TurnitinBioactivecompoundsantidiabeticandantimicrobialpotentialofpinangseeds

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Bioactive compounds, antidiabetic and antimicrobial potential of pinang seeds extract (Areca catechu l.)

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ABSTRACT

Diabetes mellitus is a chronic metabolic disorder affecting people of all ages. The critical aspect of fruits is that rich sources of antioxidants may act in combination with each other phytochemicals to provide their protective effect. Pinang (*Areca catechu* L.) fruit is edible as the local indigenous plant from West Irian Jaya (Papua) Indonesia. This study was aimed at investigating the biologically active compounds of seeds, fruits, and leaves, the α-glucosidase inhibitory and antibacterial activity of seeds of Pinang against Gram-positive bacteria *Staphylococcus aureus* ATCC 25923, and Gram-negative bacteria *Escherichia coli* ATCC 25922. Pinang fruits were extracted by using the maceration method and ethanol solvent. Identify the chemical compounds in seeds by GC-MS technique, test to a-glycosidase inhibitory effect was measured with spectrophotometric. Well, the diffusion method was employed in evaluating the antimicrobial property of extracts. The evaluation of the bioactive compound of Pinang fruits revealed the presence of Vitamin E (0.20%). The inhibition of α-glucosidase of seeds extract of IC_{50} values was 82.74 ppm, and the global standard was 0.34 ppm. This study confirmed that Pinang seeds contain glucosidase activity that has the potency to inhibit glucose. The antimicrobial activity was performed against bacteria as it showed zone inhibition. These results indicated that Pinang seed extracts exerted potent inhibitory effects against α-glucosidase and inhibited the proliferation of Gram-negative microorganisms.

Keywords: α-glucosidase, antimicrobial, Pinang (*Areca catechu* L.) extract.

ABSTRAK

Diabetes melitus merupakan kelainan metabolisme kronis yang menyerang semua usia. Aspek penting dari buah-buahan adalah sumber antioksidannya yang kaya dapat bekerja bersama dengan fitokimia lainnya untuk memberikan efek perlindungan. Buah Pinang (*Areca catechu L.*) dapat dimakan sebagai tanaman asli lokal dari Irian Jaya Barat (Papua) Indonesia. Penelitian ini bertujuan untuk mengetahui senyawa aktif biologis biji, buah, dan daun, daya hambat α-glukosidase dan aktivitas antibakteri biji Pinang terhadap bakteri Gram positif Staphylococcus aureus ATCC 25923, dan bakteri Gram negatif Escherichia coli ATCC 25922. Buah pinang diekstraksi menggunakan metode maserasi dan pelarut etanol. Identifikasi senyawa kimia dalam biji dengan teknik GC-MS, uji efek penghambatan α-glikosidase diukur dengan spektrofotometri. Nah, metode difusi digunakan dalam mengevaluasi sifat antimikroba dari ekstrak. Evaluasi senyawa bioaktif buah Pinang menunjukkan adanya Vitamin E (0,20%). Daya hambat α-glukosidase ekstrak biji kakao nilai IC50 sebesar 82,74 ppm, dan standar global sebesar 0,34 ppm. Penelitian ini menegaskan bahwa biji Pinang mengandung aktivitas glukosidase yang berpotensi menghambat glukosa. Aktivitas antimikroba dilakukan terhadap bakteri karena menunjukkan zona penghambatan. Hasil ini menunjukkan bahwa ekstrak biji Pinang memberikan efek penghambatan yang kuat terhadap α-glukosidase dan menghambat perkembangbiakan mikroorganisme Gram-negatif.

Kata Kunci: α-glukosidase, antimikroba, ekstrak Pinang (*Areca catechu L.*).

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INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder in which a person has abnormally high blood glucose levels. It is a severe, significant chronic with potency life-threatening, in which the pancreas hormone improperly regulates the homeostasis of carbohydrates, which impacts the health status. Blood glucose control is critical for preventing or reversing diabetic complicati (Doan et al., 2018; Demir et al., 2021). At least estimated in 2019 that 463 million people have dia es, projected to reach 578 million by 2030 and 700 million by 2045 (IDF 2019).

In recent years, there has been a resurge e of interest in treating diabetes mellitus (DM) using herbal remedies, mainly because of their non-toxic nature. The World Health Organization has also recommended the assessment of the effectiveness of herbal plants, particularly in cases where safe and modern pharmaceuticals are lacking. Exploring bioactive compounds in plants with potential antidiabetic properties is of utmost importance. It is well known that the presence and effectiveness of these bioactive compounds in plants can vary depending on their location. Many plant species have been documented as having hypoglycemi properties and are traditionally used for preventing and managing diabetes. Despite Sefforts to develop hypoglycemic agents from both natural and synthetic sources, diabetes and its associated complications continue to pose significant medical challenges (Arya et al., 2015; Chen H, 2021; Ansari et al., 2022). Numerous medicinal plants and their products, including active principles and crude extracts, have been reported in the literature for their traditional use in diabetes control (Hegde et al., 2016; Saad B, Kmail A, Haq SZ, 2022; Nabi M, Tabassum N, Ganai BA, 2022). Our ongoing investigation focused on assessing the inhib ry effects of Pinang (Areca catechu L.) plants on the α-glucosidase enzyme. These enzymes play crucial roles in various biochemical processes related to metabolic disorders and diseases, including diabetes. As such, there has been a concerted effort to design efficient α-glucosidase inhibitors with promising potential applications (Chipiti et al. 2015; Di Santo MC, D'Antoni CL, Rubio AP, Alaimo A, Pérez OE, 2021).

Regenerate

Figure 1. Pinang Fruits (*Areca Catechu* L.)

Alpha-glucosidase inhibitors are carbohydrates that function as competitive antagonists for the enzymes necessary for carbohydrate digestion, specifically the α-glucosidase enzymes located in the brush border of the small intestine. These intestinal α-glucosidase enzymes break down oligosaccharides, trisaccharides, and disaccharides into glucose and other monosaccharides within the small intestine. Inhibitin ese enzyme systems reduces the rate at which carbohydrates are digested, leading to decreased glucose absorption, as the carbohydrates are not broken down into glucose molecules. The short-term impact of these drug therapies in diabetic patients is a reduction in current blood glucose levels, and the long-term effect is a slight decrease in hemoglobin levels (Hussain et al. 2019: Jeong D, Priefer R, 2022; Schauer et al., 2017).

Plants produce secondary metabolites in the form of phenolic compounds, also known as polyphenols, which are known for their antioxidant properties and, as a result, offer beneficial physiological effects. Polyphenols are among the most common antioxidant phytochemicals, and they are recognized for their ability to quench singlet oxygen and scavenge free radicals, thereby retarding the oxidation of lipids. Previous research has indicated that Areca catechu L. fruits are rich in phenolics and tannins, although their antioxidant activities remain relatively unexplored. In traditional markets in Papua, Pinang seeds are frequently used to prepare beverages like coffee.

Extensive research has been conducted on the antimicrobial properties of specific plant species. The pharmacological properties of phytochemicals suggest their potential for antimicrobial and antifungal effects (Shah et al., 2018). The mechanism through which antioxidants act against bacteria involves damaging the integrity of the cell wall and cell membrane, inhibiting intracellular enzyme activity, increasing the levels of reactive oxygen species (ROS), influencing the expression of associated genes, ultimately leading to bacterial apoptosis (Hu et al., 2019; Zhang et al., 2020; Ren X, An P, Zhai X, Wang S, Kong Q, 2019). These findings offer valuable insights into identifying bioactive compounds within Pinang seeds (Areca catechu L.) using GCMS, and the inhibitory effect on α-glucosidase activity was quantified using spectrophotometry. Furthermore, the research suggests a potential antimicrobial effect against both Gram-positive

Staphylococcus aureus ATCC 25923 and Gram-negative Escherichia coli ATCC 25922, as evidenced by the observed zones of inhibition. This study holds the promise of enhancing the utility of Pinang fruits across various domains, with a particular emphasis on their applications within medicine.

RESEARCH METHOD

Plant Extraction

Dried and fully matured fruits of Areca catechu L., commonly known as Pinang, were sourced from a traditional market in Papua, Indonesia. The extraction process involved soaking the sample in ethanol for a period of 48 hours, followed by subsequent extraction. Chloroform was employed for the extraction process, yielding a chloroform-soluble extract, which was then subjected to centrifugation at 10,000 rpm for a duration of 20 minutes. The resulting clear supernatant oil was subsequently analyzed using GCMS.

Procedures for Gas Chromatography-Mass Spectrometry (GC-MS)

Chemical compound identification was carried out using the SHIMAZU Gas Chromatography 5890-11 from Japan, equipped with a fused GC column composed of polymethyl silicon, specifically OV 101, with dimensions of 0.25 mm x 50 m. The temperature programming ranged from 80°C to 200°C, with an initial hold at 80°C for 1 minute, followed by a rate of increase at 5°C/min, and a final hold at 200°C for 20 minutes. The Flame Ionization Detector (FID) was set at 300°C, while the injection temperature was maintained at 250°C. Nitrogen served as the carrier gas, flowing at a rate of 1 cm^3/min, with a split ratio of 1:75. For mass spectrum analysis, a GCMS-QP 2010 Plus instrument from Shimazu Japan was used, featuring an injector temperature of 230°C and a carrier gas pressure of 100 kph. The column had a length of 30 m, a diameter of 0.25 mm, and a flow rate of 50 m/min. The eluents were automatically directed into the Mass Spectrometer, where the detector voltage was set at 1.5 kv, and data were collected at a sampling rate of 0.2 seconds. The Mass Spectrometer was also equipped with a computer-fed Mass Spectra data bank. The centrifuge employed in the study was the HERMCE Z 233 M-Z from Germany. Reagents and solvents, such as ethanol for analytics, were sourced from Merck Germany (Iwu et al., 2018; Shahvar A, Shamsaei D, Saraji M, 2020; Kachangoon R, Vichapong J, Santaladchaiyakit Y, Srijaranai S, 2020).

Assessment of α-Glucosidase Inhibition

The initial inhibition test involved a reaction mixture comprised of 50 μL of 0.1 M Phosphate buffer (pH: 7.0), 25 μL of 0.5 mM 4-nitrophenyl α-D-glucopyranoside, 10 μL of the sample with a concentration of 500 μg mL-1, and 25 µL of α-glucosidase solution. This reagent was incubated at 37°C for 11 minutes, and the enzymatic reaction was terminated by adding 100 μL of 0.2 M Na2CO3 (200 mM). To assess the enzymatic absorbance of each mixture, a spectrophotometric measurement was performed using a microplate reader, recording the absorbance at 400 nm.

The negative control involved the reaction between the substrate and enzyme without the presence of an inhibitor. Simultaneously, a blank solution represented the reaction system where the enzyme and inhibitor were absent. This set of actions was performed in triplicate for accuracy. Acarbose was dissolved in a solution containing buffer and 2 N HCL in a 1:1 ratio and was employed as a positive control, serving as an α-glucosidase inhibitor. The calculation of % inhibition was as follows:

The formula for calculating the inhibition percentage is expressed as: % inhibition = [1 - (sample absorbance / control absorbance)] x 100%. The sample concentration and its corresponding percentage of inhibition were then plotted on a graph using the equation Y = a + bx. The term "inhibition activity value" (IC50) was defined as the concentration at which the sample could inhibit 50% of the enzyme's activity. The assessment of enzyme inhibition activities for the α-glucosidase assay was conducted with slight adjustments, as per the work of Yin et al. in 2014.

Determination of Antibacterial Activity

The antibacterial properties of the Pinang extract were assessed through the agar well diffusion method. Escherichia coli ATCC 25922 (Gram-negative) and Staphylococcus aureus ATCC 25923 (Gram-positive) bacteria strains were utilized to evaluate the antibacterial efficacy of these extracts. The extract solution was prepared by dissolving 0.1 g of the extract in 100 mL of solvent, consisting of a mixture of distilled water and absolute ethanol, resulting in a 100 mg/mL concentration. This solution was further diluted to achieve 5%, 10%, and 15% concentrations in distilled water. Subsequently, 25 μL of the extract solution was added to prepared discs measuring 6 mm in diameter, which were placed on Muller-Hilton agar as part of the sensitivity test, followed by the determination of the minimum inhibitory concentration. Chloramphenicol was employed as the positive control, and the inhibition zone produced by the extract was measured after 24 hours of incubation at 37°C (Arvind et al., 2014; Dan et al. Q, Zhang F, Wang WW, Gao JM, 2019; Ha KS, Jo SH, Mannam V, Kwon YI, Apostolidis E, 2016).

RESULTS AND DISCUSSION

The Results of Phytochemical Screening of Pinang (Areca catechu L) Plant

Analytical Gas Chromatography-Mass Spectrometry (GC-MS) was employed to determine the phytochemical components within the ethanol extracts of Pinang seeds. The identification of these compounds was confirmed by examining the molecular formula, retention time, and peak area, as illustrated in Figure 2 and detailed in Table 1.

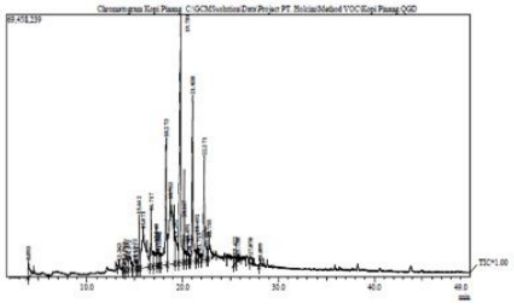

Figure 2: The components in Pinang seeds

There are 30 components metabolites in the results of Pinang seeds extract, using GCMS in Table 1

Table 1
GC-MS analysis of an extract of Pinang seeds

Peak	Retention Time (s)	Quality	Conc (%)	Name of the compound
1	3.893	48498945	1.49	Ethanol (CAS) Ethyl alcohol
2	13.363	13657573	0.42	The compound with the CAS identifier Oxiranecarboxamide, 2-ethyl-3-propyl- can be more simply referred to as Oxanamide.
3	13.864	24662469	0.76	Phenol, 2-methoxy- (CAS) Guaiacol
4	14.117	44937130	1.38	4-(1H-IMIDAZOL-4-YL)-PROPAN-1-OL
5	14.372	60537478	1.86	9-Oxabicyclo [6.1.0] nonan-4-one (CAS)4-Oxo-9-oxabicyclo [6.1.0] nonane
6	14.972	39618113	1.22	2-Noreplagiodial-D4
7	15.217	23188034	0.71	1-D1-CIS-1,2-CYCLOHEXANEDIOL
8	15.442	62348894	1.91	2-Propanol, 1-amino- (CAS) 1-Amino-2-propanol
9	15.871	3105816773	9.38	NICKEL1-AMINO-1,9-DIISOTHIOCIPNO-4,8-DI-AZAUNDECAMINE
10	16.717	1708952377	3.34	Benzenepropanenitrile. beta.-imino- (CAS) Acetonitrile, benzimidoyl-
11	17.240	96641382	2.97	2,4-Pentanediamine, 2-methyl- (CAS) 2,4-DIAMINO-2-METHYLPENTANE
12	17442	31681968	0.97	2-(5-BROMO-PENT-3-YNYLOXY)-TETRAHYDRO-PYRAN
13	17.542	37309950	1.14	Ethanamine, N-ethyl-N-hydroxy- (CAS) N, N-Diethylhydroxyamine
14	18.273	16832467	9.72	1,4-Butanediol (CAS) Sucol B
15	18.735	322898581	16.04	Tetrahydro-4H-pyran-4-ol
16	19.291	219039910	3.65	1,2,3,4-Butanetetrol, [S-(R*,R*)]- (CAS) L-ERYTHRITOL
17	19.785	144245636	16.70	Piperazine, 2-methyl- (CAS) 2-Methylpiperazine
18	20.167	7 5813436	2.33	3(2H)-Furanone, dihydro-5-methyl- (CAS) 5-METHYL-3-OXO-TETRAHYDROFURAN
19	20.491	49335566	1.51	2H-Pyranol, tetrahydro-3(or 5)-methyl- (CAS) 3-METHYL HYDROXY TETRAHYDRO PYRAN
20	20.717	12642485	0.39	5-HYDROXY-4-NITROBENZOTRIAZOLE
21	21.038	57199594 7	7.89	6H-Pyrazolo[1,2-a][1,2,4,5]tetrazine, hexahydro-2,3-dimethyl- (CAS) 1,3,4,6-TETRAAZABICYCLO
22	21.492	42383339	1.30	1,4-Butanediol (CAS) Sucol B
23	21.715	30315579	0.93	Ethanamine, N-ethyl-N-hydroxy- (CAS) N, N-Diethylhydroxyamine
24	22.272	102784108	6.22	13-Oxabicyclo[10.1.0]tridecane (CAS) Epoxycyclododecane
25	22.567	24474276	0.75	1-(CYCLOHEXYL-HYDROXY-AMINO)-PROPANE-2-OL
26	22.755	70840139	2.17	26 22.755 7 0840139 2.7 Ethanol, 2,2',2''-nitrilotris- (CAS) Triethanolamine
27	25.422	22301874	0.68	1-Piperazinecarboxylic acid, ethyl ester (CAS) Ethyl N-piperazinocarboxylate 3259080273 100.00
28	25.750	24595061	0.75	3-METHYLCYCLOPENTANONE-2,2,5,5-D4
29	27.070	30551180	0.94	1,3-Propanediamine, N, N-diethyl- (CAS) 3-DIETHYLAMINO PROPYL AMINE
30	28.099	14975956	0.46	Piperazine, 2-methyl- (CAS) 2-Methylpiperazine
		3259080273	100.00	

The observed of the ethanol extract Pinang fruits, there were 50 compounds identified. The identification was assured by observing the molecular formula, retention time, and peak area of the data shown in Figure 3 and Table 2.

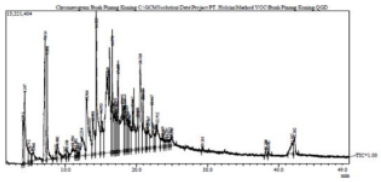

Figure 3. The components in Pinang fruits

There are 50 component metabolites in the results of Pinang fruits, using GC-MS in Table 2.

Table 2
GC-MS analysis of an extract of Pinang fruits

Peak	Retention Time (s)	Quality	Conc (%)	Name of the compound
1	3.993	4 1267771	1.84	Acetic acid, hydroxy-, methyl ester (CAS), Methyl glycolate
				5 5.466 1 6964033 0.75 Cyclopropyl carbinol
2	4.247	9 0587839	4.03	Acetic acid, hydroxy-, methyl ester (CAS), Methyl glycolate
3	4.792	1 7187970	0.76	(Methylcyclopentadienyl)
				[(2,4,6-triisopropylphenyl) phosphane] molybdenum tetrachloride
4	5.167	4316669 0	0.19 3	[2,3,5,6-tetrafluoro-4-trifluoromethyl) phenoxy] pregna-3,5-diene-20-one
5	5.466	1 6964033	0.75	5 5.466 1 6964033 0.75 Cyclopropyl carbinol
6	7.114	1 1651785	4.97	2-DEUTERO-2-PROPANOL
7	7.392	4 7463832	2.11	Oxirane (CAS) Epoxyethane
8	8.617	8 7443009	0.33	Chlorine oxide (Cl2O) (CAS) Chlorine monoxide
9	8.802	2 3545163	1.05	3-OXA-BICYCLO [3.2.0] HEPT-6-ENE-2,4-DIONE
10	9.917	5957835	0.27	1,4-Butanediol (CAS) Sucol B
11	10.150	9309750	0.41	3-Butyn-1-ol (CAS) 3-Butynol
12	10.994	24010146	1.07	L-(-)-ASPARAGIN
13	11.392	5481624	0.24	Hexatriacontane (CAS) n-Hexatriacontane
14	11.617	2727468	0.12	OCTADEC-2-YNOIC ACID
15	11.842	3723801	0.17	2,3,6,7-tetramethyl-9,10-bis(4-methylphenylsulfonyloxy)-1,4,4. alpha.,5,8,8a. beta.,9.
				10a.,9a. beta
16	12.226	4 9111091	2.19	6-DIMETHYLAMINO-3,5-DIHYDROXY-1,2,4-TRIAZINE
17	12.96 1	42 2142946	5.44	1H-Pyrrole (CAS) Pyrrole
18	13.831	4 8725150	2.17	Phenol, 2-methoxy- (CAS) Guaiacol
19	14.321	554134633	6.86	1-METHYL-OXIRANE-2-CARBOXYLIC ACID
20	14.993	6 4642977	2.88	2-(3,5-DIMETHYL-1-PYRAZOLYL)SUCCINIC ACID
21	15.922	895078510	13.13	15.92 289 5078510 13.19 TRANS- 3,5-DEUTERON HYDROXY CYCLOPENTENE
22	16.570	6 8506413	3.05	16.570 6 8506413 3.05 Phenol, 2,6-dimethoxy- (CAS) 2,6-Dimethoxyphenol
23	16.800	3 6385216	1.62	1-pentanal (CAS) n-Pentanal
24	16.992	4 1792593	1.86	HYDRAZINECARBOXAMIDE, 2-(2,6-CYCLOOCTADIEN-1-YLIDENE)-
25	17.167	3 7535112	1.67	HEXA-2,4-DIYNE-1,6-DIOL
26	17.407	5 4810399	2.44	5-ETHYL-2-METHYL-PYRIDIN-4-YLAMINE
27	17.99	121 7652166	5.24	17.99 121 7652166 5.24 1,2-(1'-METHYLTRIMETHYLENE)-DIBORANE
28	18.319	5 0954593	2.27	18.319 5 0954593 2.27 Ethanone, 1-(4-pyridinyl)-, oxime
29	18.615	3 4782251	1.55	N-[(5-METHYL-2-PYRIDYL)AMINOMETHYL]-4-NITROPHTHALIMIDE
30	18.792	1 7844319	0.79	Hexanedinitrile (CAS) Adiponitril
31	18.967	45080280	2.01	1-Propyne, 3,3'-oxy bis- (CAS) Dipropargyl ether
32	19.442	88899516	3.96	19.442 88899516 3.96 3-[(Z)-1-Butenyl]-4-vinyl cyclopentene Peak# R.Time Area
				Conc%PUSLITBANG HASIL HUTAN REPORT Name
33	20.131	41781363	1.86	2-Piperidinone, 6-methyl- (CAS) 6-Methyl-2-piperidone
34	20.518	128857593	5.73	5-ETHYL-N-METHYL-3-BUTENYLAMINE
35	20.934	57932585	2.58	6H-Pyrazolo[1,2-a][1,2,4,5]tetrazine, hexahydro-2,3-dimethyl- (CAS) 1,3,4,6-TETRAAZABICYCLO
36	21.464	15094044	0.67	7-Oxabicyclo[4.1.0]heptane(CAS) Cyclohexene oxide
37	21.667	31817404	1.42	5,5-D2-TRANS-3,4-DIHYDROXY-CYCLOPENTENE
38	22.167	48545178	2.16	Propenal dimethylhydrazine
39	22.492	12972899	0.58	5-Thiomethylfurfural
40	22.912	53046873	2.36	FORMAMIDE, N,N'-1,3-PROPANEDIYLBIS-
41	23.669	19268539	0.86	TRANS- 3,5-DIDEUTERO HYDROXY CYCLOPENTENE
42	23.942	8284749	0.37	1,3,2-Dioxaphosphorinane, 2,5,5-triphenyl-, 2-oxide (CAS) 2-OXO-2,5,5-TRIPHENYL-1,3,2-DIOXAPHOSPHORINANE

43	24.317	11892832	0.53	-Pentanol, 2-amino-4-methyl-, (S)-
44	24.767	7970280	0.35	O, N-PERMETHYLATED AC-ALA-HIS
45	24.956	13517553	0.60	Oxiranecarboxamide, 2-ethyl-3-propyl- (CAS) Oxanamide
46	29.261	1575985	0.07	1-(2-METHYL-ALLYL)-AZETIDINE 2247262259 100.00
47	38.208	4864971	0.22	Urea, N,N'-diethyl- (CAS) N,N'-Diethylurea
48	38.602	4453877	0.20	Vitamin E
49	41.917	23707032	1.05	Heptanal (CAS) n-Heptanal
50	42.262	21961642	0.98	Piperazine, 2-methyl- (CAS) 2-Methylpiperazine
		2247262259	100.00	

The identified of the ethanol extract Pinang leaves, there were 50 compounds identified. The observed was assured by observing the molecular formula, retention time, and peak area of the data shown in Figure 4 and Table 3.

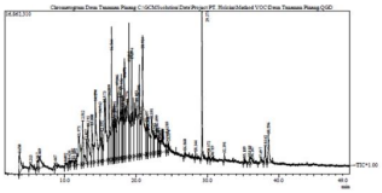

Figure 4. The components in Pinang leaves

There are 60 components metabolites in the results of Pinang leaves extract, using GCMS in Table 3:

Table 3. GC-MS analysis of an extract of Pinang leaves

Peak	Retention Time (s)	Quality	Conc (%)	Name of the compound
1	3.650	27475350	1.09	(3S,4S)-3,4-Bis(methoxy ethoxy)pyrrolidine
2	5.213	10234497	0.41	Propanenitrile, 3-(methylamino)-
3	6.167	11332584	0.45	45 N-BUTANE-1,1,1-D3
4	6.469	26579943	1.05	Cyclobutanol (CAS) Cyclobutyl hydroxide
5	8.667	5856399	0.23	Cyclobutanol (CAS) Cyclobutyl hydroxide
6	10.092	6580817	0.26	2-Pentenenitrile (CAS) 1-Cyano-1-butene
7	10.626	6532023	0.26	Formamide, N-(2-methylpropyl)- (CAS) N-Isobutylformamide
8	11.102	11998516	0.48	Ethanamine, N, N-difluoro- (CAS) ETHYL-N, N-DIFLUOROAMINE
9	11.492	6453835	0.26	Lup-20(29)-en-21-ol, 3,28-bis[(tetrahydro-2H-pyran-2-yl)oxy]-,3,3-dimethyl butanoate, (3.beta.,21 10
10	11.971	61766179	2.45	6-DIMETHYLAMINO-3,5-DIHYDROXY-1,2,4-TRIAZINE
11	12.512	44464290	1.76	2,6-Nonadien-1-ol (CAS) Cucumber alcohol
12	13.027	38331482	1.52	Pyrazine, methyl- (CAS) Methylpyrazine
13	13.292	49033980	1.95	1-Heptanamine, N-heptyl-N-nitro- (CAS) DIHEPTYL NITRAMINE
14	13.800	48746560	1.93	1,2-Hexadiene, 5-methyl- (CAS) 5-METHYL-1,2-HEXADIENE
15	14.294	100182931	3.97	1,2-DIMETHYLDIBORANE-D4
16	14.943	58918032	2.34	1-MONODEUTERO-1-HEXYNE
17	15.192	25553773	1.01	6-DIMETHYLAMINO-3,5-DIHYDROXY-1,2,4-TRIAZINE
18	15.57	1332448407	5.25	Cyclohexanone, 3-hydroxy- (CAS) 3-Hydroxycyclohexanone
19	16.219	55920181	2.22	-(6-OXA-BICYCLO[3.1.0]HEX-1-YL)-BUT-3-YN-2-ONE
20	16.544	95030755	3.77	Phenol, 2,6-dimethoxy- (CAS) 2,6-Dimethoxyphenol
21	16.742	30761807	1.22	2,2'-(1,4-PHENYLENE)BIS[4-(2,4-DICHLOROBENZYLIDENE)-4,5-DIHYDRO-5-
22	16.890	25346323	1.01	Pentane, 1-chloro- (CAS) 1-Chloropentane
23	16.992	23677774	0.94	Propanal, 2-methyl-, oxime
24	17.172	52992292	2.10	2-HYDROXY-1-ETHOXYETHYL-1-FURAN (NAME ?)
25	17.346	93298956	3.70	Pyrazine, 2-methoxy-6-methyl- (CAS) 2-Methoxy-6-methyl pyrazine
26	17.749	71507223	2.84	1-ETHYL-2-METHYL-PYRIDIN-4-YLAMINE
27	17.956	64859973	2.57	9-Oxabicyclo[6.1.0]non-4-ene (CAS) 1,2-Epoxy-5-cyclooctene
28	18.167	28806620	1.14	1-Butanamine, N-methyl-N-nitroso- (CAS) Butylmethylnitrosamine
29	18.371	86623565	3.44	FORMALDEHYDE, 1,1-DIDEUTERO-, 2,4-DINITROPHENYLHYDRAZON
30	18.77	1921523673	4.82	Butanal, 3-methyl- (CAS) 3-Methylbutanal
31	19.015	76398840	3.03	Piperazine, 2-methyl- (CAS) 2-Methylpiperazine
32	19.39	1431550732	5.22	Pregeijerene
33	20.05	1328072499	5.08	Silanediamine,1-chloro-N, N, N', N',1-pentamethyl-
34	20.390	83731457	3.32	1,6-Octadien-1-ol, 3,7-dimethyl- (CAS) 3,7-DIMETHYL 2,6-OCTADIENE-1-OL
35	20.651	46553815	1.85	3,3,3-TRIFLUORO-N-(2-FLUOROPHENYL)-2-(TRIFLUOROMETHYL)PROPIONAMIDE

36	20.92	1458289271	6.28	6H-Pyrazolo[1,2-a][1,2,4,5]tetrazine, hexahydro-2,3-dimethyl- (CAS) 1,3,4,6-TETRAAZABICYCLO
37	21.497	21046659	0.83	Piperazine, 2-methyl- (CAS) 2-Methylpiperazine
38	21.571	12075433	0.48	Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, oxime (CAS) 2-HYDROXYIMINOBORNANE
39	21.658	2606853	2.48	Cyclohexanone, 2-methyl-5-(1-methyl ethenyl)- (CAS) p-Menth-8-en-2-one
40	22.181	61268768	2.43	1,4,5,8-TETRAAZADECALIN
41	22542	11047993	0.44	7-Octynoic acid, methyl ester (CAS) METHYL-7-OCTYNOATE
42	22.899	46778771	1.86	1-Piperazinecarboxylic acid, ethyl ester (CAS) Ethyl N-piperazinocarboxylate
43	23.092	5968911	0.24	TRANS-8-EXOMETHOXYBICYCLO[4.3.0.]-3-NONENE-7-ENDO CARBOXALDEHYDE
44	23.167	15062454	0.60	1-Hepten-2-ol, (E)- (CAS) TRANS-HEPT-3-EN-2-OL
45	23.392	6531738	0.26	2-(ALLYLAMINO)-5-ETHYL-1,3,4-THIADIAZOLE
46	23.767	50112562	1.99	2,3-DIMETHYL-AZIRIDINE
47	24.318	11365084	0.45	1-Aziridineethanol (CAS) 1-Azirdineethanol
48	24.590	5973113	0.24	2,4-Pentanediamine, 2-methyl- (CAS) 2,4-DIAMINO-2-METHYLPENTANE
49	26.860	4209413	0.17	1,3-Propanediamine, N, N-diethyl- (CAS) 3-DIETHYLAMINO PROPYL AMINE
50	28.344	3639367	0.14	Phosphonic acid, diphenyl ester (CAS), Diphenyl phosphite
51	29.271	66929662	2.66	3,7-Dimethyl-1,7-octadien-3-amine
52	30.172	5573323	0.22	Ethyl N-methyl, N-ethylcarbamate
53	30719	3606344	0.14	Benzenemethanol, alpha.-(1-aminoethyl)-, (R*,R*)- (CAS) Norpseudoephedrine
54	32.391	4329790	0.17	1,4,5,8-TETRAAZADECALIN
55	35.189	3471638	0.14	1,3-Propanediamine, N, N-diethyl- (CAS) 3-DIETHYLAMINO PROPYL AMINE
56	35.918	7256057	0.29	TETRACOSANE, 1-BROMO-
57	36.267	8833182	0.35	N(2)-(t-Butoxycarbonyl)-N(6)-(benzyloxycarbonyl)-L-Lysin-4-acetamido anilide
58	37.497	5077026	0.20	Pentanal (CAS) n-Pentanal
59	38.142	15617318	0.62	Piperazine, 2-methyl- (CAS) 2-Methylpiperazine
60	38.596	34894831	1.38	Vitamin E
		2520711644	100	

The GC-MS analysis results of the extracts indicated variations in the percentage of each metabolite. The ethanol extracts of Pinang seeds contained thirty different compounds, while the fruits showed the presence of fifty compounds, and the leaves exhibited a total of sixty compounds. A noteworthy bioactive compound with antioxidant properties, Vitamin E, was detected at 0.20% in the fruits. In comparison, the analysis of Pinang leaves revealed a higher concentration of Vitamin E at 1.38%. Each of these bioactive compounds is associated with specific physiological functions. We conducted an α-glucosidase test using the Pinang seeds extract to explore the potential antidiabetic properties.

Alpha-Glucosidase Enzyme Inhibition

Numerous plant-based remedies have gained recognition for their potential in managing degenerative conditions such as diabetes, and indirectly, they serve as a source for developing prospective modern drugs, particularly for addressing hyperlipidemia. Bioactive compounds present in these plants often act as antioxidants, capable of impeding glucose absorption. In vitro analyses have confirmed the α-glucosidase inhibitory properties of ethanolic-maceration extracts. These liver glucosidase inhibitors target -1, 6-glucosidase, a glycogen-debranching enzyme within the liver. By doing so, they reduce the glycogenolytic rate, leading to increased glycogen storage in the liver. Consequently, inhibiting these enzyme systems results in decreased blood glucose levels, representing a short-term effect and a modest reduction in hemoglobin A1c levels. Given the various side effects associated with synthetic glucosidase inhibitors, researchers in the present era have a growing focus on herbal medicines (Papuc et al., 2017).

Therefore, the Pinang seeds extracts (in 80% ethanol) showed a potent α-glycosidase inhibitor in this study. The α-glucosidase inhibition of seeds may be related to the bioactive compounds properties of the fruits and leaves of this plant (Yin *et al.,* 2014). The benefit of these inhibitors would retard starch digestion, absorption, and lowering blood glucose levels. IC_{50} values of α-glucosidase inhibited seeds extract were 82.74 ppm, and the global standard was 0.34 ppm, presented in Figures 5 and 6. This research showed an inhibitory of the α-glucosidase activity of seeds of Pinang. Our results suggest that the extract of Pinang seeds is a potential candidate for developing antidiabetic agents.

Potential Antimicrobial Effect

The data indicates that the extract derived from Pinang seeds has demonstrated potential antimicrobial properties by inhibiting the growth of the Gram-negative bacterium Escherichia coli ATCC 25922, as presented in Table 4. Our findings suggest that the mechanism of action of the Pinang seeds extract against E. coli could involve membrane depolarization and increased membrane permeability, which affect intracellular enzyme activities and elevate intracellular levels of reactive oxygen species (ROS). These alterations may lead to cell apoptosis and, ultimately, the death of the bacteria (Shah et al., 2018; Zhang et al., 2020; Lin et al., 2019).

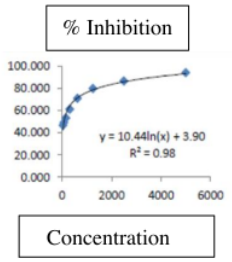

Figure 5. Values Inhibition of Pinang seeds

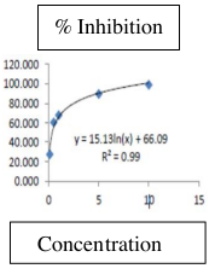

Figure 6. Values Inhibition of Acarbose

Table 4
Potential antimicrobial effect

Sample	Condition	Parameter		Result	Unit	Analysis
		E. Coli	10%	7.38	mm	diffusion
		antibacterial	5%	6.00	mm	diffusion
Pinang seeds	solid		2.5%	6.00	mm	diffusion
		S. Aureus	10%	6.00	mm	diffusion
		antibacterial	5%	6.00	mm	diffusion
			2.5%	6.00	mm	diffusion
Control (+)		E. Coli	0.1%	22.5	mm	diffusion
Chloram	solid	antibacterial				
Phenicol		S. Aureus	0.1%	24.14	mm	diffusion
		antibacterial				
Control (-)		E. Coli	20%	6.00	mm	diffusion
DMSO	liquid	antibacterial				
		S. Aeureus	20%	6.00	mm	diffusion
		antibacterial				

Disc diameter: 6.00 mm

The results of the GC-MS analysis of the seeds, fruits, and leaves of Pinang (*Areca catechu* L.) plants are essential to determine the organic compounds. Vitamin E in fruits and leaves acts as an antioxidant in this plant. Pinang seeds' concentration of bioactive compounds has potential α-glucosidase inhibited activity, impacting the delayed absorption of carbohydrates and the potent effect on diabetes treatment to increase healthy glucose blood levels. The Pinang seeds formula has the potential antimicrobial activity to inhibit the growth of *E.coli* bacteria as it showed zone inhibition. The bioactive compounds in this plant extract might prevent diabetes and antimicrobial and may serve as alternative drugs for treating various illnesses in human beings.

CONCLUSIONS AND SUGGESTIONS

The analysis results of bioactive components in seeds, fruits, and leaves Areca catechu (*Areca catechu L.*) plants have antioxidant activity. Active components in Areca nut seeds have the ability to inhibit the activity of the enzyme alpha-glucosidase, which affects carbohydrate metabolism and has the potential for alternative treatment of chronic hyperglycemia. Areca nut seeds have antibacterial bioactivity against Gram-negative bacteria E.coli, with an inhibition zone. Further research is needed to determine the benefits of the chemical composition of the Areca nut plant as an alternative medicine to help maintain health.

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