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Antidiabetic Formulation Development Based on Natural Materials As α-Glucosidase Enzyme Inhibitor

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Abstract: Salam (Syzygium polyanthum), stevia (Stevia rebaudiana), tea (Camellia sinensis), and yakon (Smallanthus sonchifolius) leaves act as inhibitors of the α-glucosidase enzyme. The aims of this research were to (i) determine the best combination of polyherbals that could inhibit the α-glucosidase enzyme, (ii) determine the phytochemical content of the teraactive fraction, and (iii) predict potential bioactive compounds that were responsible for the inhibition of the α-glucosidase enzyme. The novelty of this research was its use of several types of herbs, combined with various compositions, to obtain the IC_{50} value. Also, this research predicted the activity of the compound through a molecular docking approach with Molegro Virtual Docker software. The extraction was carried out with the maceration method with 96% ethanol and then partitioned with ethyl acetate and water. Two fractions with high inhibition percentages were combined with a ratio of 1:1, 1:2, 2:1, 1:3, and 3:1, and they were tested against the α-glucosidase inhibitory enzyme. The best fraction was identified for chemical compound content by Liquid Chromatography Mass Spectroscopy (LC-MS/MS). The compounds were analyzed in silico by using Molegro Virtual Docker software, which obtained the best reranc score. The results showed that the ethanol extract of tea leaves had the highest activity, at 65.51%, and salam, stevia, and yakon leaves had inhibition percentages of 44.11%, 34.74%, and 28.85%, respectively. The combination of water fractions of tea and salam leaves had the best activity at a ratio of 3:1 with IC_{50} of 16.53 ppm, which was almost equivalent to acarbose as a positive control with IC_{50} of 14.62 ppm. The analysis of LC-MS/MS for the water fraction of tea contained nicotiflorin with m/z 594, a reranc score of -118.24, and amino acid residues Arg, Asp, Asn, Cys, Glu, His, Thr, and Tyr. The water fraction of salam leaves contained quercetagetin with m/z 319, the reranc score was –89.123, and the amino acid residues Asn, Asp, Gln, Lys, Phe, Pro, Thr, and Trp.

Keywords: Syzygium polyanthum, Stevia rebaudiana, Camellia sinensis, Smallanthus sonchifolius, α-glucosidase enzyme inhibitor, acarbose.

以天然物质为α-葡萄糖苷酶抑制剂的抗糖尿病制剂开发

摘要：萨拉姆（蒲公英）、甜叶菊 (甜叶菊)、茶 (茶树) 和牦牛 (小花)的叶子充当 α-葡萄糖苷酶的抑制剂。这项研究的目的是 (i) 确定可以抑制 α-葡萄糖苷酶的多草药的最佳组合，(ii) 确定变异部分的植物化学成分，以及 (iii) 预测潜在的生物活性化合物，这些化合物负责抑

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制α-葡萄糖苷酶。这项研究的新颖之处在于它使用了几种类型的草药，并结合了各种成分，以获得我知道了 50 值。此外，这项研究通过分子对接方法与莫莱格罗 虚拟码头工人软件预测了该化合物的活性。用 96％乙醇浸渍法进行萃取，然后用乙酸乙酯和水分配。将两个抑制率高的馏分以 1:1、1:2、2:1、1:3 和 3:1 的比例组合，并针对 α-葡萄糖苷酶抑制酶进行测试。通过液相色谱质谱 (液相色谱-质谱/质谱) 确定化合物含量的最佳部分。使用莫莱格罗 虚拟码头工人软件对化合物进行计算机分析，获得了最佳的重新安排分数。结果表明，茶叶乙醇提取物的活性最高，为 65.51%，而萨拉姆、甜菊和雅康的抑制率分别为 44.11%、34.74% 和 28.85%。茶水组分与萨拉姆叶的组合在 3:1 的比例下具有最佳活性，我知道了 50 为 16.53 ppm，几乎相当于阿卡波糖作为阳性对照，我知道了 50 为 14.62 ppm。液相色谱-质谱/质谱分析茶的水馏分含有米/z 594、再循环分数为 -118.24 和氨基酸残基精氨酸、天冬氨酸、砷化镓、半胱氨酸、谷氨酸、他的、苏氨酸和酪氨酸的烟花苷。莴苣叶的水部分含有米/z 319 的槲皮素，重新安排评分为–89.123，氨基酸残基砷化镓、天冬氨酸、谷氨酰胺、赖氨酸、苯丙氨酸、临、苏氨酸和色氨酸。

关键词：蒲公英、甜叶菊、茶树、小花、α-葡萄糖苷酶抑制剂、阿卡波糖。

1. Introduction

Diabetes mellitus (DM) is a metabolic disease caused by chronic metabolic function failure in the human body due to lack of insulin secretion and decreased insulin tissue response to metabolize carbohydrates, proteins, and lipids. Diabetes Mellitus (DM) is a complex metabolic disorder characterized by a persistent hyperglycaemic state [1]. Diabetes mellitus has two types: type I diabetes, characterized by damage to pancreatic cells due to autoimmune diseases and causing dependence on exogenous hormones and type II diabetes, when the body becomes resistant to insulin effects or does not produce enough insulin [2]. DM is generally associated with several complications that arise secondary to diabetes, such as neuropathy, nephropathy, retinopathy, cardiovascular complications, etc. [3]. The prevalence of diabetes increases every year. In 2015, diabetes patients' number reached 415 million, and predictably, 578 million people will have diabetes by 2030. That number will jump to 700 million by 2045, with 95% of people with diabetes mellitus type-2 [1].

Several attempts have been made to reduce the prevalence of diabetes. One of the efforts made is to develop synthetic oral hypoglycemic drugs. Many synthetic oral hypoglycemic drugs have shown their inability to control DM-related complications. Oral hypoglycemic drugs have side effects: hyperinsulinemia, hypoglycemia, weight gain, diarrhea, flatulence, dehydration, and urinary tract infections [4].

Indonesian medicinal plants have been widely used for health purposes and as treatments for diabetes. Those that have antidiabetic properties include salam leaves (Syzygium polyanthum Wight Walp), tea leaves (Camellia sinensis L. Kuntze), stevia leaves (Stevia rebaudiana Bertoni), and yakon leaves (Smallanthus sonchifolius ((Poepp. & Endl) H Robinson). The tea plant (Camellia sinensis) has properties such as antioxidant, antiparkinsonian, anticancer, antihyperlipidemic, anticholesterol, antidiabetic, antiobesity, and antistroke, among others [5]. Research on several kinds of tea showed that the inhibitory activity of the enzyme α-glucosidase prepared with an infusion of white tea (IC_{50} of 43.42 ppm) was higher than that of other types of tea such as green tea, black tea, and oolong tea (IC_{50} of 44.79 ppm) [6]. Dewijanti et al. [7] showed that the boiled water extract of salam leaves (Syzygium polyanthum Wight Walp) collected from Central Java, Indonesia, had α-glucosidase enzyme inhibitory activity with an IC_{50} of 51.10 ppm. The water extract of salam leaves has a significant effect on blood sugar levels at 30 minutes (using the oral glucose tolerance method with glucose solution 1 g/kg BW). Water extract of salam leaves at a dose of 100 mg/kg BW can reduce blood glucose levels in rats by 53.45 % on the 7th day (alloxan induction 150 mg/kg BW) [8]. Stevia leaves — known as sweet or sugar leaves — have a sweetness level 200 times that of sugar. Stevia rebaudiana leaves contain alkaloids, saponins, tannins, glycosides, sterols, and triterpenes. Stevia rebaudiana offers many pharmaceutical benefits such as antibacterial, antioxidant [9], antihyperglycemic antihyperlipidemic, hepatoprotective, antitumor, nephroprotective, and anti-cancer properties [10]. Shivanna et al. [11] found that polyphenol compounds from stevia leaf extract can reduce glucose levels by

64%. Besides, stevia leaf extract can prevent and treat diabetes and is classified as safe because it can reduce oxidative stress and repair liver and kidney damage [11]. The results of an antidiabetic activity assay against a 70 % ethanol extract of yakon leaves (S. sonchifolius) produced an α-glucosidase inhibition of IC50 19.59 mg/mL [12], and Habib et al. [13] found that yacon root reduces oxidative stress in diabetogenic rats. Based on the above-mentioned research, the present study investigated salam leaves (*Syzygium polyanthum*), tea leaves (*Camelia sinensis)*, stevia leaves (*Stevia rebaudiana*) and yakon leaves (Poepp. & Endl.) H. Robinson) to determine their combined activity.

The present study was carried out as part of an effort to develop the use of medicinal plants for health care and diabetes treatment. Salam and tea plants are easily obtained, straightforward to cultivate, and are widely used as flavorings in cooking and making tea, while the various benefits of compounds found in salam leaves and tea leaves have been widely studied. By examining the effects of this combination of salam leaves and tea leaves, the researcher expected to obtain a therapeutic compound that offers optimal efficacy in treating tumors, diabetes, and cancer with minimum side effects. The effects of the extracts under investigation were tested for their activity against the inhibition of the α-glucosidase enzyme, as this test provides an indicator of antidibetic properties.

In addition, while diseases such as cancer, diabetes, etc. have been traditionally treated by mixing various herbs in various combinations, to date, no research has investigated the efficacy of combinations of the above plants in enhancing disease-healing processes. More broadly, the current study is crucial as it has an important role in the development of safe, effective, and stable herbal medicines.

2. Materials and Methods

2.1. Materials

The materials used in this research project were salam leaves (*Syzygium polyanthum*), tea leaves (*Carmellia sinensis)*, and stevia leaves (*Stevia rebaudiana*) collected from the Tawangmangu plantation, Central Java, Indonesia, and yakon leaves ((Poepp. & Endl.) H. Robinson) obtained from plantations in Wonosobo, Central Java, Indonesia.

The chemicals reagents used were 96% ethanol, ethyl acetate, n-hexane, α-glucosidase enzyme, p-nitrophenyl-□-D-glucopyranoside (PNPG) substrate, dimethyl sulfoxide (DMSO), acarbose, sodium carbonate, potassium dihydrogenphosphate, methanol, dichloromethane, TLC plate, silica gel GF254, and distilled water.

Equipment: Rotary Vacuum Evaporator (IKA), Spektrofotometer UV-Vis (Shimadzu), Ultrasonic LC 30 H Elma (Elma Schmidbawer Gmbh, Gottlieb-Daimler Straβ 17 78224, Singen, Germany), Microplate Reader Elx 800, freeze dryer, rotavapor (Heidolph, Germany), LC-MS/MS (Water Acquty Xevo G2XS, UV lamp 366, 254 nm, analytical balance (Precisa, 240A), and glassware.

2.2. Methods

2.2.1. Extraction and Partition Process

Each extract of salam, tea, stevia and yakon leaves were macerated using a 96% ethanol solution before being filtered and evaporated by using a rotary vacuum evaporator until a thick extract was obtained. All extracts were tested for their activity against the inhibition of the α-glucosidase enzyme. The extracts were partitioned by being dissolved in distilled water before adding ethyl acetate into a separating funnel. The mixture was shaken for 15 minutes and then allowed to form two separate layers (a water fraction and an ethyl acetate fraction). The addition of ethyl acetate and shaking was repeated three times. The ethyl acetate fraction was concentrated by using an evaporator and the water fraction was concentrated by using a freeze dryer.

2.2.2. Phytochemical Screening

Phytochemical screening was carried out on the most active fraction against α-glucosidase inhibition using a method based on the modified Harborne method. This included an examination of alkaloids, flavonoids, saponins, phenolics, saponins, quinones, terpenoids, and steroids [14].

2.2.3. α-Glucosidase Inhibitory Activity Assay

Plant extracts were evaluated for α-glucosidase inhibitory activity as per Elya [15] with slight modifications.

2.2.4. LC-MS/MS Analysis for the Active Fraction of Salam Water Extract and Tea Water Extract

The fraction that achieved the best activity in inhibiting the enzyme a-glucosidase (water fraction of tea and salam leaves) was analyzed by using LC-MS/MS with XEVO G2-XS QTof, UPLC Columns C18, HSS T3. LC conditions: Mobile phase: A = water/0.1% formic acid, B = acetonitrile/0.1% formic acid. Gradient: Starts at 5%, at 1 min 5% B, at 8 min 40% B, at 11 min 100%, at 13 min 100%, and at 16 min 5%. Flow rate: 0.3 mL/min, column temperature 40°C, sample temperature 20°C. Injection volume: 1 µL. MS Conditions: Source: ESI. Ion mode: Positive. V convoltage: 30 V.

2.2.5. In Silico Docking Study

To predict the active constituents present in the extract that may be responsible for the anti-diabetic activity possessed by this plant, we screened all the compounds that were previously reported to be present in the plant according to silico molecular docking studies by using Molegro Virtual Docker (MVD) software. The macromolecule of the receptor ligand of a protein consists of the enzymes α-glucosidase and α-amylase, information on which was taken from the Protein Data Bank (PDB) [30]. Validation is done by determining the value of RMSD (Root Mean Square Deviation) until the value of < 2 is obtained. Docking was carried out on the compounds to obtain the MolDock Score, Rerank Score, and H-bond, as well as the RMSD (Root Mean Square Deviation) value. 3-D structures of all the molecules were prepared using Chemdraw 17.1 software, and the 3TOP crystalline structure was obtained from the PDB. Ligands were imported to MVD, and their energies were set to the possible minimum. The solid surface of the 3TOP receptor protein was created, and a maximum of five different binding cavities were selected on it, followed by their energy minimization. The screening process was then initiated by docking five poses of 10 ligand conformations onto the 3TOP receptor. The result of the best run was taken as the final observation, ligand-receptor interaction image was generated, and docking score was expressed as ligand-receptor interaction energy (kcal/mol).

3. Results and Discussion

3.1. Yield Extract

The maceration extraction method with 96% ethanol aims to attract chemical compounds that are non-polar to polar. The partition process is carried out to separate the non/semi-polar and polar fractions because compounds that have α-glucosidase inhibitory activity are mostly phenolic compounds and flavonoids [16]. The yield of each extract and fraction can be seen in Table 1.

Table 1 The yield extract of polyherbal (tea, salam, stevia, and yakon leaves)

No	Dry sample	Yield (%)		
		EtOH extract	Ethyl acetate fraction	Water fraction
1	Tea	18,78	6,19	12,59
2	Salam	12,94	4,3	5,84
3	Stevia	9,06	8,44	0,61
4	Yakon	5,64	4,87	0,77

The yield of the ethanol extract and the partition showed that the aqueous extract of salam and tea leaves gave higher amounts than the extracts of stevia and yakon leaves. This is due to a large number of polar compounds (polyphenols, flavonoids) extracted by ethanol and water solvents [17]. These polar compounds include flavonoids, their derivatives, and polyphenol derivatives such as tea containing catechin, epi-catechin, epi-gallocatechin, and theaflavin; salam containing quercetin, quersetagetin, retusin, and kaemferol-3-rutinoside, lowering blood glucose levels and inhibiting the α-enzyme, glucosidase, and α-amylase in digestion [16].

3.2. α-Glucosidase Inhibitory Activity Assay

The inhibitory activity of the α-glucosidase enzyme for four extracts can be seen in Table 2. The results showed that 96% ethanol extract of tea leaves had the highest inhibition, followed by salam, stevia, and yakon extracts.

Table 2 Inhibition of α-glucosidase enzyme (%) for Simplicia (single and combination extracts)

No	EtOH extracts	Inhibition (%)
1	Tea (A)	65.57
2	Salam (B)	44.11
3	Stevia (C)	34.74
4	Yakon (D)	28.85
	Combination of ethanol extracts with a ratio of 1:1	
6	Tea : salam (A:B)	59.31
7	Tea : stevia (A:C)	53.36
8	Tea : yakon (A:D)	52.58
9	Salam : stevia (B:C)	47.57
10	Salam : yakon (B:D)	46.00
11	Stevia : yakon (C:D)	41.15
	Combination of ethanol extracts with a ratio of 1:1:1	
12	Tea : salam : stevia (A:B:C)	52.11
13	Tea : salam : yakon (A:B:D)	53.83
14	Tea : stevia : yakon (A:C:D)	49.29
15	Salam : stevia : yakon (B:C:D)	44.60
	Combination of ethanol extracts with a ratio of 1:1:1:1	
16	Tea : salam : stevia : yakon (A:B:C:D)	46.00

Table 2 shows that a single extract of tea leaves has better activity than other single extracts because tea leaves contain main polyphenolic compounds, especially flavonoids with an important role in the inhibitory activity of the α-glucosidase enzyme such as catechins (catechin 3-gallate (CG), gallocatechin-3-gallate (GCG), epi-catechin 3-gallate (ECG), epigallocatechin 3-gallate (EGCG)), unbound catechins (gallocatechin (GC), epicatechin (EC), epi-gallocatechin (EGC)), and theaflavins [18].

A single extract in medicine, usually in addition to having activity, also has side effects. For this reason, a combination is carried out to reduce side effects without reducing its activity [19]. The combination of tea and salam is the best, with the inhibitory activity not much different from that of a single extract of tea. Salam contains flavonoid compounds such as quercetin, qoniferin, retusine, and quercitrin [7].

The extracts were combined to obtain better activity using lower concentrations and to know whether the combination was synergistic, antagonistic, or additive. Synergists allow the use of extracts in small amounts but have higher activity.

The 96% ethanol extracts of tea and salam were partitioned with ethyl acetate and water to separate polar and semi-polar compounds. The inhibitory activity of the α-glucosidase enzyme as a result of partitioning tea and salam extracts can be seen in Table 3.

Table 3 The IC_{50} for α-glukosidase enzyme inhibition

No	Sample	IC_{50}
1	Fr. Ethyl of salam	75.95
2	Fr. Ethyl of tea	114.64
3	Fr. H_2O of salam	63.64
4	Fr. H_2O of tea	68.57
	Combination of water extracts in the ratios	
5	Salam : tea (1:1)	24.08
6	Salam : tea (2:1)	70.27
7	Salam : tea (3:1)	16.53
8	Salam : tea (1:2)	80.37
9	Salam : tea (1:3)	25.53
10	Acarbose	14.62

Table 3 shows that the water fraction has better activity than the ethyl acetate fraction. These results indicate that polar compounds tend to exert α-glucosidase inhibitory activity. The water fraction of salam and tea leaves with a composition of 3:1 has the activity with the best IC50 of 16.53 ppm shows that the combination has a synergistic effect because the IC50 of the combination is smaller than the IC50 of 63.64 ppm and 68.57 ppm for a single extract (salam and tea) [19].

This study hypothesizes that polyherbal combination has a better α-glucosidase enzyme inhibitory activity than single herbs. It has been proven that the water fractions of salam leaves and tea leaves have inhibitory activity with IC_{50} values of 63.64 and 68.57 ppm. The combination of water fractions of salam leaves and tea leaves with a composition (3:1) has an IC_{50} value of 16.53 ppm. According to [20], IC_{50} values under 25 ppm are in the very active category, and 50-100 ppm are less active in inhibiting α-glucosidase enzymes.

3.3. Phytochemical Content for Water Fraction of Tea and Salam Leaves

The water fraction of salam leaves and tea leaves has the best activity in inhibiting the α-glucosidase enzyme. The phytochemical content that supports these activities can be seen in Table 4

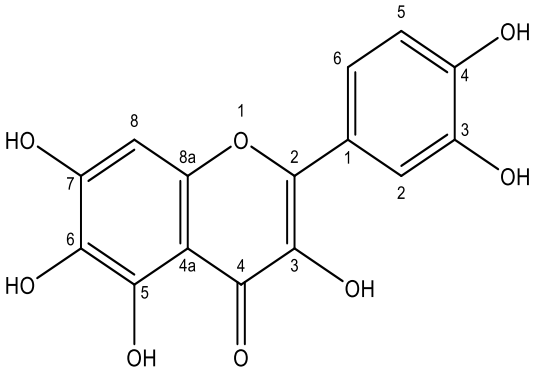
Table 4 Phytochemical content of H_2O fraction of salam and tea

No	Test	Salam Fraction		Tea Fraction	
		Ethyl	H_2O	Ethyl	H_2O
1.	Alkaloid	+	+	+	+
2.	Flavonoid	+	+	+	+
3.	Tannin	+	+	+	+
4.	Saponin	-	+	-	+
5.	Triterpenoid	-	-	-	+
6.	Fenol	+	+	+	+
7.	Steroid	+	+	+	-

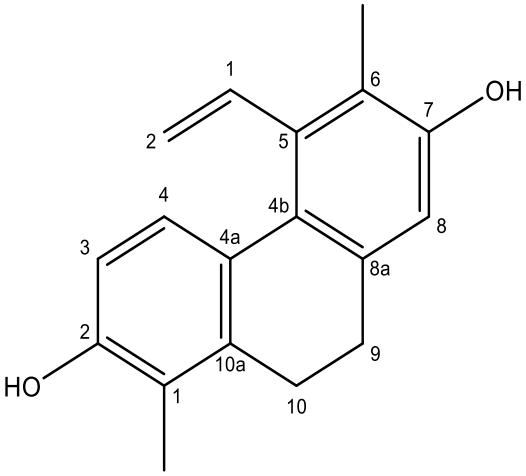
In the ethyl acetate fraction, saponins were not detected, possibly because the saponins contained in salam leaves had a polar nature so they could only be attracted to ethanol and water solvents. Dewijanti et al.'s research [7] reported that in the water extract of salam leaves, triterpenoids were not detected because triterpenoids are non-polar compounds so cannot be attracted by polar solvents.

3.4. LC-MS/MS Analysis for Water Fraction of Tea and Salam Leaves

LC-MS/MS is a high-resolution analytical technique that can be used for the identification of chemical structures. LC-MS/MS provides a very useful approach in determining the profile of a secondary metabolite [21]. Interpretation of LC-MS/MS for the water fraction of tea and salam leaves showed that the water fraction of salam contained quersetagetin (1) and juncusol (2), while the water fraction of tea contained taxifolin (3), gallocathecin-(4alpha8)-epicathecin (4), and nicotiflorin (5). Quercetagetin is known as an antimicrobial, anti-inflammatory, antioxidant and antidiabetic that inhibits the α-glucosidase enzyme [22, 23]. Although all extracts were thought to contain polyphenol and flavonoid compounds, the extract's level of activity in inhibiting the α-glucosidase enzyme was different. It shows that there are interactions between chemical compounds that can be antagonistic, synergistic or act as an additive.



(1)



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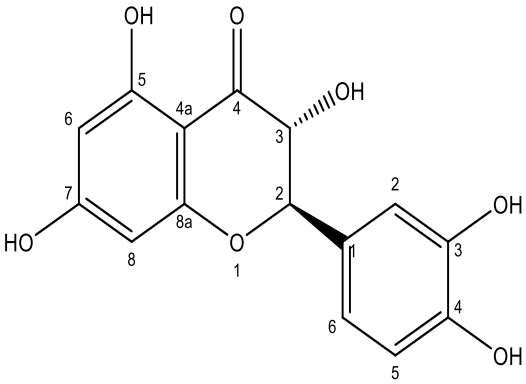

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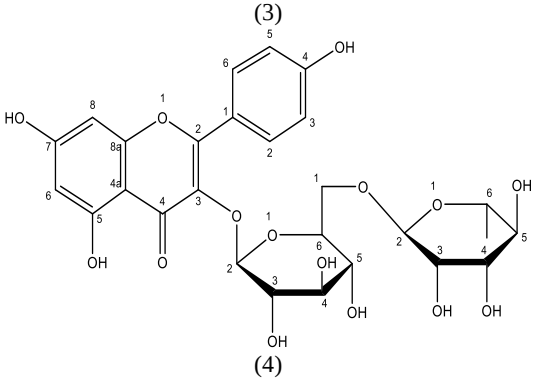


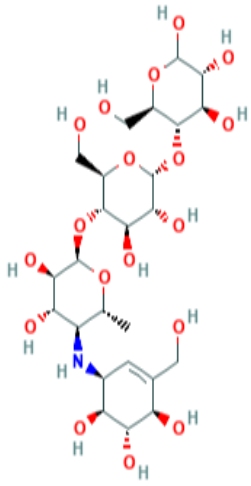
(4)

Fig. 1 Chemical structure of quercetagetin (1), juncusol (2), taxifolin (3), and gallocathecin-(4alpha8)-epicathecin (4)

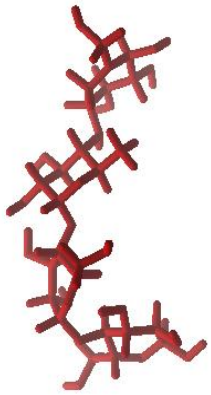
3.5. Molecular Docking Analysis of Chemical Compounds in the Aqueous Fraction of Tea and Salam Leaves As α-Glucosidase Enzyme Inhibitor

The chemical structure of each tea and salam water fraction was analyzed by docking using 3TOP protein obtained from PDB. The 3TOP protein is a hydrolase enzyme inhibitor in humans that functions to hydrolyze α-1,4-linked oligosaccharide substrates that play an important role in glucose production in the human lumen and act as an efficient drug target for type 2 diabetes and obesity [24]. The crystal structure of the 3TOP protein with acarbose complex was chosen based on its resolution with an RMSD value of 1.07Å. The 3D structure of the enzyme 3TOP (maltase-glucoamylase) with acarbose complex can be seen in Fig. 2.

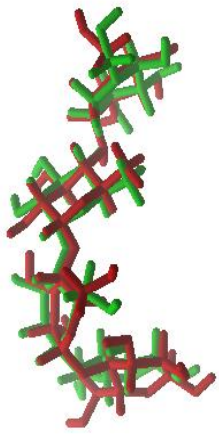
(a)



(b)



(c)



(d)

Fig. 2 Protein complex 3TOP with ligand (a), 2D acarbose structure (b), native ligand 3D (c), and native ligand validation (d)

The validation process is carried out to ensure a match between the protein structure, software and devices used in the docking process. The docking process for chemical compounds contained in the water fraction of salam and tea gave a reranc score as shown in Table 4.

Table 5 Molecular docking analysis of compounds from water fractions of salam and tea with 3TOP protein

No.	Compounds	*m/z*	MolDock Score [GRID](*)	MolDock Score	Reranc Score
Fr. H_2O of Salam					
1.	Quercetagetin	319	-98.65	-93.32	-89.67
2.	Juncusol	265	-75.29	-74.20	-62.68
3.	Gallocathecin-(4alpha8)-epicathecin	594	-143.40	-135.16	-108.71
Fr. H_2O of Tea					
1.	Nicotiflorin	594	-161.26	-146.21	-118.24
2.	Taxifolin	304	-94.68	-90.57	-84.35
Native Ligand					
	Acarbose	645	-170.96	-15614	-139.92

A smaller reranc score indicates a more stable bond. The more stable the ligand binding to the receptor, the greater the activity can be predicted [24]. Table 4 shows that the quercetagetin has a higher reranc score than juncusol. This proves that the compounds contained in salam leaves are polar, such as flavonoids, and have activity as inhibitors of the α-glucosidase enzyme. Nicotiflorin is more active than gallocathecin-(4alpha8)-epicathecin and taxifolin. Nicotiflorin is a flavonoid derived compound and gallocathecin-(4alpha8)-epicathecin is a polyphenolic compound and biflavonoid derived from (+)-gallocatechin and (-)-epicatechin. Quercetagetin is a flavonoid derived from quercetin with other names 6-hydroxyquercetin and 3,3',4',5,6,7-hexahydroxyflavone, which has anti-inflammatory, antiviral, antioxidant, and antidiabetic activities [23]. Nicotiflorin, with another name kaemferol-3-rutinoside, has antioxidant, hepatoprotective, anticancer, and antidiabetic activities [25].

Solomon reported that nicotiflorin had α-glucosidase enzyme inhibitory activity with an IC_{50} of 8 times better than acarbose. The hydroxyl group at the ortho position in the nicotiflorin structure is the active site in inhibiting the α-glucosidase enzyme.

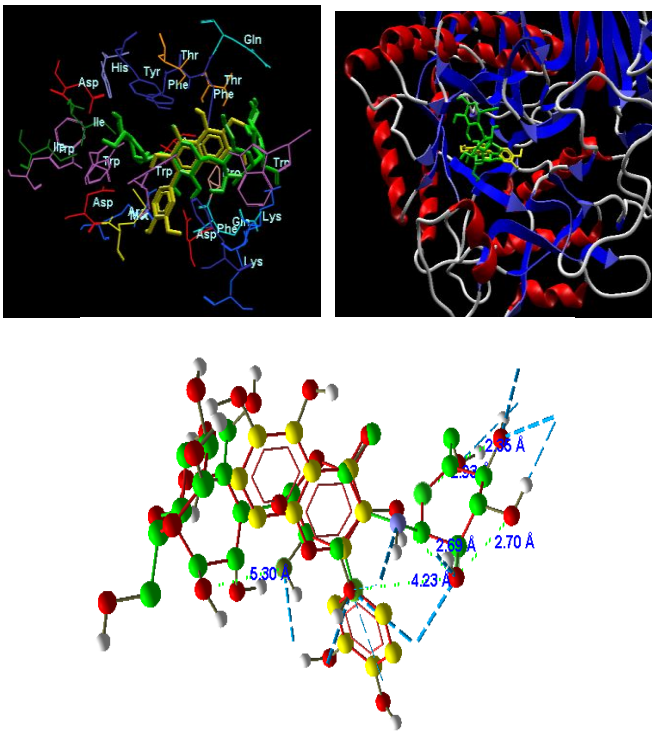


Fig. 3 Electrostatic interaction of quercetagetin with amino acid residues of 3TOP

Quersetagetin interacts with amino acids through 5 hydrogens and two silicates. Hydrogen-activated amino acids include Arg1510 and Asp1526 (4.23Å), His1584 (2.69Å), Asp1157 (5.3Å), Asp11 (2.34Å), and Asp1279 (2.93Å). The amino acids are controlled by steric Met1421(2.70Å) and Pro1159 (2.3Å).

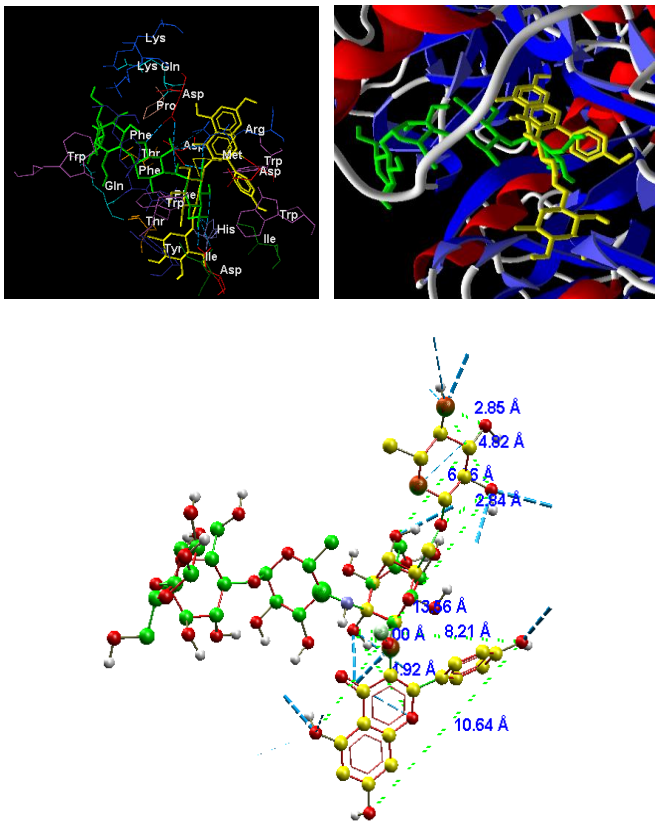


Fig. 4 Electrostatic interaction of nicotiflorin with amino acid residues of 3TOP

The docking results show that nicotiflorin has interactions with amino acids including Thr1586, Asn1585, Arg1250 (2.84Å), Tyr1251 (2.85Å), Hys1584, Asp1420 (4.92Å), Arg1510 (3.00Å) Arg1582, Trp1418 (8.21Å), Glu1423 (10.64Å) Asp1157 (4.82Å), Asp1279 (6.86Å), and Thr1586, Asn1585, Arg1250 (13.56Å).

Nicotiflorin has a higher reranc score than quercetagetin; it can be seen from a large number of hydrogen bonds and interactions with amino acids, where the more interactions occur, the better the activity. The interaction of amino acids Arg has the largest bond distance of 13.56Å. This causes nicotiflorin to have greater activity than quercetagetin, with the highest bond distance of 4.23Å. Previous research that docked nicotiflorin with protein 5NN8 using AutoDock Vina software obtained free energy G of 8.00 (kcal/mol) with amino acid interactions Arg125, Glu206, Tyr547, Tyr666, Glu205, Tyr662, and Phe357.

The results of Sulastri's research [26] reported that the components of chemical compounds in tea leaves with the highest reranc score value are theaflavin compounds with a reranc score of -104,033, and the bay leaf compound is gallic acid with a value of -71,3957. The difference was due to using a different macromolecular protein coded 4L3W with the miglitol assay ligand (CID 441314) and different instrument specifications [26].

These differences occur because the software and the type of protein used are different. The Molegro software was chosen due to its better accuracy compared to other applications. The Molegro has a cavity, so it is easier to know exactly where the ligand interacts with the receptor [27]. Quercetagetin and nicotiflorin are flavonoid derivatives. According to the report of Proenca [28], flavonoid and polyphenols are inhibitors of the α-glucosidase enzyme compound which are the most effective in reducing postprandial hyperglycemia (Post Prandial Hyperglycemia (PPHG)) in the management of diabetes mellitus type 2. Flavonoids act as good modulators of α-glucosidase enzyme activity [28]. Flavonoids have two catechol groups in rings A and B and a hydroxyl group in ring C, the most active groups in inhibiting α-glucosidase enzymes. Flavonoids have the potential as an alternative in the regulation of PPHG [29].

Molecular docking is a very efficient tool for predicting the receptor-specific activity of molecules by evaluating their binding interaction with the target protein. Docking outcome provides us with the ligand-protein interaction energy (kcal/mol), the number of interactions, and amino acids involved in it, based on which biological activity of various molecules is predicted. MVD software is used for in silico docking studies, and we used it to screen compounds in Camellia sinensis and Syzygium polyanthum for their interaction with receptor protein (3TOP).

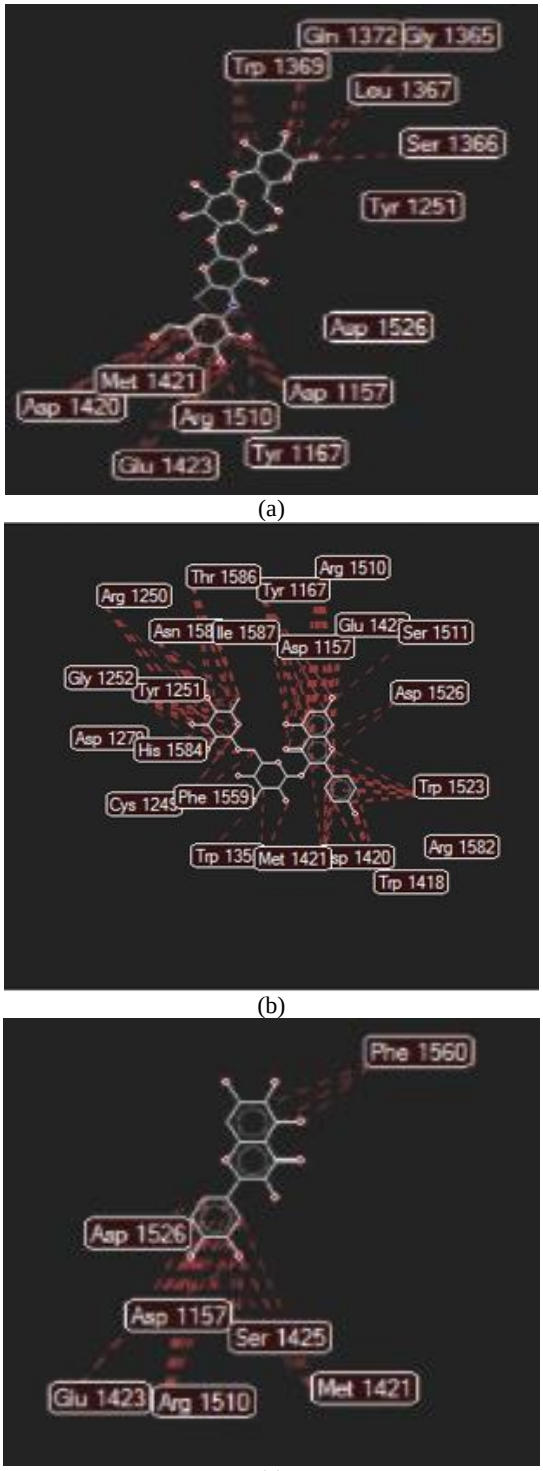


Fig. 5 Isosteric interactions and hydrogen bonds between amino acids and acarbose (a), nicotiflorin (b), and quercetagetin (c)

Compounds having minimum ligand-receptor binding energy and maximum interactions with the receptor (<6 Å bond lengths) were predicted to be most effective. Results of the docking studies showing the best two molecules are depicted in Table 4. Compounds showing interaction with the 3TOP receptor may efficiently modulate hyperglycemia through insulin signaling. Quercetagetin and nicotiflorin were predicted to be the most efficient molecules as depicted by the lowest interaction energy, bond length, and the number of ligand-protein

interactions (Fig. 3). These molecules showed exceptionally good interaction with 3TOP and can be considered potential molecules that may prove beneficial in diabetes through their direct action on the insulin receptor. However, these molecules need to be further screened extensively through in vitro and in vivo experiments before reaching any deceive conclusion.

4. Conclusion

The combination of the water fractions of salam and tea in the ratio 3:1 has the inhibitory activity of the α-glucosidase enzyme of IC_{50} 16.53 ppm. The water fractions of salam and tea containing quercetagetin and nicotiflorin had a reranc score of -89.65 and -118.24, respectively, and acarbose had a reranc score of -139.92.

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