharides, and disaccharides into glucose [5].

Pharmacological properties of n-hexadecanoic acid have been reported as antioxidant, hypocholesterolemic, nematicide, anti androgenic, hemolytic inhibitor including potential application of radioprotective phytochemicals effect [6], it possess potential antimicrobial effect [7], antimicrobial effect and anti-fungal activity [8], potency of anti hyperglycemic and anti hyperlipidemic activity of antioxidant in Torbangun (*Coleus amboinicus* Lour) leaves ethanol extracts [9].



Figure 1 *Gadung* (*Dioscorea hispida* Dennst)

The leaves and tuber of *gadung* are spread all over Indonesia known as *umbi gadung* is one of sources of food in supporting food diversification. *Gadung* is a creeper plant that can grow in any type of soil, and it has become the second stock food besides rice in Indonesia. The highest nutrient content as a source of carbohydrate, it is one alternative by utilizing local food. This plant contains dioscorin, evodiamine and rutaecarpine, it can be used as an insecticide [10]. We have to perform a pretreatment to remove dioscorin and cyanide acid, by immersing it in a salt solution for 3 days prior to consuming. These findings provide insight to determine the organic compounds in leaves and *gadung* tuber (*Dioscorea hispida* Dennst) by GCMS and inhibition of α -glucosidase. Antibacterial tests were performed on plants with potential antibacterial effect toward against bacteria gram positive *Staphylococcus aureus* ATCC 25923 and bacteria gram-negative *Escherichia coli* ATCC 25922. Antimicrobial activity was performed to evaluate the effectiveness of tubers of *gadung*. The present study has provided us with promising results for developing a wide range of folklore an herbal medicine.

2. Methods

2.1. Plant material and extract

Leaves and tuber of *gadung* (*Dioscorea hispida* Dennst) were collected on July 2019 from the Sarolangun, Jambi Indonesia. *Gadung* leaves and tuber were separated, water cleaned and air-dried in order to make simplisia powdered. The sample was macerated in ethanol 96%. The powdered leaves, tuber of *gadung* weighing 70 g, it was extracted with 600 ml of 95% ethyl alcohol for 72 hours for each batch with stirring. The final is concentrated with evaporator (Buchi, Switzerland), to obtain the extract.

Chemical compounds were identified using the Clarus 500 Gas Chromatography/Mass Spectrometer analysis was carried out on 7890 A, allows a carrier-gas flow of Electron Ionization (EI), the instruments consist of the following brand Shimadzu Type GCMS-QP2010. The column Elite-1 fused silica capillary type Rtx-5MS; 60 m; 0.25 mmID; helium (99.999%); column temperature 50 °C; inlet press (kPa) 0.85; a split ratio of 10 : 1, an injection volume 2 ml; SPL temperature 280 °C; MS interface 280 °C; ion source 200 °C. Each component was calculated by comparing its average peak area to the total area [11].

2.2. Determination of α -glucosidase Enzyme Inhibition

Inhibition test was done first for reaction mixture consisted 50 μ l of 0.1 M phosphate buffer (pH:7.0), 25 μ l of 0.5 mM 4-nitrophenyl α -D-glucopyranoside, 10 μ l of test sample (concentration: 500 μ g mL⁻¹), and 25 μ l of α -glucosidase solution. Each reaction was incubated at 37°C for 30 minute. Test terminated by adding 100 μ l of 0.2 M sodium carbonate solution. The enzymatic absorbance for each mixture was measured at 400 nm using microplate reader. Acarbose/glucobay was used as a positive control.

The inhibition percentage of α -glucosidase was calculated using a formula:

$$\% \text{ inhibition} = [1 - (\text{sample absorbance} / \text{control absorbance})] \times 100\%$$

Sample concentration and % inhibition were plotted: $Y = a + bx$. IC₅₀ value (inhibition activity) was defined as sample concentration that can inhibit 50% of enzyme activity, was calculated. The enzyme inhibition activities for α -glucosidase assay was evaluated with minor modification [12].

2.3. Determination of inhibition

Tests of antibacterial effect of leaves and tuber of *gadung* against bacteria gram positive *Staphylococcus aureus* ATCC 25923 and bacteria gram negative *Escherichia coli* ATCC 25922 were performed commonly by using agar well diffusion method. The dilution from 50 mg/mL to obtain 5%, 10%, 20%, in distilled water and 25 μ L of extract was added to prepared disc on Muller-Hilton agar as it was done for sensitivity test followed (minimum inhibitory concentration). The positive control used chloramphenicol for 24 hours incubation time at 37 °C the inhibition zone of the extract was measured [13].

3. Result and Discussions

3.1. Phytochemical screening

Phytochemical components in ethanolic extract of *gadung* leaves. Compounds identified from the leaves ethanol extract of *gadung* using GC-MS. These compounds were identified based on the Concentration (%), Retention Time (RT) and the Name of components. There are 18 components in the results, known as the Name, Quality, Concentrations, Retention Time and Peak on Table 1.

Table 1 Total ionic chromatogram (GCMS) of *gadung* leaves

Peak	Retention Time (s)	Quality	Conc (%)	Name
1	26.569	95	2.90	4. Neophytadiene
2	31.251	95	5.09	5. Phytol
3	32.740	99	5.67	6. n-Hexadecanoic acid
4	32.837	95	5.94	7. Heptadecane
5	32.988	64	2.18	8. 5-Cholestene-3-ol, 24-methyl
6	33.140	95	3.54	9. Campesterol
7	33.257	94	5.91	10. Campesterol
8	33.988	64	2.47	11. 15-Crown-5-
9	34.298	80	11.82	12. dl-Chimyl alcohol
10	34.436	97	5.11	13. Stigmasterol
11	34.560	99	6.24	14. Stigmasterol
12	34.609	99	3.19	15. Stigmasterol
13	34.671	99	9.55	16. Stigmasterol
14	34.029	99	10.84	17. 10 (E), 12(E)-Conjugated linoleic acid
15	35.795	53	3.55	18. Octaethylene glycol monododecyl ether
16	53.123	99	4.60	19. Vitamin E
17	53.143	99	2.56	20. Vitamin E
18	54.343	97	2.74	21. gamma.-Tocopherol

Compounds identified from the tuber ethanol extract of *gadung*. The 19 compounds were identified based on the Concentration (%), Retention Time (RT) and the Name of components. There are 19 components in the results, known as the Name, Quality, Concentrations, Retention Time and Peak on Table 2.

Table 2. Total ionic chromatogram (GCMS) of *gadung* tuber

Peak	R.Time	Quality	Conc%	Name
1	29.479	98	1.40	Hexadecanoic acid, ethyl ester
2	30.686	94	4.07	5-hydroxymethylfurfural
3	30.906	99	2.28	Linoleic acid ethyl ester
4	32.485	99	3.20	2-((8Z,11Z)-Heptadeca_8,11-dien-1-yl)-4,5-dihydrooxazole
5	32.533	76	2.74	Glycidyl palmitat
6	32.727	99	18.85	n-Hexadecanoic acid
7	33.133	35	23.16	7-Azabicyclo [4.1.0] heptane, 1-methyl-
8	32.251	49	2.66	Tridecenyl angelate, 2E
9	33.968	78	1.21	Octaethylene glycol monododecyl ether
10	34.209	90	2.60	Octadecanoic acid
11	34.499	68	1.42	Ethanol, 2-[2-(2-ethoxyethoxy) ethoxy]-
12	34.657	93	2.42	Butyl 9, 12 – octadecadienoate
13	35.002	99	13.73	10 (E), 12(Z)-Conjugated linoleic acid
14	35.333	53	1.88	1,4,7,10,13,16-Hexaoxacyclooctadecane
15	36.567	70	1.09	1,4,7,10,13,16-Hexaoxacyclooctadecane
16	39.767	76	1.39	18-Crown-6,[2-(tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]
17	40.242	46	1.22	2-[2-[2-[2-[2-[2-[2-[2-(2-hydroxyethoxy)
18	42.966	64	4.34	1,4,7,10,13,16-Hexaoxacyclooctadecane
19	53.040	97	3.07	Vitamin E

The present study revealed that *gadung* tuber extract possess potential antimicrobial effect against gram positive bacteria like *Staphylococcus aureus* ATCC 25923 on Figure 2, and gram- negative

Escherichia coli ATCC 25922 as indicated on Figure 3. The antimicrobial effect of *gadung* leaves was shown on Figures 4 and 5. This may be due to the ability of ethanol extracts semi polar dissolved component that have active properties were compared with control positive (chloramphenicol) and control negative (DMSO).

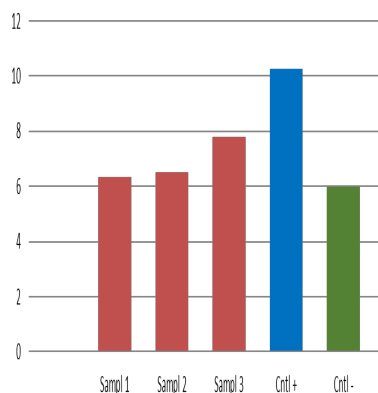


Figure 2 Zone of inhibition against *S. aureus* by *gadung* tuber. Statistically significant: $P < 0.05$; (One-way ANOVA).

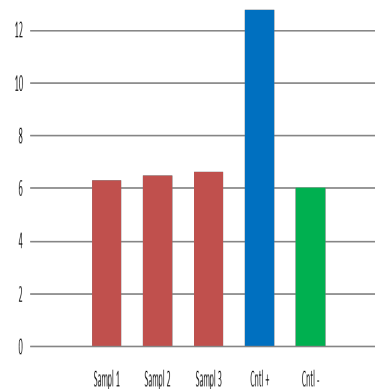


Figure 3. Zone of inhibition against *E. coli* by *gadung* tuber. Statistically significant: $P < 0.05$; (One way-ANOVA).

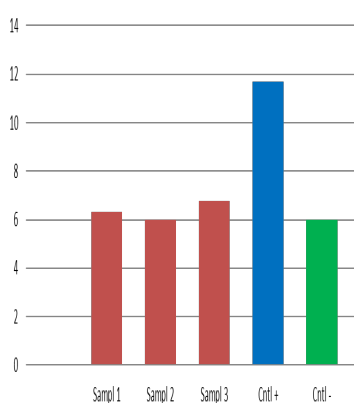


Figure 4 Zone of inhibition against *S. aureus* by *gadung* leaves. Statistically significant: $P < 0.05$ (One-way ANOVA).

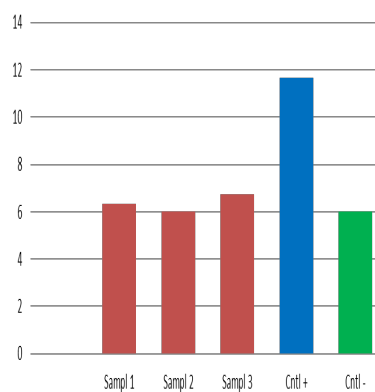


Figure 5 Zone of inhibition against *E. coli* by *gadung* leaves. Statistically significant: $P < 0.05$; (One-way ANOVA).

Alpha glucosidase inhibition activities involved in the biochemical process of carbohydrates metabolism, related to metabolic disorders such as hyperglycemia and bacterial infections. The inhibitors would retard the starch digestion and lowering blood glucosa level. The enzymatic was monitored by p-nitrophenol released in the reaction mixture at 400 nm using microplate reader, in triplicate carried out. Based on these IC_{50} values, *gadung* tuber extract was >300 ppm and glucobay standard value was 0.210 ppm. The inhibition can be increased by various bioactive compounds in the plant. The ethanolic extract of *gadung* tuber possession of antimicrobial activity and promise as effective antibacterial agents.

The secondary metabolites identified in the plant materials used in this study could be responsible for healthy biomodulatory activity exhibited by these plants. This research showed a lower inhibitory of

the α -glucosidase activity. The potential inhibited by many bioactive compounds by hydrolytic cleavage of *gadung* (*Dioscorea hispida* Dennst) plant may have beneficial effects on people's health.

4. Conclusion

The phytochemical analysis of the leaves and tuber of *gadung* (*Dioscorea hispida* Dennst) plant are important results to determine the organic components. The results concluded that *Gadung* has great potential to invade some human pathogenic bacteria as it showed zone inhibition. The concentration of tuber of *gadung* compound has a low inhibition of alpha glucosidase activity, impacting the carbohydrate metabolism. It is also important to undertake a long-term study of this plant to evaluate the herbal medicine and therapeutic action of this plant.

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