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Original Article

INHIBITION ACTIVITY OF LIQUID SMOKE *COCOS NUCIFERA* L. ON DPP-IV AND AGE-RAGE *IN SILICO* AND *IN VITRO*: ANTIDIABETIC AND ANTI-INFLAMMATORY ACTIVITY

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ABSTRACT

Objective: The research aims to predict the inhibitory activity of liquid smoke compounds from coconut shells (*Cocos nucifera* L.) *In silico* and to determine the activity on reduction of glucose levels by the Nelson-Somogyi method and anti-inflammatory effect on the inhibition of protein denaturation *in vitro*.

Methods: This research used biological activity prediction, physicochemical prediction, molecular docking, and *in vitro* analysis using a UV-Vis spectrophotometer.

Results: There were 13 liquid smoke compounds from Gas Chromatography-Mass Spectrometry (GCMS) result and shows that every liquid smoke compound has wound-healing activity and complies with Lipinski's Rule of Five. Urea did not fulfil the AMES Toxicity parameter, and four compounds had the highest level of toxicity. From the docking results, the binding affinity score between liquid smoke compounds and DPP4 inhibitors ranged from-5.3 to-3.0. Meanwhile, the Advance Glycation End Products Receptors (AGE-RAGE) receptor went from -2.5 to -1.5. 13 compounds had inhibitory activity on Dipeptidyl Peptidase 4 (DPP4); meanwhile, there are 12 compounds on AGE-RAGE *In silico*. The activity of liquid smoke antidiabetic at 10 µg/ml was 31.26%, while quercetin was 46.36%. In the anti-inflammatory analysis, the IC50 value of the liquid smoke compound was 22.41 µg/ml, while diclofenac sodium was 0.42 µg/ml.

Conclusion: The result shows that 13 liquid smoke compounds had inhibitory activity on DPP4, while 12 compounds on AGE-RAGE were *In silico*. The *in vitro* results found that liquid smoke compounds have glucose-reducing activity, and from the IC50 value, it is concluded that both compounds have potent anti-inflammatory activity.

Keywords: DPP4 inhibitor, AGE-RAGE, Liquid smoke, Nelson-somogyi, Protein denaturation

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INTRODUCTION

People with diabetes can have complications that consist of both microvascular and macrovascular complications. Microvascular complications in people with diabetes are characterized by nerve damage, which can decrease neural sensitivity and lead to gangrene wounds. The incapability of macrophages causes the failure of wound healing in people with diabetes to phagocytose pathogenic organisms and the occurrence of inhibition of the phenotype transition of M1 macrophages (pro-inflammatory) to M2 macrophages (anti-inflammatory). It causes M1 macrophages to produce more pro-inflammatory cytokines, so the wound fails to progress into the next phase [1].

Coconut shells are one of the natural materials that can be used for wound healing in people with diabetes. The coconut tree (*Cocos nucifera* L.) is one of the most common plants in Indonesia, but its utilisation is not popular among the national society. Coconut shells can be made into Liquid Smoke by pyrolysis. Liquid smoke can be used as a topical agent in the treatment of wounds by increasing fibroblast proliferation and capillary formation [2, 3].

The receptors that interact with liquid smoke are dipeptidyl peptidase 4 (DPP4) and Advance Glycation End Products Receptors (AGE-RAGE). DPP4 is accountable for breaking down incretins, particularly glucagon-like Peptide I (GLP-1) and Glucose-Dependent Insulinotropic Peptide (GIP). GLP has a role in increasing endothelial synthesis, stimulating angiogenesis, being an antioxidant and anti-inflammatory, and promoting cell proliferation and migration. GLP production also assists the wound healing process in the remodelling phase by stimulating Transforming Growth Factor (TGF) production through increased collagen synthesis and extracellular matrix remodelling. High DPP4 production indicates prolonged inflammatory status, so DPP4 inhibitors can help reduce pro-inflammatory cytokines [4, 5].

In contrast, Advanced Glycation End product receptors (RAGE) are a heterogeneous group of molecules that form continuously in the body, increasing oxidative stress. Excessive buildup of AGE-RAGE in injured skin could hinder wound healing by impairing macrophages' phagocytic function. The method of inhibiting AGE-RAGE signalling can decrease the pro-inflammatory function (M1) and increase the anti-inflammatory function (M2), enhance angiogenesis, increase tissue granulation, and promote faster re-epithelialization of wounds [6].

Computer simulations are developing quickly, making it easier for humans in various aspects, including research. The *In silico* approach is one method to predict molecular interactions between a compound and a protein. The advantages of the *in silico* method are that it can reduce the use of tools, materials, and experimental animals and is more affordable [7].

This research will also analyse *in vitro* using the nelson-smogyi method to observe the reduction of glucose levels in liquid smoke and the anti-inflammatory effect on the inhibition of protein denaturation. The nellson-somogyi method measures glucose level reduction using Nelson reagent and arsenomolybdate. This method has the principle of oxidising glucose using the Nelson reagent, and adding arsenomolybdate solution will form a greenish-blue molybdenum complex that can be measured to determine glucose levels. Coconut shell liquid smoke exhibits potent antioxidant properties that help regulate blood glucose levels and prevent diabetic problems. Liquid smoke also contains several phenolic compounds that can potentially be antidiabetic [8].

The anti-inflammatory treatment utilised the protein denaturation

inhibition technique with Bovine Serum Albumin (BSA). Protein denaturation in tissues is one of the causes of inflammation. So that inhibition of denaturation can help reduce inflammation. Liquid smoke extract was topically applied to inflamed rat skin for two w. Administration of the extract significantly reduced the expression of biomarkers associated with Tetracanoylphorbol (TPA-induced) inflammation [9].

Based on the description above, it is known that research on liquid smoke compounds in DPP4 and AGE-RAGE inhibition has never been done. Also, tests on the effect of liquid smoke on reducing blood sugar levels and the anti-inflammatory effect of inhibiting protein denaturation have never been done. So, it is crucial to research the potential content of liquid smoke compounds such as DPP4 and AGE-RAGE inhibitors *in silico* and *in vitro* using a UV-Vis spectrophotometer.

MATERIALS AND METHODS

Research material

The tools used for the *in silico* method are hardware devices such as laptops with 11th Gen Intel® CoreTM i7-1165G7 processor specifications, 3.20 GHz, 16 GB RAM, and 512 GB SSD. Software such as Biovia Discovery Studio, Autodock Vina, IBM SPSS Statistic Viewer, PASS online website, Lipinski's rule of five website, pre-ADMET website, Pymol, PubChem website, Protein Data Bank website, and Protox-II website. The compounds in this research are liquid smoke compounds from GCMS results that have a similarity index value>95%, which are propylene sulfide, diethyl ether, urea, acetic acid, propionic acid, 1-hidroxy-2-butanone, 2-cyclopenten-1-one, phenol, guaiacol, 4-methoxyphenol, 2-propanone, butyric acid, 2-methyl-2-cyclopentenone. The comparator is linagliptin and aminoguanidine, and the receptor is DPP4 Inhibitor (PDB: 4A5S) and AGE-RAGE (PDB: 6XQ7).

The equipment used for the *in vitro* method for antidiabetic activity is the Nelson-Somogyi method, and anti-inflammatory activity is used using the protein denaturation inhibition method in vare laboratory equipment (Pyrex glass), analytical balance, drop pipette, 20 ml vial bottle, and UV-Vis spectrophotometer (Hitachi U-2900). The materials used for this research are liquid smoke compounds, arsenomolybdate reagent (ammonium molybdate, $H2SO_4$, sodium arsenate dibasic heptahydrate), nelson A reagent (sodium carbonate, potassium sodium tartrate, sodium bicarbonate, sodium sulfate), Nelson B reagent ($CuSO_4$ and $H2SO_4$), Tris Buffer Saline (TBS); Bovine Serum Albumin (BSA); diclofenac sodium.

In silico

Studies prediction of biological activity using pass online (Prediction of activity spectra substances)

Biological activity prediction was carried out by submitting the SMILES of each liquid smoke compound.

The Lipinski's rule of five test

The Lipinski's Rule of Five test was carried out by submitting the 3D structure of the liquid smoke compound in *pdb format.

Pre-ADMET test

The pre-ADME test was carried out using two websites, namely the pkCSM online tool and the Protox-II website. The SMILES of each liquid smoke compound are submitted to the website, and the ADMET test results are obtained for each compound.

Ligand and receptor preparation

Ligand preparation was done by downloading and converting the liquid smoke compounds from *sdf 3D format into *pdb format using Biovia Discovery Studio. Receptor preparation was done by downloading the file receptor in 3D format with *pdb format. Receptor preparation was carried out using AutodockVina to remove water molecules, native ligands, chain B bonds, and hydrogen atoms, Kollman Charges and Compute Gasteiger were added (fig. 1).

Docking validation

The docking method was validated by docking the receptor with the natural ligand. The docking findings will be further analysed using the Pymol application to get the RMSD value.

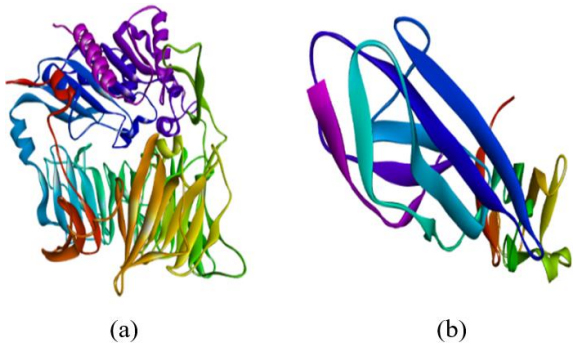

Fig. 1: Prepared receptor (a) DPP4 Inhibitor (b) AGE-RAGE

Molecular docking

Molecular docking was done by docking the receptor with liquid smoke compounds using auto dock vina. The result of the docking will be saved in *pdb format. The docking results will be visualised using Biovia Discovery Studio, resulting in amino acid residues between the receptor and the liquid smoke compounds.

Antidiabetic activity

Antidiabetic activity testing uses the Nelson-Somogyi method to measure glucose reduction using UV-Vis spectrophotometry. Determination of the wavelength by making a standard solution using glucose in water at 25 µg/ml. The absorbance of the solution is measured at visible wavelengths, and the operating time procedure is continued. Antidiabetic measurements were carried out by preparing samples and quercetin concentrations of 2, 4, 6, 8, and 10 µg/ml. The negative control was added with a 45 µg/ml concentration at each concentration and homogenised. 1 ml of each solution was taken, then 1 ml of Nelson's reagent was added and then heated for 10 min. Next, the solution was added with arsenomolybdate reagent into a 10 ml measuring flask. The solution was incubated at 37 °C in dark conditions for 20 min. Absorbance was measured at a maximum wavelength of 760.0 nm with an operating time range of 29-42 min [8].

Anti-inflammatory activity measurement

Anti-inflammatory activity testing uses the protein denaturation inhibition method using UV-Vis spectrophotometry. Anti-inflammatory activity was measured by making a solution of 50 mg of sample (liquid smoke) and positive control (diclofenac sodium) in 5 ml of methanol. Solutions were made in 10000, 1000, 100 and 10 µg/ml concentrations. From each solution, 50 µl** was taken, and 4950 µl** of BSA was added. Each solution was incubated at 25 °C for 30 min. Then, it was heated for 5 min at 72 °C and kept for 25 min at 23 °C. Absorbance was measured at a maximum wavelength of 660 nm [10]. The percentage of deposition inhibition (protein denaturation) was determined based on the percentage relative to the negative control using the following equation:

$$\% \text{ Inhibition of protein denaturation} = \frac{\text{Absorbance of negative control} - \text{Absorbance of sample}}{\text{Absorbance of negative control}} \times 100$$

RESULTS AND DISCUSSION

GC-MS results and biological activity prediction results using PASS online

The results of the activity prediction on coconut shell liquid smoke compounds were carried out using the Pa (probability for active molecule) parameter to find the potential compounds for diabetic wound healing. This research found that every compound has biological activity in diabetic wound healing, with Pa scores above 0.7 (table 1).

Table 1: GCMS results and biological activity of liquid smoke compounds

Liquid smoke compound	Similarity index (%)	Biological activity	Pa value (Probable activity)
Urea	98	-	-
Acetic Acid	98	-	-
2-Cyclopenten-1-one	98	Membrane integrity agonist	0.85
		NADPH peroxidase inhibitor	0.81
		Macrophage colony-stimulating factor agonist	0.71
		5 Hydroxytryptamine release stimulant	0.70
Phenol	98	Membrane integrity agonist	0.94
		NADPH peroxidase inhibitor	0.92
		5 Hydroxytryptamine release stimulant	0.89
		GST A substrate	0.81
		MAP kinase stimulant	0.80
		Macrophage colony-stimulating factor agonist	0.77
		MMP-9 expression inhibitor	0.73
Guaiacol	98	Membrane integrity agonist	0.92
		5 Hydroxytryptamine release stimulant	0.88
		NADPH peroxidase inhibitor	0.84
		MAP kinase stimulant	0.82
		MMP-9 expression inhibitor	0.78
		GST A substrate	0.70
2-Propanone	98	5 Hydroxytryptamine release stimulant	0.87
		NADPH peroxidase inhibitor	0.88
		Membrane integrity agonist	0.88
		GST A substrate	0.86
		Macrophage colony-stimulating factor agonist	0.84
Propylene Sulfide	97	NADPH peroxidase inhibitor	0.80
Propionic Acid	97	NADPH peroxidase inhibitor	0.89
		Macrophage colony-stimulating factor agonist	0.87
		Membrane integrity agonist	0.87
		GST A substrate	0.86
		Antihypoxic	0.75
1-Hidroxy-2-Butanone	97	Membrane integrity agonist	0.88
		Macrophage colony-stimulating factor agonist	0.87
		5 Hydroxytryptamine release stimulant	0.86
		NADPH peroxidase inhibitor	0.85
		GST A substrate	0.82
Diethyl Ether	96	Membrane integrity agonist	0.91
		NADPH peroxidase inhibitor	0.88
		5 Hydroxytryptamine release stimulant	0.87
		GST A substrate	0.76
4-Methoxyphenol	96	Membrane integrity agonist	0.93
		5 Hydroxytryptamine release stimulant	0.89
		NADPH peroxidase inhibitor	0.86
		MAP kinase stimulant	0.83
		MMP-9 expression inhibitor	0.77
		GST A substrate	0.71
2-Methyl-2-cyclopentenone	96	Membrane integrity agonist	0.80
		MMP-9 expression inhibitor	0.73
Butyric Acid	95	GST A substrate	0.89
		Macrophage colony-stimulating factor agonist	0.88
		Antihypoxic	0.80
		Membrane integrity agonist	0.88
		NADPH peroxidase inhibitor	0.87
		5 Hydroxytryptamine release stimulant	0.77

PASS prediction results can be read and explained as the following: (1) If the Pa value is>0.7, the probability of compound activity is high, and it can be used as an analogue of an existing drug; (2) If the Pa value is between 0.5<Pa 0.7, the probability of identifying compound activity is lower, and the molecule is not very similar to existing drugs, (3) If the Pa value is<0.5, the probability of identifying compound activity is very low so that it cannot be used as a new drug analogue [11].

The wound healing process consists of four phases: homeostasis, inflammation, proliferation, and maturation. Every stage includes different methods of operation in cell membranes, such as structural restoration and regrowth. When a wound occurs, membrane integrity agonists will maintain the integrity of the damaged cell membrane and regulate normal cell physiology and function. Eight liquid smoke compounds exhibit this process [12].

Individuals with diabetes experience challenges during the inflammatory phase. The macrophage colony-stimulating factor agonist enhances the inflammatory phase's healing mechanism by boosting macrophage populations near the wound. This aids in phagocytosis to eliminate foreign objects, germs, and damaged tissue components from the wound [13]. Compounds that have biological activity as macrophage colony-stimulating factor agonists are shown the compounds Propionic Acid, 1-hydroxy-2-butanone, 2-cyclopenten-1-one, Phenol, 2-propanone, and Butyric Acid.

In patients with diabetic wounds, there is an increase in the number of free radicals caused by hyperglycemia. ROS can damage blood supply, metabolism, and peripheral nerve structures. NADPH peroxidase is one of the enzymes that affect the increased production of ROS in diabetic blood vessels. NADPH peroxidase is also one of the causes of endothelial dysfunction, so it is necessary to

inhibit the enzyme [14]. Eight liquid smoke compounds carry out this activity.

Furthermore, one of the things that can also increase free radical production in wounds is the lack of oxygen in the wound area (hypoxia), which is caused by microvascular and macrovascular damage. Antihypoxic biological activity can prevent oxygen deficiency in wounds. Liquid smoke compounds that have this activity are Propionic Acid and Butyric Acid [15].

In the wound-healing phase, there is a decrease in the density of blood vessels. The formation of new blood vessels can replace the damaged blood vessels required to provide oxygen and nutrients to the cells. MAP kinase stimulant is involved in angiogenesis and endothelial cell growth [16]. Three liquid smoke compounds have this activity: Phenol, Guaiacol, and 4-methoxyphenol. Additionally, the biological activity of GST A substrate can support cell proliferation and migration to produce collagen and extracellular matrix and form new tissues [17]. Compounds that have biological activity as GST A substrates are Diethyl Ether, Propionic Acid, 1-Hydroxy-2-Butanone, Phenol, Guaiacol, 4-methoxyphenol, 2-propanone, and Butyric Acid. Increase skin tissue growth and skin homeostasis can be assisted by 5 Hydroxytryptamine release stimulants, which are found in the Diethyl Ether, Propionic Acid, 1-Hidroxy-2-Butanone, 2-Cyclopenten-1-one, Phenol, Guaiacol, 4-Methoxyphenol, 2-Propanone, and Butyric Acid compounds [18].

Chronic wounds have higher concentrations of MMP-9, which can inhibit fibroblast function. In addition, TIMP levels, which regulate MMP-9 activity, are also reduced in chronic wounds. The imbalance in the ratio of MMP and TIMP can impair the wound-healing process. So, it is crucial to inhibit the biological activity of the MMP-9 expression inhibitors. It was found in 4 liquid smoke compounds: Phenol, Guaiacol, 4-methoxyphenol, and 2-methyl-2-cyclopentenone [19].

Lipinski's rule of five test

The physicochemical properties of liquid smoke compounds can be seen in table 2, and all 13 liquid smoke compounds passed Lipinski's Rule of Five tests. Lipinski's Rule of Five tests predict a chemical's solubility and permeability through biological membranes. Prediction of solubility and permeability is essential to prevent failure in developing drug-candidate compounds, especially for drug candidates whose use is orally. Based on Lipinski's rules, a compound is said to meet the criteria if it meets five regulations or has a violation of no more than one. Lipinski's rules include molecular weight below 500 Da, Log P below 5, the number of hydrogen bond donors (HBD) below 5, the number of hydrogen bond acceptors (HBA) below 10, and a molar refractivity value between 40 to 130 [20].

Table 2: Physicochemical properties of liquid smoke compounds

No	Parameter	Standard	Observed	Conclusion
1	Molecular Weight	<500 Da	60-124 Da	Complies
2	Log P	<5	-0.04-1.40	Complies
3	HBD	<5	0-4	Complies
4	HBA	<10	0-3	Complies
5	Molar Refractivity	40-130	13.30-34.65	All compounds do not comply

Pre-ADME test

The pre-ADME test is a test carried out to predict the absorption, distribution, metabolism, and excretion activities of a compound consisting of testing Human Intestinal Absorption (HIA), Skin Permeability, VDss, log Blood Brain Barrier (BBB), and total clearance and can be seen in table 3. The HIA value represents the number of active compounds absorbed in the intestine through the accumulation of bioavailability and absorption evaluated through the ratio of excretion by urine, bile, and faeces [21]. The HIA results obtained for liquid smoke compounds were in the range of 93.055% to 100%. All liquid smoke compounds meet the HIA parameters as compounds with good absorption categories.

Table 3: Pre-ADME test of liquid smoke compounds

No	Parameter	Standard	Observed	Conclusion
1	HIA	Low: 0-20% Medium: 20-70% High: 70-100%	79.49%-100%	Complies
2	Skin Permeability	Low:>-2.5	-4.204 --1.924	Five compounds do not comply
3	VDss	High:>0.45 Low:<-0.15	-0.421-0.174	Five compounds at the low level and eight compounds at the medium level
4	LogBBB	-1>logBBB>0.3	-0.292-0.121	Complies
5	Total clearance	-	0.208-0.723	Complies

A chemical with a log Kp value higher than-2.5 is categorised as having low skin permeability. Medicinal compounds with high skin permeability are essential for creating new drugs that can be administered through the skin [22]. Five smoke components failed to meet the skin permeability criteria.

The steady-state volume of distribution refers to the volume in which a medicine can achieve a consistent concentration in the blood plasma. A higher VDss value signifies a more excellent distribution of medicines in the tissue compared to the plasma [23]. The VDss prediction results indicated that five liquid smoke compounds had low VDss levels, whereas eight had moderate VDss values.

Considering a drug's capacity to cross the blood-brain barrier is crucial for minimising adverse effects and toxicity. If the logarithm of the BBB value is more significant than 0.3, the compound can pass the blood-brain barrier. If the logarithm of the BBB value is less than-1, the compound cannot be distributed effectively [21].

Total clearance is a combination of hepatic clearance and renal clearance, which is related to bioavailability. Predicting the complete clearance of a compound is essential in determining the dose of a compound to reach steady state concentration. Table 3 predicts the compounds' excretion rate where the total clearance values at liquid smoke ranged from 0.208-0.723 log ml/min/kg. The higher the total clearance value, the faster the compound excretion rate [23].

Toxicity test parameters include AMES Toxicity, LD50, hepatotoxicity, and skin sensitisation, which can be seen in table 4. The AMES Toxicity test evaluates the mutagenic properties of a substance by utilising microorganisms. Positive results from the AMES Toxicity test indicate that the compound has potential mutagenic and carcinogenic effects [24]. From table 4, it was found that there was one positive compound for AMES toxicity, urea.

Table 4: Toxicity test of liquid smoke compounds

No	Parameter	Standard	Observed	Conclusion
1	AMES Toxicity	No	One compound tested positive	One compound does not comply
2	LD50 (mg/kg)	Class 1: ≤ LD50 Class 2: 5<LD50 ≤ 50 Class 3: 50<LD50 ≤ 300 Class 4: 300<LD50 ≤ 2000 Class 5: 2000<LD50 ≤ 5000 Class 6: LD50>5000	91-63509 mg/kg	Three compounds in class III Six compounds in class IV Two compounds in class V One compound in class I
3	Hepatotoxicity	No	No	Complies
4	Skin Sensitisation	No	-	Four compounds do not comply.

The results of LD 50 testing on liquid smoke compounds showed that the compounds were in classes III to VI. Toxicity classes IV and V have relatively low toxicity levels, toxicity class III has a high toxicity level, and toxicity class II has a very high toxicity level [25].

In the table, it can also be observed that none of the liquid smoke compounds have hepatotoxic effects. This means that all compounds are predicted not to cause liver damage, which is a vital organ in metabolising substances such as drugs. However, it was found that four compounds have skin sensitisation effects: 2-methyl-2-cyclopenten-one, 4-methoxyphenol, Guaiacol, and Phenol. This means that four compounds can cause an allergic response on the skin.

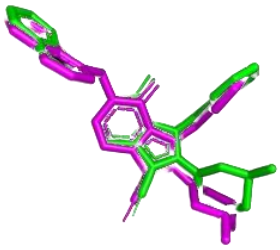

Fig. 2: Validation of native ligand (green) and redocking ligand (purple)

Molecular validation and docking

From the results of docking validation on the DPP4 inhibitor receptor, the Root mean Square Deviation (RMSD) value is 1.020 Å (fig. 2).

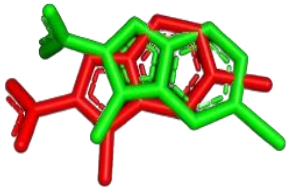

Fig. 3: Validation of native ligand (green) and redocking ligand (red)

While the results of docking validation on the AGE-RAGE receptor, the RMSD value of 1.086 Å (fig. 3). RMSD is a parameter that describes the amount of interaction change between protein and ligand before and after docking. The docking method is valid if the RMSD value is ≤ 2 Å. The lower value of RMSD shows that the conformation used is getting closer to the appropriate position. The smaller RMSD value also indicates a low deviation so that errors in predicting interactions can be reduced [26].

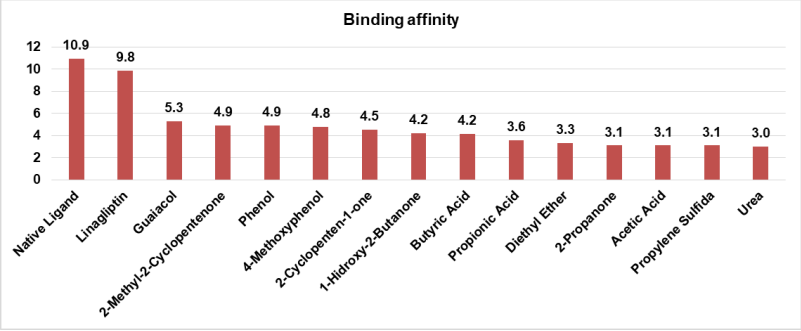

Fig. 4: Binding affinity of liquid smoke compounds and DPP4 inhibitor

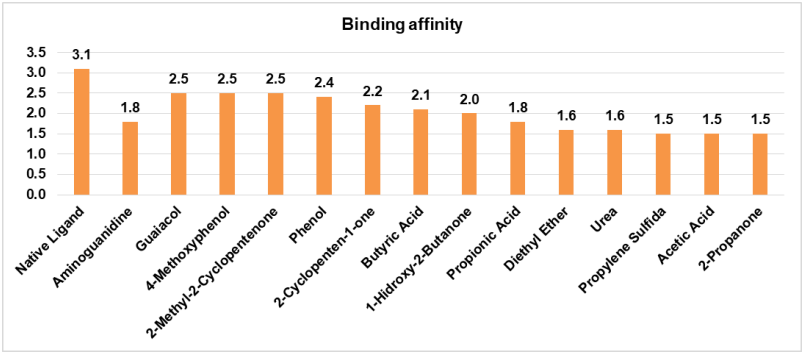

Fig. 5: Binding affinity of liquid smoke compounds and AGE-RAGE

Fig. 4 and 5 provide the binding affinity values of liquid smoke compounds with DPP4 inhibitors ranging from-5.3 to-3.0. Meanwhile, the critical affinity values of liquid smoke compounds with AGE-RAGE went from -2.5 to -1.5. Binding affinity is a parameter used to measure the bond between a ligand and a receptor. The binding affinity value is dependent on the number of bonds formed. The more bonds formed show a negative critical affinity value and indicate that the bond is getting stronger to create a more stable bond [27].

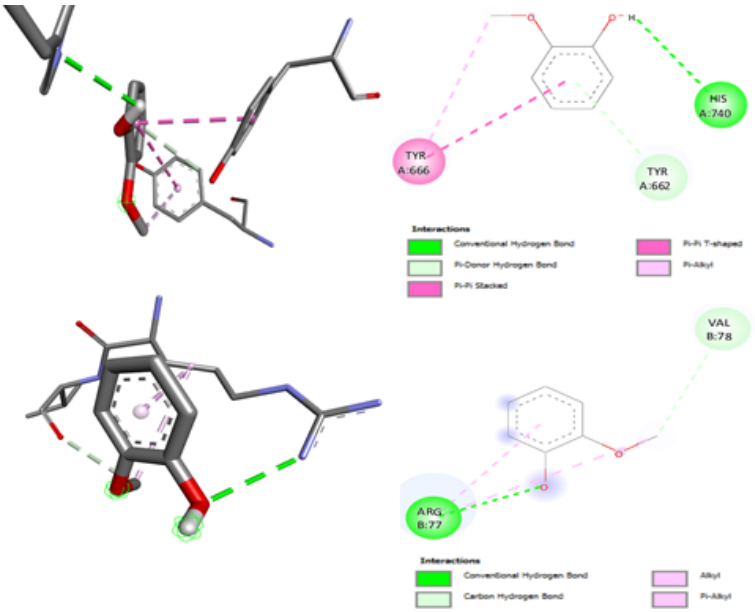

Fig. 6: Visualisation of binding interaction

Visualisation of docking results between liquid smoke compounds with DPP4 inhibitor receptors and AGE receptors can be seen in fig. 6. In DPP4 inhibitor receptors, between native ligands and comparator drugs, the identical two amino acid residues were obtained in hydrogen bonds, which are TYR 662 and SER 630, while in hydrophobic bonds, the similar two amino acid residues were obtained, which are TRP 627 and TRP 629. Based on research by Rozano [28] on compounds that have potential as DPP4 inhibitors, the amino acid residues are SER 630 and TYR 662 in hydrogen bonds and in hydrophobic bonds, such as TYR 662, TRP 629 and TYR 631. There are 13 liquid smoke compounds between DPP4 inhibitor receptors and liquid smoke compounds, which are Guaiacol, 2-methyl-2-cyclopentenone, Phenol, 4-Methoxyphenol, 2-Cyclopenten-1-one, 1-Hydroxy-2-Butanone, Butyric Acid, Propionic Acid, Diethyl Ether, 2-Propanone, Acetic Acid, Propylene Sulfide, and Urea that have similar hydrogen and hydrophobic amino acid residues with native ligands, comparator, and previous study [28].

In AGE receptors, the binding affinity sequence obtained from the smallest to the largest is a native ligand, liquid smoke compounds, and drug comparator. The amino acid residues in the native ligand, such as PRO B 80, LEU B 79, ARG B 77, and PHE B 85, were obtained. From the results, there are 12 compounds, which are guaiacol, 2-methyl-2-cyclopentenone, phenol, 4-methoxyphenol, 2-cyclopenten-1-one, 1-hydroxy-2-butanone, Butyric Acid, Propionic Acid, Diethyl Ether, Propylene Sulfide, and Urea that have similar hydrogen and hydrophobic amino acid residues with native ligands and comparator (aminoguanidine).

The binding site is an area of the protein that is the place where the ligand and protein will bind. The active side comprises amino acid residues that will act as donors to the ligand interaction. The more residues interacting with the ligand, the stronger the binding between the ligand and the target protein. The ligand and the receptor bond will produce a tissue response such as activation or inhibition [29].

Besides the affinity energy (ΔG) value, interactions between residues and ligands, such as hydrogen bonds, are also considered. Hydrogen bonds are crucial for protein structure as they directly impact the stability of the structure. The greater the number of hydrogen bonds present, the stronger the binding between the ligand and receptor. Hydrogen bonding can influence compounds' chemical and physical characteristics, including boiling temperature, melting point, water solubility, chelate formation ability, and acidity. Hydrophobic interactions contribute to the stability of the ligand binding to the receptor. Hydrophobic interactions occur on the inner side of the protein structure to avoid watery environments. Hydrophobic bonds reduce the contact between nonpolar residues and water [26].

Antidiabetic activity analysis

The results of the maximum wavelength of 25 µg/ml Glucose solution resulted in a maximum absorbance wavelength of 761 nm. Based on the literature, the maximum wavelength of glucose is 761 nm. Operating time is optimal for a compound to interact with other compounds to create a stable compound condition, which aims to determine the regular sample measurement time. Based on the research that has been done, the operating time was obtained to be 29 to 42 min [8].

The decrease in absorbance value occurs because liquid smoke has the potential to reduce glucose and cholesterol concentrations, increase insulin and glycogen in the liver, and increase glucose tolerance, which can be helpful as an antidiabetic [3]. According to Tarawan [30], Liquid Smoke contains flavonoids with nonpolar or semi-polar properties. In flavonoid compounds, there is a hydroxyl group where sugar is attached, which can increase the solubility of flavonoids in water. In flavonoids, the hydroxyl group of flavonoids can be attached to one or more sugars by forming hemiacetal bonds that are non-acid resistant and produce glycosides that make flavonoids more soluble in water [30]. Flavonoid hydroxyl groups binding to glucose will decrease the absorbance of the glucose standard solution.

In this research, the method used is the Nelson-Somogyi method, which is based on the amount of cupro-oxide precipitate that is reduced and reacted with arsenomolybdate into a blue molybdine blue complex that can be measured using a UV-Vis spectrophotometer. A red-brick-colored cupro-oxide precipitate will form due to the interaction between the residual glucose and the

Nelson reaction. Then, the solution will be heated and covered with cotton to optimise the response. The purpose of the heating is to increase the kinetic energy of the molecules so that it will increase the speed of the reaction. The heated solution will be stabilised, and after that, the addition of arsenomolybdate reagent was carried out/Cupro-oxide will reduce arsenomolybdate again to molybdine blue, which has a blue-green color and will measured by UV-Vis spectrophotometer [8].

Table 5: Glucose level reduction values

Concentration (µg/ml)	Glucose level reduction value (%)	
Negative control (45 µg/ml glucose standard solution)	**43.0546±0.11**	
	Liquid smoke	Quercetin
2	16.44±0.08	39.73± 0.07
4	19.90± 0.16	41.31± 0.11
6	24.67± 0.10	43.09± 0.04
8	27.62± 0.20	44.31± 0.11
10	31.26± 0.32	46.36± 0.08

n = 3; values are expressed as mean±SD

The results of data on the decrease in glucose levels show a percentage decrease with the addition of an increasing concentration of the test solution. The higher the concentration, the more glucose is bound, and the less glucose remains, so it decreases glucose levels significantly. The reduction in glucose level can be seen in table 5.

Anti-inflammatory activity analysis

This research was conducted using Bovine Serum Albumin (BSA). BSA measurement eliminates the use of live specimens in the drug development process. The compounds with anti-inflammatory activity have a protein denaturation percentage of>20%. This research used diclofenac sodium, which has an inhibitory activity of 92.81% at a concentration of 100 ppm, as a positive control [31, 32].

Anti-inflammatory tests were carried out on Liquid Smoke and sodium diclofenac, where both compounds had an inhibition percentage of>20%. Increasing the concentration of both compounds will also increase the percent inhibition.

Table 6: Anti-inflammatory activity result of liquid smoke compound and diclofenac sodium

No	Sample	Concentration	% Inhibition*	IC50 (µg/ml)
1	Liquid Smoke	0.1	29.93± 0.00	22.43
		1	32.65± 0.00	
		10	32.99± 0.00	
		100	67.01± 0.00	
2	Diclofenac Sodium	0.1	46.57± 0.00	0.42
		1	50.68± 0.00	
		10	54.79± 0.00	
		100	92.81± 0.00	

*n = 3; values are expressed as mean±SD

Table 6 displays the anti-inflammatory effects of liquid smoke and diclofenac sodium. Liquid smoke includes phenols, tannins, and flavonoids that function as antioxidants, antimicrobials, and anti-inflammatories. Liquid smoke phenols exhibit antioxidative activity that helps limit lipid oxidation by stabilising free radicals, enhancing blood flow to damaged tissue, and reducing scar formation. Liquid smoke has a system that detoxifies reactive oxygen species (ROS) to protect the body from oxidative stress. Liquid smoke tannin content plays a role in increasing epithelialisation, while the flavonoid content of liquid smoke reduces lipid peroxidase, which will prevent necrosis, improve vascularisation, and increase the strength of collagen fibres [31, 32].

The regression equation between log concentration (X) and probit percent inhibition (y) was calculated to obtain the IC50 value of Liquid Smoke and diclofenac sodium, and the linear regression from the regression equation above was obtained that the IC50 value of the Liquid Smoke was 22.41 µg/ml. In comparison, diclofenac sodium was 0.42 µg/ml. The IC50 results above show that both compounds have potent anti-inflammatory activity [31, 32].

CONCLUSION

The results have shown that all the Liquid Smoke has biological activity in diabetic wound healing, and all of the compounds have met Lipinski's Rule of Five. One compound, namely urea, does not comply with the AMES Toxicity parameter, and Butyric Acid, Phenol, Propylene Sulfide, and Propionic Acid are at the highest level of toxicity. From the binding affinity analysis, the value ranged from-5.3 to-3.0. There are 13 liquid Smoke, which are guaiacol, 2-methyl-2-cyclopentenone, phenol, 4-methoxyphenol, 2-cyclopenten-1-one, 1-hydroxy-2-butanone, Butyric Acid, Propionic Acid, Diethyl Ether, 2-Propanone, Acetic Acid, dane Sulfide, and Urea were found to have potential as DPP4 inhibitor based on on the analysis of the amino acid residue of the native ligand, linagliptin, and previous study. In AGE receptors, the value ranged from-2.5 to-1.5, and there are 12 compounds, which are guaiacol, 2-methyl-2-cyclopentenone, phenol, 4-methoxyphenol, 2-Cyclopenten-1-one, 1-Hydroxy-2-Butanone, Butyric Acid, Propionic Acid, Diethyl Ether, Propylene Sulfide, and Urea were found to have potential as AGE-RAGE inhibitor based on on the analysis of the amino acid residue of the native ligand, and aminoguanidine linagliptin. The *in vitro* results found that Liquid Smoke has glucose-reducing activity, while Liquid Smoke has lower activity than quercetin. In the anti-inflammatory analysis, the IC50 value of the liquid smoke compound is 22.41 µg/ml, while diclofenac sodium is 0.42 µg/ml. From the IC50 results above, it can be seen that both compounds have potent anti-inflammatory activity.

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AUTHORS CONTRIBUTIONS

All authors significantly contributed to the conception, design, data acquisition, analysis, and interpretation. They were involved in drafting and revising the article for important intellectual content. All authors agreed to submit the work to the current journal,

approved the final version for publication, and agreed to be accountable for all aspects of the research.

CONFLICT OF INTERESTS

The authors declare no conflict of interest

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