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A STUDY OF DRUG RELATED PROBLEMS IN CHRONIC KIDNEY DISEASE PATIENTS IN HOSPITAL

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ABSTRACT
Patients with chronic kidney disease experience decreased kidney function (as an organ of elimination) and receive various drugs, so they are susceptible to Drug Related Problems (DRP). This study aimed to identify the type of potential DRP and analyzed the influence of gender, age, number of drugs, co-morbidities and length of stay on the incidence of DRP in hospitalized chronic kidney disease patients. This study was a cross-sectional study with retrospective data collection. The subjects of the study were chronic kidney disease patients who underwent hospitalization at a hospital in Surakarta at 2016-2021. The incidence of DRP was analyzed descriptively using the PCNE V9.1 algorithm, while the associations between risk factors and the incidence of DRP was analyzed statistically using the Fisher's Exact Test. Data were obtained from 54 patients whose progress was followed up through medical record. The results showed that 36 patients (66.67%) had DRP while 18 patients (33.33%) did not. In the Problem category there were 22 events while in the Cause category there were 34 events. The results of statistical analysis using the Fisher's Exact Test showed that there were no significant associations between the risk factors (gender, age, number of drugs, number of co-morbidities, length of stay) and the incidence of DRP in hospitalized chronic kidney disease patients.

Keywords: Drug related problems; Chronic kidney disease; Hospital patients

1. INTRODUCTION

The kidney is the main organ for eliminating drugs. Patients with chronic kidney disease experience a decrease in kidney function which is characterized by uremia, in which glomerular filtration and active tubular secretion decrease due to nephron damage. Chronic kidney disease (CKD) is defined as the presence of kidney damage or an estimated glomerular filtration rate (eGFR) less than 60 ml/min/1.73 mt^2, persisting for 3 months or more, irrespective of the cause (KDIGO, 2013). Creatinine clearance describes the function of the kidney to excrete the drug. Low creatinine clearance may increase the risk of drug toxicity or decrease the therapeutic effect. Management of chronic kidney disease is long-term treatment and often uses more than one medication. Patients with chronic kidney disease most often are secondary to diabetes mellitus and cardiovascular disease, thereby increasing the variety of drugs used.

Decreased kidney function as an organ of drug elimination and the variety of drugs used in patients with chronic kidney disease can lead to Drug Related Problems (DRP). A Drug-Related Problem is an event or circumstance involving drug therapy that actually or potentially interferes with desired health outcomes (PCNE, 2020). Several classification systems of DRP have been used in clinical practice, among them were the most widely used Pharmaceutical Care Network (PCNE). Previous studies have determined numerous risk factors for DRPs in

general patients. The major causes contributing to DRPs were adverse drug reactions and noncompliance (Al Hamid et al., 2014). In addition, the major risk factors associated with DRPs were old age, the number of drugs, heart rate ≥ 80 bpm and comorbidities (Saldanha et al., 2020; Sell & Schaefer, 2020).

There are still limited studies regarding the risk factors for DRP in patients with chronic kidney disease. Patients with chronic kidney disease require special attention because of a decrease in kidney function which further causes changes in the pharmacokinetic and pharmacodynamic profile of drugs. In this population, drug safety is an important concern in order to achieve rational pharmacotherapy. Identification of risk factors plays an important role in preventing DRP. DRP are an important problem in clinical practice and many of them are preventable, so the specific risk factors that facilitate the occurrence of DRP are of considerable interest (Kaufmann et al., 2015). As part of the implementation of pharmaceutical services, pharmacist has responsibility for identifying actual and potential DRP, preventing potential DRP, and overcoming DRP that occurred.

Based on the description above, the purpose of this study was to identify the type of DRP and to analyze the factors of age, gender, the number of drugs, co-morbidities and length of stay that can affect the incidence of DRP in patients with chronic kidney disease in a hospital in Surakarta City. By knowing the DRP and the risk factors for the occurrence of DRP, it is hoped that rational pharmacotherapy will be achieved for the treatment of patients with chronic kidney disease.

2. METHODS

2.1. Study Design

This study was an observational study with a cross-sectional design. Data were taken retrospectively at a hospital in Surakarta City from August 2021 – December 2021. Ethical *approval* was *obtained* from Ethics Commission of Kusuma Husada University (No.91/UKH.L.02/EC/IX/2021).

2.2. Population and Sample

The subjects of this study were chronic kidney disease patients who were hospitalized in a hospital in Surakarta City between 2016-2021 who met the inclusion and exclusion criteria. The inclusion criteria for this study included patients with a diagnosis of chronic kidney disease, at least 18 years old and receiving drug therapy. Exclusion criteria included patients with a history of impaired liver function, patients with Human Immunodeficiency Virus (HIV) acquired immunodeficiency syndrome (AIDS), patients undergoing chemotherapy, patients with kidney transplants.

2.3. Data Collection Method

The data were taken from the medical records of patients with primary diagnosis of chronic kidney disease. Subsequently, the data were tabulated in Excel data sheets followed by descriptive analysis and statistical analysis from SPSS. Data were included patient characteristics, medical history, history of drug use, and general clinical variables comprised body temperature, leukocyte values, blood pressure, blood sugar level, allergies and medical specialty. Patient characteristics included age, gender, patient diagnosis, the number of drugs, type of co-morbidities and length of stay in hospital. Another material used in this research is a research worksheet and Pharmaceutical Care Network Europe (PCNE) V9.1 algorithm.

2.4. Data Analysis

The DRP was analyzed by descriptive evaluative method. DRP was classified according to the PCNE V9.1 algorithm. DRP that were detected, classified as potential DRP. The classification was only conducted in the *Problems* and *Cause* categories. To find out the associations between risk factors and the occurrence of DRP, the statistical Fisher's Exact Test

(95% CI; α = 0.05) was used because the 2x2 table did not meet the Chi Square criteria, that was, more than 20% of cells had an expected value of less than 5.

3. RESULTS AND DISCUSSION

A total of 54 hospitalized chronic kidney disease patients were followed by their progress notes in the medical record. Data describing the characteristics of the research subjects were in **Table 1**, including gender, age, number of drugs, number of co-morbidities, types of co-morbidities, and length of stay.

Table 1. Patient characteristics (N=54)

No.	Characteristics	Frequency	Percentage (%)
1	**Gender**		
	Men	16	29.63
	Women	38	70.37
2	**Ages (years)**		
	18-25	2	3.70
	26-35	6	11.11
	36-45	2	3.70
	46-55	16	29.63
	56-65	8	14.81
	≥65	20	37.04
3	**Number of co-morbidities**		
	< 3	20	37.04
	≥ 3	34	62.96
4	**Type of comorbidity**		
	Cardiovascular disease	30	55.56
	Diabetes Mellitus (DM)	2	3.70
	Cardiovascular disease and DM	12	22.22
	Other than cardiovascular disease and DM	10	18.52
5	**Number of drugs**		
	1-4	2	3.70
	5-10	24	44.44
	>10	28	51.85
6	**Length of stay**		
	< 7 days	30	57.69
	≥ 7 days	24	46.15

Based on **Table 1**, the number of women was greater than that of men. These results indicated that the prevalence of cases of chronic kidney disease in these hospitals was higher in women than in men. It has been reported internationally that chronic kidney disease (CKD) is more prevalent among women than men (USRDS, 2020). The differences in the underlying pathophysiology of disease might account for these dissimilarities between the sexes (Carrero et al., 2018). Women are more at risk for kidney failure because they are more susceptible to urinary tract infections that can damage kidneys. They also have increased risk for kidney disease due to hypertensive disorders of pregnancy (preeclampsia and gestational hypertension) (Barrett et al., 2020). Menopausal women are at higher risk for CKD than premenopausal women because estrogen may have protective effect on the kidneys (Harris & Zhang, 2020).

Our study found that most patients experienced CKD were geriatric (≥65 years old). It has been known that estimated glomerular filtration rate (eGFR) will declines along with increasing age. Elderly people with advanced CKD have a higher risk of death, kidney failure, myocardial infarction, and stroke than similar people who have a normal or slightly low eGFR (Tonelli & Riella, 2014). The length of stay was mostly for < 7 days (57.69%). The increase in length of stay can be caused by the age factor, because the elderly experience a decrease in physiological functions such as decreased metabolic ability, so that the healing process for a disease will take longer (Fatimah et al., 2023).

The number of comorbidities in chronic kidney patients was dominated by ≥ 3 comorbidities. The highest comorbidities suffered by patients were cardiovascular disease as much as 55.56%, followed by cardiovascular disease and Diabetes Mellitus (DM) as much as 22.22%. Cardiovascular disease was the main cause of death in ESRD (End Stage Renal Disease) patients and contributes to more than half of deaths. Older ESRD patients tend to have a higher prevalence of cardiovascular disease. However, the prevalence of cardiovascular disease is also high among the ages of 22-44 years, although a much higher prevalence occurs at the age of 45 years or older (USRDS, 2020). Patients with chronic kidney disease (CKD) exhibit an increased cardiovascular risk associated with coronary artery disease, heart failure, arrhythmias, and sudden cardiac death. CKD can cause a chronic and systemic proinflammatory state that contributes to vascular and myocardial remodeling processes resulting in atherosclerotic lesions, vascular calcification, and vascular senescence as well as myocardial fibrosis and heart valve calcification. Thus, CKD can mimic accelerated aging of the cardiovascular system (Jankowski et al., 2021).

Almost all patients received more than 5 drugs (polypharmacy). CKD patients are at high risk of drug related problems due to polypharmacy and impaired renal excretion. DRP can lead to decreased quality of life, increased length of hospital stays, increased overall health care costs, and even increased risk of morbidity and mortality (Garedow et al., 2019). Therefore, complex treatment regimens consisting of several types of medications are usually required to treat chronic kidney disease (CKD) and related comorbidities. Patients with CKD require several drugs to treat medical conditions that accompany the development of CKD (such as diabetes mellitus, hypertension, and prevent the development of chronic kidney disease), as well as common complications of CKD (such as hyperlipidemia, anemia, bone and mineral disorders) (Mason & Bakus, 2010).

DRP analysis was based on PCNE V9.1. This study was only carried out in the Problems and Cause categories. Based on the k of the study, it was found that 36 of 54 patients (66.67%) were experienced DRP. A total of 56 cases were found and categorized as Problems and Causes. In the Problem category there were 22 cases while in the Cause category there were 34 cases. The full number of DRP events in the Problem Category and Causes can be seen in **Table 2**.

Table 2. Number of DRP events in the category of problems and causes (N=60)

Category	Code V9.1	Primary Domains	Case Number	Total (N)
Problems	P1	Treatment effectiveness	14	22
	P2	Treatment safety	1	
	P3	Other	7	
Causes	C1	Drug selection	34	34
	C2	Drug form	0	
	C3	Dose selection	0	
	C4	Treatment duration	0	
Total				**56**

3.1. The Problems

The problems consist of treatment effectiveness, treatment safety, and other problems. Based on the analysis of DRP according to PCNE V9.1, the results obtained were 22 events where the most problems were in the category of treatment effectiveness by 14 events (63.64%), followed by treatment safety for 1 event (4.55%), and other problems 7 events (31.82%). The explanation of Problem Category can be seen in **Table 3**.

In the P1.1 subdomain (No effect of drug treatment despite correct use), it showed that all treatment of hospitalized patients with chronic kidney disease had therapeutic effect. In subdomain P1.2 (Effect of drug therapy not optimal) there were 7 events as shown in **Table 4**. The effect of suboptimal drug therapy was dominated by patients who received a combination of antihypertensive drugs but the patient's blood pressure was still high, ranging from 160/100

mmHg to with 217/101 mm Hg. Based on the JNC 8 algorithm, the blood pressure target for hypertensive patients with CKD is <140/90 mmHg (James et al., 2014). The selection of drugs for 3 patients (case No. 1, 21, 26) was correct, namely the ACEI or ARB group combined with other drug classes, while for the patient with case no. 17 the combination of captopril and ramipril could not control the blood pressure because the two drugs had the same mechanism of action on the renin-angiotensin-aldosterone system. In other wise, blood pressure that had not met the target might be caused by psycho-social factors such as lifestyle and medication adherence. Non-pharmacologic lifestyle interventions should be encouraged as adjunctive therapy for hypertension such as regular physical activity, weight control, smoking cessation, stress reduction, and avoiding excessive alcohol intake (Oliveros et al., 2020). In this study, the patient's medication adherence and lifestyle could not be known because data collection was carried out retrospectively. Intervention and strategies such as the use of single pill combination, electronic pill monitors, lowering economic barriers, and collaborative care between pharmacist and primary care provider or cardiologist have been shown to increase adherence rates (Neiman et al., 2017).

Table 3. The problems

Code V9.1	Primary Domains	Code V9.1	Problem (subdomains)	Case Number	Percentage (%)
P1	Treatment effectiveness	P1.1	No effect of drug treatment despite correct use	0	63.64
		P1.2	Effect of drug treatment not optimal	7	
		P1.3	Untreated symptoms or indication	7	
P2	Treatment safety	P2.1	Adverse drug event (possibly) occurring	1	4.55
P3	Other	P3.1	Unnecessary drug-treatment	7	31.82
		P3.2	Unclear problem/complaint. Further clarification necessary (please use as escape only)	0	
Total cases				22	100.00

Table 4. Subdomains of problems P1.2 (Effect of drug treatment not optimal)

Case Number	Drug	Explanation
1	Ramipril 1x10 mg, amlodipine 1x10 mg, clonidine 2x0.15 mg, bisoprolol 1x5 mg	Blood pressure was still high
6	Na diclofenac po 2x50 mg	Painkillers were inadequate because patients still felt pain after surgery
8	Atorvastatin po 1x20 mg	Antihyperlipidemic drugs were inadequate because total cholesterol, triglyceride and LDL levels were still high
10	Metformin po 3x500 mg	Blood glucose was still high
17	Captopril po 1x25 mg, ramipril po 1x10 mg	Blood pressure was still high
21	Ramipril po 1x10 mg, amlodipine po 1x10 mg, HCT po 1x25 mg, clonidine po 2x0.15 mg	Blood pressure was still high
26	Ramipril po 1x10 mg, amlodipine po 1x10 mg, bisoprolol 1x5 mg, clonidine po 3x0.15 mg	Blood pressure was still high

In the P1.3 subdomain (Untreated symptoms or indications) there were 7 events as shown in **Table 5**. This subdomain was dominated by patients who experienced nausea and vomiting but had not received antiemetic therapy. Patients received one or a combination of omeprazole, sucralfate, ranitidine while they had not received antiemetic therapy. Nausea and vomiting in kidney disease are not associated with excess stomach acid. Serum creatinine and urea in CKD patients can cause patients to lose their appetite, nausea, vomiting, loss of energy and protein,

decrease in carnitine production which causes a decrease in energy production for the skeleton and results in fatigue (Artom et al., 2014).

Table 5. Subdomains of problems P1.3 (Untreated symptoms or indications)

Case Number	Complain	Explanation
3	Diarrhea	The patient had not received antidiarrheal therapy
9	Blood pressure was 150/80 mmHg	The patient's blood pressure was still high, but antihypertensive drugs are no longer given
10	Nausea, vomitting	Had not received antiemetic drugs
14	Nausea, vomitting	Had not received antiemetic drugs
15	Pain during urination	Had not received pain medication yet
16	Nausea	Had not received anti-nausea therapy, because nausea and vomiting in kidney disease were not related to excessive stomach acid
25	Dermatitis	Had not received medicine to treat dermatitis yet

In the P2.1 subdomain (Adverse drug event (possibly) occurring), there was 1 event where the patient was hypotensive. This hypotension most likely occurred due to ADR (adverse drug reactions) from the use of the antihypertensive drug, namely carvendilol and at the same time the patient received tamsulosin to treat BPH. Tamsulosin is an alpha blocker that works selectively on α_{1A} receptors in the prostate, while α_{1B} receptors are found in blood vessels. A study on the use of tamsulosin for BPH in patients aged 40-85 years in the USA showed that there was a associations between the use of tamsulosin and the incidence of severe hypotension (Bird et al., 2013). Therefore, the use of tamsulosin in elderly patients must be wary. In this study, the patient was 77 years old, so the occurrence of hypotension could not be ruled out related to the use of carvendilol and tamsulosin together in elderly patients.

In the P3.1 subdomain (Unnecessary drug-treatment) there were 7 events as shown in **Table 6**. There were several drugs prescribed, but without clear indications or diagnosis. These drugs included suppressors of stomach acid production, the Proton Pump Inhibitor and H-2 blockers. Both of these drug classes usually indicated to reduce gastric acid secretion (Katzung, 2018). Nausea and vomiting is very common in kidney patients and has many causes. These causes include the buildup of uremic toxins and medications metabolites because kidney can't eliminate them, gastroparesis, high level of blood sugar, vestibular dysfunction, motion disorder and increased intra-cranial pressure. Serum creatinine and urea in CKD patients can cause patients to lose their appetite, nausea, and vomiting (Artom et al., 2014). Dimenhydrinate is mostly efficacious for nausea/vomiting caused by vestibular dysfunction, motion disorder and increased intra-cranial pressure. For patients with gastric stasis/gastroparesis, metoclopramide or domperidone may relieve nausea and vomiting (Camilleri et al., 2013).

Unnecessary prescribing of antibiotics also occurred, levofloxacin and ceftriaxone were given to patients without signs of infection, normal temperature was 37 °C, and leukocyte values were still in the range of 4500-11,000/mm^3.

3.2. The Causes

The Causes consist of primary domains, C1 – C9. Each of these primary domains has subdomains or secondary domains. Information from patient medical records could only be used to identify domains C1 – C4. We could not identify the C5 – C9 domains because we did not follow the patient's progress during treatment.

Based on the results of identification of DRP problems according to PCNE V9.1 domain C1 - C4, it was found that there were 34 incidents where the most problems were in the Drug Selection domain (100%). The causes can be seen in **Table 7**. In the causes of DRP, the secondary domain that occurred most frequently was domain C1.2 (No indication for drugs), which was 32.35%.

Table 6. Subdomains of problems P3.1 (Unnecessary drug-treatment)

Case Number	Drug	Explanation
8	Levofloxacin	There was no indication of infection, the temperature was 37 °C (normal), leukocyte values were still in the range of 4500-11.000/mm^3
9	Ranitidine, omeprazole	Ranitidine and omeprazole are drugs to reduce stomach acid secretion. However, the patient had no indications of dyspepsia, nor complaints related to the stomach or digestive system
13	Omeprazole, sucralfate	There were no indications of dyspepsia, nor complaints related to the stomach or digestive system
15	Ceftriaxon	There was no indication of infection, the temperature was 37 °C (normal), leukocyte values were still in the range of 4500-11.000/mm^3
17	Omeprazole	There were no indications of dyspepsia, nor complaints related to the stomach or digestive system
23	Echinacea	Echinacea is an immune-boosting supplement, however, the patient did not have a viral or bacterial infection that required an increased in the immune response
25	Paracetamol	There were no complaints of pain or fever. According to IONI (2020) the indication for paracetamol is as an analgesic and antipyretic

Table 7. The causes

Code V9.1	Primary Domain	Code V9.1	Cause (subdomain)	Case Number	Percentage (%)
C1	Drug selection	C1.1	Inappropriate drug according to guidelines/formulary	4	11.76
		C1.2	No indication for drug	11	32.35
		C1.3	Inappropriate combination of drugs, or drugs and herbal medications, or drugs and dietary supplements	3	8.82
		C1.4	Inappropriate duplication of therapeutic group or active ingredient	5	14.71
		C1.5	No or incomplete drug treatment in spite of existing indication	7	20.60
		C1.6	Too many different drugs/active ingredients prescribed for indication	4	11.76
C2	Drug form	C2.1	Inappropriate drug form/formulation (for this patient)	0	0.00
C3	Dose selection	C3.1	Drug dose too low	0	0.00
		C3.2	Drug dose of a single active ingredient too high	0	0.00
		C3.3	Dosage regimen not frequent enough	0	0.00
		C3.4	Dosage regimen too frequent	0	0.00
		C3.5	Dose timing instructions wrong, unclear or missing	0	0.00
C4	Treatment duration	C4.1	Duration of treatment too short	0	0.00
		C4.2	Duration of treatment too long	0	0.00
Total cases				34	100.00

In the subdomain C1.1 (Inappropriate drug according to guidelines/formulary) there were 4 incidents which the use of ondansetron in patients with kidney disease to treat nausea and vomiting did not comply with the National Formulary because it was not nausea and vomiting caused by chemotherapy or surgery. In subdomain C1.2 (No indications for drugs) there were 11 events, the full details of which can be seen in **Table 8**. The drugs prescribed without any indication included antibiotic, proton pump inhibitor, H-2 antagonist, antiemetic and analgetic. A patient prescribed several kinds of drugs to treat digestive disorders, those were sucralfate,

curcuma, ondansetron, and ranitidine. Even the patient had not experienced nausea and vomiting anymore but these drugs were still given. The C1.2 subdomain was dominated by PPI and H2-blocker prescribing without any indication of gastric or gastrointestinal disorders, as well as administration of antibiotics without establishing a diagnosis of infection. Paracetamol was also prescribed but the patient did not complain of dizziness, pain or fever. According to PIONAS paracetamol is indicated as an analgesic and antipyretic (BPOM RI, 2020).

Table 8. Subdomain of causes C1.2 (No indication for drug)

Case Number	Drug	Explanation
8	Levofloxacin	No indication of infection. Patient complained of low back and shoulder pain, flatulence, dizziness, fatigue, tingling from the back of the neck, shoulders to spine. Patient diagnosed with CKD, CHF, Hypertension Heart Disease, DM, Dyslipidemia, PVC
9,23	Ranitidine	There was no indication of peptic ulcer or other digestive disorders
9,13,17	Omeprazole	There was no indication of peptic ulcer or other digestive disorders
13	Sucralfate	There was no indication of peptic ulcer or other digestive disorders
15	Ceftriaxone	There was no indication of infection, the patient did not complain of fever, normal body temperature, normal leukocyte count
23	Ondancetron	The patient did not experience nausea and vomiting
23, 25	Paracetamol	The patient did not complain of dizziness, pain or fever. Paracetamol is indicated as an analgesic and antipyretic

In the subdomain C1.3 (Inappropriate combination of drugs, or drugs and herbal medications, or drugs and dietary supplements) there were 3 events as shown in **Table 9**. In this subdomain there were 2 cases where ceftriaxone iv and calcium carbonate were given together. This could be fatal because combination of calcium-containing preparations with intravenous ceftrixone can cause precipitation of ceftriaxone in the lungs and kidneys which is very dangerous. If the two drugs will be administered together, they must be separated for at least 48 hours (Suwandi & Pahlavi, 2016).

Table 9. Subdomain of causes C1.3 (Inappropriate combination of drugs, or drugs and herbal medications, or drugs and dietary supplements)

Case Number	Drug	Explanation
13,15	Ceftriaxone iv and calcium carbonate	Concurrent use of calcium-containing preparations with intravenous ceftrixone can cause very dangerous precipitation of ceftriaxone in the lungs and kidneys. Their use must be separated by at least 48 hours
14	Aspilet, clopidogrel, warfarin, heparin	The concomitant use of antiplatelet and anticoagulant drugs can trigger bleeding

In the C1.4 subdomain (Inappropriate duplication of therapeutic group or active ingredient) there were 5 events, the full details of which can be seen in **Table 10**. There were 2 cases where combination of ramipril and captopril were administered to patients with hypertension. Ramipril and captopril are antihypertensives in the same class of therapy, namely ACE inhibitors, so they are not appropriate when used together. The combination of antihypertensives in the same class also occurred in a patient, that was the use of bisoprolol and carvendilol together. Both are antihypertensive drugs belonging to the beta blocker. The combination of the two could increase the antihypertensive effect in the form of beta-adrenergic receptor blockade. According to JNC 8, first-line antihypertensive drugs in hypertensive patients with CKD are ACE inhibitors or ARBs alone or in combination with other therapeutic class drugs (James et al., 2014). If the blood pressure target is not reached, it can be titrated up to the maximum dose or combined with other therapeutic class drugs.

Table 10. Subdomain of causes C1.4 (Inappropriate duplication of therapeutic group or active ingredient)

Case Number	Drug	Explanation
4,12	Ramipril and captopril	Ramipril and captopril are antihypertensives in the same therapy group, namely ACE inhibitors, so they are not appropriate when used together
9	Ketorolac and mefenamic acid	Ketorolac and mefenamic acid are anti-inflammatory and analgesics in the NSAID category. The concomitant use of them was inappropriate and could triggers GI bleeding. The doctor also prescribed ranitidine and omeprazole in this patient possibly to prevent GI bleeding
10	Antalgin injection and ketorolac injection	Ketorolac and antalgin are anti-inflammatory and analgesics in the NSAID category. The concomitant use of them was inappropriate and could triggers GI bleeding.
14	Bisoprolol dan carvedilol	Bisoprolol dan carvedilol are antihypertensive drugs in the same class, namely beta blockers. The combination of the two can increase the antihypertensive effect in the form of beta-adrenergic receptor blockade

In the subdomain C1.5 (No or incomplete drug treatment in spite of existing indication) there were 7 events as shown in **Table 11**. There were 3 cases of patients who experienced nausea and vomiting but had not received antiemetic therapy. Patient with case number 10 received sucralfate and ranitidine, patient with case number 14 only received curcuma, patient with case number 16 received ranitidine. Nausea and vomiting in patients with kidney disease frequently caused by high levels of creatinine in the blood. Dialysis is an effective way to reduce blood creatinine levels. Through dialysis, the various waste products and metabolites are removed from the body (Daugirdas et al., 2015). For patients with multiple comorbidities, a shift is made to conservative management using all proper treatments apart from dialysis. For patients with gastric stasis/gastroparesis, metoclopramide or domperidone may relieve nausea and vomiting (Camilleri et al., 2013).

Table 11. Subdomain of causes C1.5 (No or incomplete drug treatment in spite of existing indication)

Case Number	Complain	Explanation
3	Diarrhea	The patient had not received antidiarrheal therapy
10,14,16	Nausea, vomitting	The patient had not received antiemetic therapy such as domperidone or metoclopramide
15	Pain	The patient had not received analgesic therapy to treat pain during urination
16,19	Cough	The patient had not received cough medicine

In the subdomain C1.6 (Too many different drugs/active ingredients prescribed for indication) there were 4 events as shown in **Table 12**. More than 5 drugs prescribed to treat digestive disorders such as sucralfate, curcuma, ondansetron, ranitidine, omeprazole injection, hyoscine injection, metoclopramide, and lansoprazole. The use of excessive medications can increases the risk of adverse drug effects, including falls and cognitive impairment, potential drug-drug interactions, and drug-disease interactions, in which a medication prescribed to treat one condition worsens another or causes a new one. As the number of drugs taken increases, the risk of ADR increases exponentially. Polypharmacy may also lead to decreased medication compliance, poor quality of life, and unnecessary drug expenses (Dagli & Sharma, 2014). Patient diagnosed with ischemia compensated cordis was given 4 blood thinning drugs those were aspirin, clopidogrel, warfarin, and heparin. Concomitant use of blood-thinning drugs must be careful because they can trigger intracranial hemorrhage (Mohammed et al., 2013).

Table 12. Subdomain of causes C1.6 (Too many different drugs/active ingredients prescribed for indication)

Case Number	Indication	Drug
7	Nausea	Sucralfate, curcuma, ondansetron, ranitidine. Patients who were not nauseous were still given these drugs
10	Nausea, vomiting, abdominal pain	Omeprazole injection, hyoscine injection, sucralfate, ranitidine
14	compensated cordis (extensive anterior ischemia)	Many blood thinners: aspirin, clopidogrel, warfarin, heparin. Concomitant use of blood thinners can trigger a brain hemorrhage or intracerebral hemorrhage
26	Nausea, vomiting	Metoclopramide, ondancetron, lansoprazole, sucralfate

In C2, C3, and C4 domains there were no DRP events, those meant the dosage form, dose selection and duration of therapy in hospitalized chronic kidney disease patients were appropriate. Patients with chronic kidney disease require special attention because of a decrease in kidney function which further causes changes in the pharmacokinetic and pharmacodynamic profile of drugs. Impaired renal function can affect the pharmacokinetics and pharmacodynamics of drugs through several mechanisms. Kidney disease can affect the process of drug distribution through the mechanism of changes in volume of distribution and drug-plasma protein binding. The drug will compete with the urea toxin to bind to the plasma protein albumin (Klammt et al., 2012). Patients with chronic kidney disease are susceptible to changes in both clearance (CL) and volume of distribution (Vd) (Lea-Henry et al., 2018). Prescribing to patients with kidney disease requires knowledge about the drug especially the pharmacokinetics of drugs and how much the patient's physiology changes. Generally, drugs in patients with uremia or kidney impairment have prolonged elimination half-lives and a change in the apparent volume of distribution (Shargel & Yu, 2016). All disturbances in the pharmacokinetics of drugs can result in changes in the therapeutic response and safety of the drug. Changes in therapeutic response can be seen from the Problem Category, sub-category P1 (Effectiveness of Therapy), where a total of 14 cases were found, while drug safety can be seen from the Problem Category, sub-category P2 (Therapeutic Safety), where a total of 1 case was found.

3.3. Risk Factors for DRP

It has been reported that the major risk factors associated with DRP were old age, polypharmacy and comorbidities (Al Hamid et al., 2014). The multipathological conditions that are often experienced by CKD patients require that patients consume large amounts of drugs. Thus, can increase the risk of DRP events. On the other hand, aging also affects kidney function. The kidneys gradually experience a decrease in their physiological functions with increasing age. The liver's ability to metabolize drugs decreases so that drugs tend to stay longer in the geriatric patient's body and will prolong the effects of the drug and increase the risk of ADR (Alomar, 2014). **Table 13** presents the results of the analysis using the Fisher's Exact Test.

In this study, there was no significant associations between age and the incidence of DRP, so age was not a risk factor for DRP (p value > 0.05). Even though there was a difference in the proportion, the difference in the proportion was less than 20%, which means that was not clinically significant. The number of co-morbidities and polypharmacy were also not a risk factor for DRP (p > 0.05). It has been reported that the number of diseases, advanced age, and polypharmacy were associated with a great prevalence of DRP (Garin et al., 2021). Geriatric patients are at high risk of DRP exposure due to several factors, such as multiple disease, receiving many drugs, and cognitive factors (Sell & Schaefer, 2020). The multipathologic condition experienced by CKD patients requires the patient consume large amounts of drugs. Prescription more than 7 drugs in a day was known to increase the risk of DRP (Saldanha et al., 2020). Most DRPs encountered were prevalent among adult patients taking medicines for cardiovascular diseases and diabetes (Al Hamid et al., 2014). The greater number of drugs will increase the risk of drug interactions, synergism, and duplication (Alomar, 2014). The results of

this study were different from previous studies, possibly because the majority of subjects received polypharmacy (n = 52; 96.30%) where polypharmacy is a risk factor for DRP. The lack of variation in the subjects causes inadequate statistical results.

Table 13. Factors associated with DRP among CKD patients

Risk factor	Number of patients		P value	OR
	DRP	Not DRP		
Age (years)				
Geriatrics (≥ 65)	14 (70.0%)	6 (30.0%)	0.771	1.273
Non geriatrics (<65)	22 (64.7%)	12 (35.3%)		
Gender				
Males	12 (75.0%)	4 (25.0%)	0.532	1.75
Females	24 (63.2%)	14 (36.8%)		
Number of Co-morbidities				
< 3	12 (60.0%)	8 (40.0%)	0.552	0.625
≥ 3	24 (70.6%)	10 (29.4%)		
Type of Co-morbidities				
Cardiovascular and DM	30 (68.2%)	14 (31.8%)	0.715	1.429
Non cardiovascular and DM	6 (60.0%)	4 (40.0%)		
The numbers of drug				
<5	2 (100.0%)	0 (0.0%)	0.547	1.529
≥5	34 (65.4%)	18 (34.6%)		
Length of Stay (days)				
< 7	14 (58.3%)	10 (41.7%)	0.264	1.964
≥ 7	22 (73.3%)	8 (26.7%)		

Information: Significant if $p < 0.05$ using Fisher's Exact Test

The proportion of male patients who experienced DRP was 75%, while the women was 63.2%. In this study, gender was not a risk factor for DRP because the p value was > 0.05. The results of this study were in line with previous studies. Gender was not associated with a higher risk of developing DRP (Garin et al., 2021). However, there is controversy on the impact of gender on the risk of developing DRP especially in clinical practice (Alomar, 2014; Garin et al., 2021; Kaufmann et al., 2015).

Length of stay shows the efficiency of treatment and the effectiveness of therapy. Problems involving medication are associated with a higher number of hospitalizations, long-term hospitalizations, admission to emergency services, additional visits to the doctor's office, additional prescriptions and death (Al Hamid et al., 2014). In this study, length of stay was not a risk factor for DRP (p value > 0.05). The proportion of patients with length of stay <7 days who experienced DRP was 73.3%, while patients with length of stay ≥7 days who experienced DRP was 58.3%. Even though there was actually a difference in proportions, based on statistical analysis the difference was not significant. The result of this study was in line with previous study that the patients who were hospitalized for 5–10 days were found to have the highest number of DRPs compared to the patients with length of stay <4 days. However, no association was found between the length of stay and incidence of DRPs with the Chi-square test (Movva et al., 2015).

Pharmacists who have the responsibility of carrying out pharmaceutical service duties can assist clinicians in exploring patient complaints and evaluating whether there are duplications or the presence of drugs that may be used to treat unwanted effects from other drugs. Thus can reduce unnecessary drug prescribing. With the PCNE algorithm, the nature, prevalence, and incidence of DRP can be identified. It is also intended to help health professionals, especially pharmacists, to document DRP information in the pharmaceutical service. Identification of risk factors also plays an important role in preventing DRP. By knowing the factors that triggers the incidence of DRP, it will make it easier for clinicians to provide appropriate therapy to patients and make it easier for pharmacists to provide education to patients. In a further step, these risk factors will serve as the basis for a screening tool to identify patients at risk for DRPs.

4. CONCLUSION

Based on the results of this study, of the 54 patients whose progress was followed through medical records, it was found that 36 patients (66.67%) experienced potential DRP while 18 patients (33.33%) did not experience potential DRP. In the Problem category there were 22 incidents while in the Cause category there were 34 incidents. The results of statistical analysis using the Fisher's Exact Test showed that there were no significant associations between the risk factors for gender, age, number of drugs, number of co-morbidities, and length of stay for the incidence of potential DRP in hospitalized chronic kidney disease patients.

5. AUTHOR DECLARATION

Authors' Contributions and Responsibilities

The authors made substantial contributions to the conception and design of the study. The authors took responsibility for data analysis, interpretation, and discussion of results. The authors read and approved the final manuscript.

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FORMULATION AND ANTIOXIDANT ACTIVITY TEST OF FACE TONER EXTRACT PREPARATION FROM 70% ETHANOL OF CUCUMBER FRUIT (*Cucumis sativus* L.)

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ABSTRACT
Cucumber (*Cucumis sativus* L.) is a natural ingredient with potential therapeutic properties for addressing various skin-related problems. The study aims to identify a suitable formulation for a facial toner extract that exhibits favourable physical properties as determined by organoleptic, homogeneity, pH, and hedonic testing. The present study employed an experimental approach to produce a 70% ethanol extract of cucumber fruit through the maceration method, utilising 70% ethanol solvent. Subsequently, the extract derived from the cucumber fruit developed four formulas to prepare face toners. The concentrations of these formulas were designated as F0 (0%), FI (0.5%), F2 (1%), F3 (1.5%), and F4 (utilising brand x toner as a positive control). The physical evaluation test yielded the fulfilment of the physical criteria for the toner, encompassing the organoleptic, homogeneous, pH, and hedonic tests. The IC50 values obtained from the antioxidant activity test conducted on face toner preparations containing cucumber fruit extract at concentrations of 0.5%, 1%, and 1.5% were found to be 128 ppm, 91.017 ppm, and 62.218 ppm, respectively. The SPSS analysis reveals a significant difference in the IC50 value among the various formulas. Specifically, F3 exhibits a smaller IC50 value than the other formulas, indicating a stronger antioxidant activity. It is important to note that a smaller IC50 value indicates a stronger antioxidant activity. The study's findings suggest a positive correlation existing between the extract's concentration and its antioxidant activity, specifically, the extract denoted as F and administered at a concentration of 1.5%.

Keywords: Cucumber; Face toner; Antioxidant

1. INTRODUCTION

Individuals aspire to possess a hygienic, healthy, and meticulously maintained facial appearance. However, sometimes, the facial region encounters various issues, including a lacklustre and dishevelled appearance. A sense of insecurity may arise due to the lack of vibrancy in one's facial appearance. A facial freshener is necessary to cleanse and revitalise the countenance effectively to prevent a dull appearance. Toner is a liquid solution that removes residual dirt and makeup from the facial skin and provides a refreshing effect as well. Toner is a skincare product applied after the cleansing step and before applying facial moisturiser. Toners can eliminate impurities and purify facial skin while regulating sebum production without causing dehydration in delicate skin types. To mitigate facial oiliness, it is necessary to utilise a facial toner to impart a refreshed and immaculate appearance (Khanza & Mardhiyah, 2018)

Toner is a cleansing agent that is commonly retailed in cosmetic establishments, albeit at a relatively high cost. Because chemical toners can be costly, some women in their twenties and beyond who have oily skin may forego this particular facial treatment (Karyanto *et al.*, 2022). The toner available in the market is typically composed of alcohol and is marketed as a cosmetic product for removing makeup. However, its use may lead to skin dryness and enlargement of

pores. Hence, there is a requirement for alternative formulations devoid of alcohol, and the present trend is to employ natural ingredients for this purpose. Recently, the public, particularly among women, favours toners containing natural ingredients due to their organic composition and absence of significant adverse reactions compared to chemical toners (Chasanah, 2019). Cucumbers are a commonly utilized plant in the cosmetic industry due to their high concentration of chemical compounds such as terpenoids, phenolics, flavonoids, and alkaloids. The flavonoid compounds present in cucumber possess antioxidant properties and exhibit the potential to disrupt free radical chains (Agustin & Gunawan, 2019)

The researchers decided on cucumber ingredients due to their lack of alcohol content and high levels of vitamin C, antioxidants, and water. These properties make cucumbers an ideal choice for refreshing facial skin. Another objective of utilizing herbal toners is to enhance efficiency and cost-effectiveness. Cucumbers offer notable benefits, including a remarkable cooling sensation on the skin. Subsequently, these naturally occurring constituents facilitate the cleansing of facial pores by removing surplus sebum and impurities. In addition to its refreshing properties, cucumber toner has demonstrated efficacy in moisturizing the skin and regulating facial oil balance (Chasanah, 2019).

According to a study by (Badriah et al., 2021) entitled "Formulation and Antioxidant Effectiveness Test of Handbody Lotion 70% Ethanol Extract of Cucumber Fruit (*Cucumis sativus* L.)" states that 70% ethanol extract of cucumber fruit has antioxidant activity with an average IC_{50} value of 27.56 µg/ml and enters the category of powerful antioxidants. Based on the background that has been described, this research aims to formulate and carry out antioxidant activity tests preparation of face toner extract from ethanol 70% cucumber (Cucumis sativus L.)

2. METHODS

2.1. Tools

The tools used in this study were a set of glassware, mortar and stamper, analytical balance (ohaus), furnace, karl fischer (Metrohm), moisture balance (Bel engineering), rotary evaporator (IkA), pH meter (Ohaus), water bath, UV-Vis's spectrophotometry (Shimadzu UV-I900i).

2.2. Materials

The materials used in this study were Cucumber (*Cucumis sativus* L.), DPPH, 70% ethanol, glycerine, propylene glycol, nipagin, nipasol, distilled water, and vitamin C. The part of the plant used was the selected green and fresh cucumber (*Cucumis sativus* L.).

2.3. Work Procedures

2.3.1. Plant Determination

Determination of cucumber (*Cucumis sativus* L.) was carried out at Herbarium Bogoriense, Botany Division, Centre for Biological Research and Development, National Research and Innovation Agency, Jl. Raya Jakarta-Bogor KM.46 Cibinong Bogor, 16911-West Java.

2.3.2. Production of Cucumber Simplicia (Cucumis sativus L.)

35 kg of freshly harvested cucumbers (*Cucumis sativus* L.) underwent wet sorting to eliminate extraneous matter, including undesired plant components and other impurities in the sample. Subsequently, the newly acquired samples were subjected to a washing procedure. Then, the cucumbers were sliced and segregated from the seedless part of the contents. After that, the desiccation procedure is executed using an oven set at a temperature range of 40-50^0 C. After the drying stage, a dry sorting procedure is implemented to eliminate extraneous substances that may have been acquired during the aforementioned drying process. The uncontaminated specimens were blended using a blender until they achieved a powdered consistency and subsequently filtered through a No. 40 mesh sieve to ensure uniformity in size. The next step is to measure and document the weight of the simplicia. Subsequently, the simplicia quality parameter examination

was conducted, encompassing distinct parameters such as organoleptic assessments and non-specific parameters such as moisture content, ash content, and drying shrinkage (Heniarti *et al*., 2016).

2.3.3. Preparation of 70% Ethanol Extract of Cucumber Fruit (Cucumis sativus L.)

The extraction process was carried out for 5 days and was divided into 2 parts, the first was the 3-day maceration, and the second was maceration for 2 days. Simplicia was weighed as much as 600 grams, put into a container, and then soaked using 70% ethanol solvent for as much as 6000 ml covered with aluminium foil for 3 days (stirred every day). Then, it was filtered using filter paper and obtained macerate 1 and dregs 1. The dregs were re-soaked using 3000 ml of 70% ethanol solvent for 2 days (stirred every day), then filtered using filter paper and obtained macerate 2 and dregs 2. Furthermore, macerates 1 and 2 are combined into one, and then concentrated using a rotary evaporator until a thick extract is obtained. After that, a parameter test for extract quality consisting of specific parameters, including organoleptic observations, and non-specific parameters, including water content, ash content, and residual solvent was conducted.

2.3.4. Phytochemical Screening

Phytochemical screening was carried out to determine secondary metabolite compounds present in 70% ethanol extract of cucumber (*Cucumis sativus* L.). The Phytochemical screening included:

Alkaloid - The experimental procedure involved crushing a 2 g sample in a mortar, followed by adding 5 mL of 25% ammonia. Subsequently, 20 millilitres of chloroform were introduced into the mortar and subjected to vigorous crushing. Then, the amalgam was subjected to filtration, and the resultant filtrate was subsequently applied onto filter paper, followed by the application of Dragendorff reagent. The observed orange colour indicates the presence of alkaloids in the sample. The residual filtrate underwent two extractions in a separatory funnel utilizing a 10% hydrochloric acid solution. The aqueous layer is distinctively partitioned from the organic layer. The water layer was extracted and subsequently transferred into a test tube for analysis via the Mayer and Dragendorff reactions. A positive outcome was indicated by a white precipitate upon reaction with Mayer's reagent and a red precipitate upon reaction with Dragendorff's reagent (Aprilliani, 2018).

Flavonoid - The experiment involved heating a sample weighing 2 g in 100 mL of water for 15 minutes, followed by filtration to collect the resulting filtrate. A volume of 5 mL filtrate was combined with 0.1 g of magnesium powder, 1 mL of hydrochloric acid, and 5 mL of amyl alcohol. The resulting mixture was agitated and subsequently allowed to undergo phase separation. The development of red, yellow, or orange colour on the amyl alcohol layer is indicative of the presence of flavonoids with positive characteristics (Aprilliani, 2018)

Saponin - 2 g of sample was heated in 100 mL of water for 15 minutes, then filtered, and then the filtrate was collected. 10 mL of the filtrate was put into a test tube. The tube is shaken vigorously for 10 seconds, if foam forms which can last for 1 minute, it is suspected to contain saponins. Then drop the 2 N HCl solution into the test tube. If the foam does not disappear, this indicates that the sample is positive for saponins (Aprilliani, 2018)

Tanin - One gram of the sample was introduced into 100 mL of heated water, subjected to boiling for 15 minutes, and subsequently filtered. The experimental protocol entails the preparation of three test tubes, each of which should be filled with 5 mL of filtrate solution. In this experiment, three tubes were utilized. Tube 1 underwent a reaction with a 1% iron (III) chloride solution, and the presence of polyphenol compounds was confirmed by the formation of blue ink or black-green colour. Gelatine was added to the second tube, and the formation of a white precipitate indicated the presence of tannin. In the third tube, stiasny reagent (30% formaldehyde: HCl 2:1) was added, and the mixture was heated in a 90°C water bath. The

formation of a pink precipitate indicated a positive result for simplicia containing tannins. The precipitate in the third tube was filtered, and the filtrate was mixed with a 1% iron (III) chloride solution. The presence of error tannins was confirmed by the formation of blue ink or greenish-black colour in positive samples (Aprilliani, 2018)

Steroid/Triterpenoid - A total of 1 g of sample was macerated with 20 mL of n-hexane for 2 hours, then filtered and then the filtrate was collected. 5 mL of the filtrate was evaporated in an evaporating cup. The Liebermann-Burchard reagent was added to the evaporated residue. The formation of red to violet colour indicated the presence of triterpenoids in the sample (Aprilliani, 2018).

2.3.5. Formulation of Face Toner of 70% Ethanol Extract of Cucumber Fruit (Cucumis sativus L.)

The formula for face toner preparations comes from (Hilmarni et al., 2022) Utilization of Aromatic Water/Hydrosol of Torbangun Leaves (*Plectranthus Amboinicus* L). In this case the renewal of the formula for the preparation of Face toner can be seen in the following **Table 1**.

Table 1. Formula of 70% ethanol extract face toner cucumber fruit (*Cucumis sativus* L.)

Materials	F0(-)	F1	F2	F3	F4(+)
Cucumber extract	-	0.5	1	1,5	
Nipagin	0.02	0.02	0.02	0.02	Antioxidant
Nipasol	0.02	0.02	0.02	0.02	Toner
Glycerine	10	10	10	10	Brand X
Propylene glycol	10	10	10	10	
Distilled water	Ad 100 ml	Ad 100 ml	Ad 100 ml	Ad 100 ml	

Note:
F0 = Face toner preparation without cucumber fruit extract (*cucumis sativus* L.) 0%
F1 = Face toner preparation of cucumber fruit extract (*cucumis sativus* L.) 0,5%
F2 = Face toner preparation of cucumber fruit extract (*cucumis sativus* L.) 1%
F3 = Face toner preparation of cucumber fruit extract (*cucumis sativus* L.) 1,5%
F4 = Brand X antioxidant toner

Cucumber fruit extract (*Cucumis sativus* L.) was formulated into a liquid preparation for use as a facial toner. Preparation of cucumber fruit extract is performed following the concentration that has been determined, with sufficient amounts of distilled water added beforehand. Nipagin and nipasol are weighed, then dissolved in water and stirred until homogenous. Earlier, glycerine and propylene glycol were measured and added to the solution. Then, cucumber extract was added, followed by the rest of the distilled water, and stirred until uniform. Finally, they were placed in a container.

2.3.6. Physical Evaluation of 70% Ethanol Extract of Cucumber Fruit (Cucumis sativus L) Face Toner

Organoleptic Test - Organoleptic examination is carried out by visually looking at the physical form, which includes the colour, shape, and smell of the preparation.

Homogeneity Test - Homogeneity testing is carried out by taking 10 ml of the toner formula preparation, then putting the toner into the glass beaker and then observing the arrangement of coarse particles or inhomogeneity in the toner preparation.

pH test - The pH measurement begins with pH meter calibration. Calibration is performed with a buffer solution of pH 4.01 and pH 6.86, then the pH meter is turned on by pressing the on button, the pH meter is inserted into the container containing the toner preparation to be tested, dipped into the water containing the toner preparation, and the scale will move until the numbers stop moving.

Hedonic test - Hedonic test or preference test for face toner preparation is conducted by pouring it on cotton and paying attention to aroma, colour, and texture. A test is performed on the

prepared substance, which is then poured onto cotton or the palm of the hand and applied to the face. Twenty panellists who previously utilized face toner was examined.

2.3.7. Antioxidant Activity Test of Cucumber Fruit Extract (Cucumis sativus L.) With DPPH Methods

Preparation of solution DPPH 0,05 mM - A total of 1.97 mg of DPPH was weighed and then put into a 100 ml volumetric flask added with methanol until the boundary mark was shaken homogeneously, so that a DPPH solution with a concentration of 0.05 mM was obtained (Hasanah *et al*., 2017).

Maximum wavelength determination - Maximum absorption is achieved by determining the longest wavelength. Using a visible spectrophotometer, the absorbance of up to 4 ml of a 0.05 mM DPPH solution in a cuvette was measured at wavelengths between 400 and 800 nm to determine the maximum wavelength. The maximum absorbance value yields the maximum wavelength (Hasanah et al., 2017)

Determination of operating time - Operating time was determined by taking 50 µL of the test solution plus 4.0 ml of 0.05 mM DPPH solution then vertexing and measuring at 0, 5, 10, 15, 20, 25 and 30 minutes at the maximum wavelength obtained from the predetermined wavelength. The minute that produces the most stable absorbance (0.2-0.8) of DPPH free radical immersion is the operating time (Mulangsri *et al*., 2017)

Preparation of blank solutions - 2 ml of DPPH solution (0.05 mM) was put into a test tube and added by 2 ml of methanol p.a., which is then covered with aluminium foil. Then it was homogenized with a vortex and incubated in a dark room for the operating time (Fathurrachman, 2014). Then the absorption is measured at the maximum wavelength from the results of the measurements that have been made.

Preparation of Vitamin C Solution - An accurate weigh of 10 mg of vitamin C powder is dissolved in 100 mL of methanol p.a in a 100 mL volumetric flask to obtain a concentration of 100 ppm (mother liquor). Then from the mother liquor, a concentration series of 1, 2, 3, 4, and 5 ppm was made in a volumetric flask, and the volume was made up with methanol p.a up to 5 mL. Each concentration of 2 mL of vitamin C comparison solution was put into a test tube, and then added by 2 mL of 0.05 mM DPPH solution homogenized with a vortex. Then, it was incubated in a dark room during the operating time (Fathurrachman, 2014). Then, the absorption is measured at the maximum wavelength from the results of the measurements that have been made (Zaky *et al*., 2021)

Solution Preparation and Antioxidant Activity Testing of 70% Ethanol Extract of Cucumber Fruit (Cucumis sativus L.) - 2.5 mg of extract was weighed and dissolved in 10 ml of methanol p.a until homogeneous to obtain a concentration of 250 ppm (mother liquor). The main solution was pipetted as much as 0.08; 0.16; 0.24; 0.32, and 0.4 ml into a 10 ml volumetric flask to obtain a test solution concentration of 2; 4; 6; 8, and 10 ppm. Then, the volume was made up of 10 ml with 2 ml of methanol p.a. for each sample solution put into a test tube, then added by 2 ml of 0.05 mM DPPH solution, homogenized with a vortex. It was then incubated in a dark room during the operating time (Fathurrachman, 2014). Then the absorption is measured at the maximum wavelength from the results of the measurements that have been made.

Preparation of Solutions and Antioxidant Activity Test of Face Toner Preparations - Toner has weighed carefully as much as 10 mg and dissolved in 10 mL of methanol p.a until homogeneous, so that a concentration of 1000 ppm was obtained. This solution was then made into a series of concentrations of 50, 100, 150, 200, and 250 ppm in a 10 ml volumetric flask and the volume was made up with methanol p.a up to 10 ml. 2 ml of each test solution was pipette into a test tube, and 2 ml of the main DPPH solution was added, then homogenized, and measured with UV-Vis's spectrophotometry.

3. RESULT AND DISCUSSION

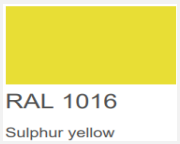
3.1. Plant Determination

The results of the determination carried out at the Herbarium Bogoriense, Botany Division of the Centre for Biological Research and Development-BRIN (National Research and Innovation Agency) Cibinong, showed that the cucumber obtained from Sodong Village RT 002/ RW 003, Tigaraksa sub-district, Tangerang regency, is a cucumber fruit plant with the Latin name Cucumis sativus L in the tribe of Cucurbitaceae.

3.2. Preparation of Cucumber Simplicia

The harvested cucumber (*Cucumis sativus* L.) fruit was subjected to wet sorting to separate the impurities or plant parts not used in the study and collected as much as 35 kg. After that, it was washed with running water. Clean cucumbers are then chopped and separated from the seedless part of the contents to reduce the particle size and increase the surface area. During the extraction process, direct drying is carried out for 7 days using sunlight covered with a black cloth and dried. It used the oven at 50ºC. After drying, dry sorting was carried out and pulverized with a blender to obtain simplicia powder, then sifted using mesh No.40. The simplicia powder was weighed and a yield of 700 g was obtained. After that, a simplicia quality parameter test was carried out to determine the characteristics of the simplicia and ensure that the simplicia used met the specified quality. The results of simplicia quality parameters can be seen in **Table 2.**

Table 2. The results of testing the quality parameters of cucumber (*Cucumis sativus* L.) simplicia

Parameter	Characteristics	Result ± SD	Condition
Non specific	Water content	6.44	<8.5%
	Ash content	5.39 ± 0.37%	<7.2%
	Drying shrinkage	8.0± 0.3%	<10%
Parameter	**Characteristics**	**Condition**	
Specific	Organoleptic:	Powder	
	• Shape		
	• Colour		
	• Aroma	Cucumber typical Bitter	
	• Taste		

3.3. Preparation of 70% Ethanol Extract of Cucumber Fruit (*Cucumis sativus* L.)

As much as 700 grams of cucumber simplicia were macerated using 10 L of 70% ethanol for 3x24 hours and stirred every morning and evening. After the maceration process, filtering is carried out to obtain macerate and dregs. The filtered dregs are re-macerated for 2x24 hours. The macerate obtained was concentrated using a rotary evaporator, and then it was concentrated again using a water bath until a thick extract was obtained. The results of the thick extract obtained were 196.23 grams. The yield of the extract obtained was 32.70%. After that, an extract quality parameter test was carried out to determine the characteristics of the extract and ensure that the extract used met the specified quality. The results of simplicia quality parameters can be seen in **Table 3**.

3.4. Phytochemical Screening

Phytochemical screening aims to determine secondary metabolite compounds contained in cucumber rind simplicia and 70% ethanol extract of cucumber fruit. The results of the phytochemical screening can be seen in **Table 4**. The results of the phytochemical screening of 70% ethanol extract of cucumber fruit (Cucumis Sativus L.) revealed that it contains flavonoids, saponins, and alkaloids.

Table 3. The results of testing the quality parameters of 70% ethanol extract of cucumber fruit

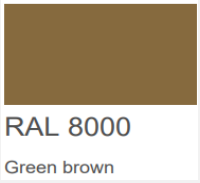
Parameter	Characteristic	Result ± SD	Condition
Non specific	Water content	18.31	<30%
	Ash content	8.15 ± 0,17%	<9.2%
	Residual Solvent	0.89	<1%
Parameter	**Characteristic**	**Condition**	
Specific	Organoleptic: • Shape • Colour • Aroma	Thick  Cucumber typical	

Table 4. Phytochemical screening results

No	Phytochemical Test	Result
1	Flavonoid	+
2	Saponin	+
3	Steroid	-
4	Tanin	-
5	Alkaloid	+

3.5. Physical Evaluation of 70% Ethanol Extract of Cucumber Fruit (*Cucumis sativus* L) Face Toner

Physical evaluation of the preparation is a parameter set to determine the stability of the preparation including organoleptic, pH, homogeneity and hedonic tests. Results of dosage formulation Cucumber extract face toner can be seen in **Figure 1**.

Figure 1. Face toner preparation of 70% ethanol extract of cucumber Fruit (*Cucumis sativus* L.)

3.5.1. Organoleptic Test

Organoleptic tests were conducted to determine the physical appearance of the antioxidant toner preparations, including their shape, colour, and odor. The preparations were stored for three weeks at three-week intervals, with various concentrations exhibiting relatively stable colour, shape, and odor stability. Face toner does not undergo any changes during storage. The results of the organoleptic test indicate that the higher the extract concentration in each formula, the more concentrated the colour concentration, which has a significant/visible difference in colour intensity between each formula. Formula 3, with a 1.5% extract concentration, has a darker brown hue than other formulas (Saratiana, 2020).

3.5.2. Homogeneity Test

The homogeneity test is performed to determine whether or not the antioxidant toner ingredients are well mixed. After 3 weeks and a time interval of homogeneity test results on face toner preparations, it was determined that the toner base and the active ingredient were evenly mixed.

3.5.3. pH test

The purpose of the pH test is to determine whether the preparation's pH is compatible with the pH of the skin. A pH meter is used to determine the pH of toner formulations. The test was conducted for three weeks with a weekly observation interval for all formulas. The pH test results are presented in **Table 5**.

Table 5. Face toner pH test results

Formulation	pH Testing, Week -		
	I	II	III
F0 (0%)	6.1	5.9	5.7
F1 (0,5%)	5.5	5.5	5.3
F2 (1%)	5.3	5.2	5.1
F3 (1,5%)	5.0	4.9	4.7

Note:
F0: Formula with concentration without cucumber fruit extract 0 %
F1: Formula with concentrated cucumber fruit extract 0.5 %
F2: Formula with concentrated cucumber fruit extract 1 %
F3: Formula with concentrated cucumber fruit extract 1.5 %

Based on **Table 5** of the pH test, the weekly pH decreases proportionally to the concentration of cucumber fruit extract. This indicates that the pH of cucumber fruit extract (Cucumis sativus L.) is low enough to affect the formulation. In this study, the overall pH of the face toner formulations varied from week 1 to week 3 between 4.7 and 6.1. These results are still within the 4.5-8 pH range recommended by SNI for healthy skin. It will likely not be irritating. In conclusion the face toner containing cucumber fruit extract met the pH requirements for skin preparation.

3.5.4. Hedonic Test

Hedonic test or preference test for face toner preparations is conducted by paying attention to aroma, colour, and texture. Examination was carried out on 20 panellists. The hedonic test results are shown in **Table 6**.

Table 6. Face toner hedonic test results

Sample	Parameter	Dislike	Quite Like	Like	Really Like
F0	Colour	4	5	6	5
	Smell	4	7	7	2
	Texture	4	6	5	5
F1	Colour	0	4	12	5
	Smell	1	5	9	5
	Texture	1	3	10	6
F2	Colour	0	5	11	4
	Smell	2	5	5	8
	Texture	0	5	9	6
F3	Colour	5	3	5	7
	Smell	2	7	9	2
	Texture	1	6	10	3

According to the table, the hedonic test results for face toner preparations obtained in terms of the colour of the formula that panellists liked the most was F1. The preference factor for each

panellist formula that affected it was the addition of cucumber fruit extract. The higher the concentration, the darker the resulting colour. However, with only a small amount of the product, the colour intensity will appear natural and attractive.

Regarding aroma, F1 (Cucumber Fruit Extract Face Toner), the most popular formula among panellists, possesses a distinct cucumber aroma. In the term of texture, the panellists preferred F1 because the resulting texture was soft and not excessively sticky.According to the results of the hedonic test, the preferred formula among the panellists is F1 with a 0.5% extract concentration.

3.6. Ascorbic Acid Antioxidant Activity Test as a Comparison

The use of ascorbic acid as a comparison control in testing antioxidant activity is to find out how strong the antioxidant potential of the extract when compared to ascorbic acid. If the IC50 value of the sample is equal to or close to the IC_{50} value, it can be said that the sample has the potential to be one of the very strong antioxidant alternatives. **Figure 2** reveals that the IC_{50} value of ascorbic acid was 7.768 µg / ml. A very strong antioxidant category, namely IC_{50} values are in the range of 0-50 µg / ml. This suggests that ascorbic acid has a very strong power of antioxidant activity (Zaky *et al*., 2021).

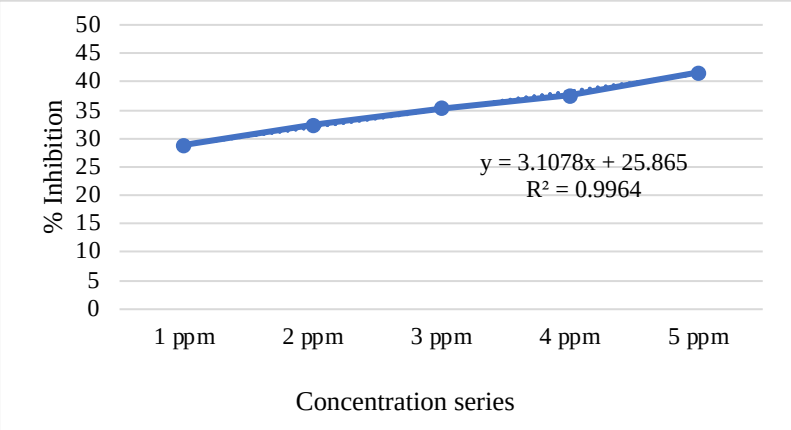


Figure 2. Curve % inhibition of ascorbic acid antioxidant test as a comparison control

3.7. Antioxidant Activity Test of 70% Ethanol Extract of Cucumber Fruit (*Cucumis sativus* L) Face Toner

The face toner preparation was formulated using a concentration of 0.5%, 1% and 1.5% cucumber fruit extract. This concentration was chosen because it was a cosmetic preparation for skin care. Extract antioxidant activity test was carried out to ensure that cucumber fruit extract has antioxidant activity. This is evidenced by the IC_{50} value of cucumber fruit extract of 21.22 µg/ml. The IC_{50} value belongs to the powerful antioxidant category.

The formula was made by increasing the concentration of the extract, starting from a concentration of 0.5; 1; and 1.5%. With an increase in the concentration of the extract in the formula, the greater the % inhibition obtained. For F1 which contained 0.5% cucumber fruit extract, the IC_{50} was 128 µg/ml—in F2, cucumber fruit extract of 1% received IC_{50} of 91.017 µg/ml. Whereas for F3 cucumber fruit extract of 1.5% obtained IC_{50} of 62.218 µg/ml. From the results of tests that have been carried out on formulas that were given extracts, it can be concluded that formulas with extract concentrations of 0.5; 1; and 1.5% still has antioxidant activity and is classified as a strong to moderate antioxidant, where the strong to moderate antioxidant category is 50-150µg/ml. In other words, the less antioxidant effect in the formula, the stronger the antioxidant activity.

Measurement results absorbance and % inhibition of cucumber fruit extract can be seen in **Table 7**.

Table 7. Test results of antioxidant activity of cucumber fruit extract (*Cucumis sativus* L.)

Sample	Concentration (ppm)	Absorbance			
		U 1	U 2	U 3	Mean
Cucumber fruit extract	blank	0.307	0.307	0.307	0.307
	2 ppm	0.283	0.284	0.284	0.284
	4 ppm	0.271	0.271	0.271	0.271
	6 ppm	0.257	0.257	0.257	0.257
	8 ppm	0.243	0.243	0.244	0.243
	10 ppm	0.230	0.230	0.230	0.230

The results show that cucumber fruit extract has antioxidant activity with very strong antioxidants, namely with an average value of IC_{50} 21.22 µg / ml. This is because the active substance used in the preparation, cucumber fruit extract, has compounds that act as antioxidants, namely flavonoids. The content of this secondary metabolite is antioxidant that affect the results of antioxidant activity tests. Based on research by Badriah *et al*. (2021) entitled "Formulation and Test of the Effectiveness of Antioxidant Handbody Lotion Ethanol Extract 70% Cucumber (Cucumis sativus L.)" states that 70% ethanol extract of cucumber fruit has antioxidant activity with an average value of IC_{50} which is 27.56 µg / ml and is included in the category of very strong antioxidants. Measurement results absorbance and % inhibition F3 can be seen in **Table 8**.

Table 8. F3 Antioxidant activity test results (extract 1.5%) of cucumber fruits

Sample	Concentration (ppm)	Absorbance			
		U 1	U 2	U 3	Mean
F3 (extract 1.5%) cucumber fruits	blank	0.540	0.540	0.540	0.540
	50 ppm	0.275	0.275	0.275	0.275
	100 ppm	0.255	0.256	0.256	0.256
	150 ppm	0.239	0.239	0.239	0.239
	200 ppm	0.223	0.223	0.223	0.223
	250 ppm	0.204	0.205	0.205	0.205

The result for F3 toner was determined by IC_{50} value of 62.218 g/ml. This shows that F3 as the highest concentration for facial toner preparations of cucumber fruit extract has relatively high antioxidant activity. To sum up, the greater the concentration of cucumber fruit extract added, the greater the antioxidant activity obtained.

Table 9 indicates the IC_{50} analysis value of ascorbic acid results of 7.768µg / ml. The results show that vitamin C has a very strong antioxidant activity. For the yield of cucumber fruit extract (Cucumis sativus L.) of 21.22µg / ml, it shows that cucumber fruit extract has a very strong antioxidant activity. While for the preparation, it can be seen that the antioxidant activity of the F0 face toner preparation test solution without extract yields an IC_{50} value of 213.72 µg / ml, indicating that F0 without the addition of 70% ethanol extract face toner preparation extract has the strength of antioxidant activity which is relatively weak as a face toner preparation because there are only excipients. F1 face toner preparation with 0.5% extract concentration has an IC_{50} value of 128 µg / ml, indicating that F1 with the addition of 0.5% extract of 70% ethanol extract face toner preparation of cucumber fruit has also weak power of antioxidant activity as a face toner preparation. F2 face toner preparation with a concentration of 1% has an IC_{50} value of 91.017 µg / ml, indicating that F2 with the addition of 1% extract of 70% ethanol extract face toner preparation of cucumber fruit has the power of antioxidant activity which is classified as a strong face toner preparation. Likewise, F3 face toner preparation with a concentration of 1.5% has an IC_{50} value of 62.218 µg / ml, showing that F3 with the addition of 1.5% extract of 70% ethanol extract face toner preparation of cucumber fruit has the power of antioxidant activity which is classified as strong as a face toner preparation. It can be said that the higher the concentration of cucumber fruit extract added, the higher the antioxidant activity obtained. F4 toner preparation brand x has an IC_{50} value of 58.444 µg / ml, this shows that formula 4 brand x

has the power of antioxidant activity which is classified as strong as a positive control toner preparation. research on the effectiveness of handbody lotion preparations had the highest antioxidant activity in the third formula with a concentration of 1.5% at 91.657 ppm. It indicates that toner preparations have higher antioxidant activity than lotions. In conclusion, the higher the concentration of antioxidant activity, the greater the antioxidant activity (Aprilliani *et al*., 2022). Results can be seen in the **Table 9**.

Table 9. Linear regression results and IC_{50} values obtained for Face Toner Preparation 70% Ethanol Extract of Cucumber Fruit

Test Solution	%Inhibition	IC_{50}
Vitamin C	41.666 44.444 47.037 49.074 51.851	7.768
Cucumber fruit extract	41.666 44.444 47.037 49.074 51.851	21.22
F0 (0%)	45.74 48.518 51.296 53.888 57.222	213.72
F1 (0.5%)	45.74 48.518 51.296 53.888 57.222	128
F2 (1%)	47.407 50.925 53.703 56.296 59.444	91.017
F3 (1,5%)	49.074 52.592 55.74 58.703 62.037	62.218
F4Toner Merk X	49.629 52.037 54.259 56.481 58.888	58.444

Based on the results, the face toner preparation in formula 3 with a concentration of 1.5% had a strong IC_{50} value of 62.218 g/ml while the results of the cucumber fruit extract itself had a very strong IC_{50} value of 21.22 g/ml. The difference in these results was due to the fact that the extract has only a small amount as a preparation compared to pure extract. Thus, this is one of the causes for the disparity between the IC_{50} values of face toners and pure extracts.

The statistical data analysis of antioxidant activity test for each formula with the DPPH method was conducted by using the IBM Statistical Product and Service Solution (SPSS) 25.0 application with the One-Way ANOVA method. It aims to determine whether there are significant differences between treatment groups among the five treatment group formulas. The One-Way

ANOVA parameter test has several requirements to meet, including the data obtained must be normally distributed and the variance of the data obtained must be homogeneous.

The normality test results of the One-Sample Kolmogorov-Smirnov Test show that the data is not normally distributed because it has a significance value or p=0.039, meaning that a significant value of p<0.05. Meanwhile, the homogeneity test results showed a p-value or significance of p = 0.062, indicating that the data is homogeneous because of the significance value of p <0.05.

Subsequent analysis used the one-way analysis of variance method to determine whether there is a significant difference in each treatment group. The hypothesis of ANOVA is that the results obtained a significance value of 0.000, indicating that there is a significant difference from each formula due to the p value <0.05. However, since the data is not normal and homogeneous, it is continued using the Kruskal Wallis test. It is to determine whether there is a difference between variables for data that does not meet the requirements of the ANOVA test. The results of the Kruskal-Wallis's test showed a significant difference between the five treatment groups. The Mann-Whitney test was carried out in the next step to find out which group had a significant difference.

The Mann-Whitney test revealed a significant difference between the negative control (F0) and the treatment group that received extracts F1, F2, and F3, with a significance value of less than 0.050, indicating a significant difference. This difference is evident from the fact that the IC_{50} value for F0 is greater than the IC_{50} value for the group that received the extract, indicating that the lower the IC_{50} value, the greater the antioxidant activity.

Further comparisons between extracts F1 and F2, F1 and F3, and F2 and F3 revealed a significance value of less than 0.050, indicating that there was a significant difference. It can be seen in the IC_{50} value obtained for F3, which is smaller than those obtained for F1 and F2 or the smaller the IC_{50} value obtained, the greater the antioxidant activity. To conclude, the extract's antioxidant activity increases with its concentration.

The significance value of the comparison between the positive controls (F4) and the treatment group (F0, F1, F2, F3) is less than 0.050, indicating a significant difference. This distinction is evident from the IC_{50} obtained for F4, which has an IC_{50} value. It is smaller than the treatment group, meaning that the greater the antioxidant activity, the smaller the IC_{50} value. The treatment group has not been able to outperform the positive control group.

4. CONCLUSION

Physical evaluation tests show that it meets the physical requirements of toner (organoleptic, homogeneous, pH, and hedonic tests). Antioxidant activity cucumber fruit extract face toner with concentrations of 0.5%, 1% and 1.5% has IC_{50} values of 128 ppm, 91.017 ppm, and 62.218 ppm respectively. The SPSS analysis reveals a significant difference in the IC_{50} value, by which F3 has a smaller IC_{50} value than other formulas, meaning that the smaller the IC_{50} value, the stronger the antioxidant activity. In conclusion, the higher the concentration of the extract, the stronger the antioxidant activity.

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6. AUTHOR DECLARATION

Authors' Contributions and Responsibilities

The authors made substantial contributions to the conception and design of the study. The authors took responsibility for data analysis, interpretation, and discussion of results. The authors read and approved the final manuscript.

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MIGRATION INHIBITION ACTIVITY BY METHANOL EXTRACT *Hibiscus tiliaceus* Linn. ON 4T1 BREAST CANCER

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ABSTRACT
The prevalence of breast cancer cases in Indonesia is increasing along with the ability of cancer cells to migrate or move from the primary tumor mass and form new colonies elsewhere. The migration of cancer cells has encouraged the develop anticancer drugs from natural ingredients. Waru leaves have been shown to have cytotoxic activity. This study aims to determine the inhibition activity of migration of breast cancer cells 4T1 from methanol extract of waru leaves. Waru leaves methanol extract was obtained using the maceration method. Cytotoxic test of methanol extract of waru leaves (MEWL) was the migration test used in the scratch wound healing method at concentrations 162.5, 325, and 650 µg/mL at 0, 18, 24, and 42 hours after treatment. Analysis of IC_{50} using linear regression, while large areas were analyzed using Image-J software. The percentage of data closure was analyzed statistically with the Anova Repeated Measure test. All concentrations of Methanol Extract of Waru Leaves had significant inhibition of cell migration ($p<0,05$) compared to control at each observation time at 0, 18, 24, and 42 hours after treatment. So, MEWL is able to inhibit migration in 4T1 cells.

Keywords: Methanol Extract of Waru Leaves (*Hibiscus tiliaceus* Linn.); 4T1 cells; MTT Assay; Scratch wound healing

1. INTRODUCTION

Breast cancer is a crucial and unavoidable health problem for most women. Based on data from the Global Cancer Observatory in 2020, breast cancer cases are ranked first, namely 65.858 cases or 30.8% of the total 213.546 cancer cases that occur in women. The mortality rate for breast cancer is in second rank among all cancer cases (Globocan, 2021). Meanwhile, the incidence of breast cancer is 42.1 per 100.000 population, with an average death rate of 17 per 100.000 population (Kemenkes RI, 2019).

The leading cause of the high death rate in breast cancer is related to the ability of cancer cells to move from their primary tumor mass and form new colonies elsewhere, or cells can metastasize (Sopik & Narod, 2018). The process of cell migration mediates breast cancer cell metastasis (Medeiros & Allan, 2019). The migration of cancer cells has encouraged the development of anticancer drugs from natural ingredients. One part of the potential as an anticancer is waru leaves (*Hibiscus tiliaceus* Linn.), but research on waru leaves is rarely carried out.

Waru leaves (*Hibiscus tiliaceus* Linn.) is a wild plant used in traditional medicine. Waru leaves are widely used in Indonesia as an anti-inflammatory, laxative in urine, sputum, and reduce fever and tonsils (Dalimartha, 2006). Methanol extract from waru leaves contains chemical compounds such as tannins, flavonoids, alkaloids, and saponins (Surahmaida et al., 2020). Based

on studies, methanol extract of waru leaves has a selective cytotoxic effect on MDA-MB-435S breast cancer cells (Uddin et al., 2011). 4T1 cells and MDA-MB-435S cells are categorized into the triple negative Breast Cancer (TNBC) group because they have the same characteristics (Tao et al., 2008; Vuletic et al., 2015). 4T1 cells are cancer cells isolated from the mammary glands of mice (Mus musculus) from the BALB/cfC3H strain, which have characteristics similar to advanced/metastatic breast cancer (BCRJ, 2023). So far, the existing research is only related to cytotoxic activity, so this research was carried out to determine the resulting migration effect. The large number of cases of cancer cell metastasis encourages the importance of research into antimigration. This study aimed to determine the migration inhibition activity of the methanol extract of waru leaves (*Hibiscus tiliaceus* Linn.) against 4T1 cells.

2. METHODS

2.1. Materials

The primary material used is fresh green waru leaves obtained from Kunduran Village, Blora Regency, Central Java. Determination of the waru plant was carried out at the Ecology and Biosystematics Laboratory, Department of Biology, Mathematics and Natural Sciences, Diponegoro University, Semarang. The 4T1 cell test subjects were obtained from the collection of the In Vitro Cell Culture Laboratory, Faculty of Medicine and Health Sciences, Muhammadiyah University, Yogyakarta.

2.2. Extract Preparation of War Leaves

Methanol extract of waru leaves was prepared by maceration method, using methanol solvent with a ratio 1 : 10. As much as 500 gram of waru leaves powder is soaked in 3.750 mL of methanol for three days, stirring occasionally twice a day. After three day of maceration, the extract is filtered, and the dregs are filtered to obtain macerate (filtrate I). and then remaceration is carried out by adding 1.250 mL of methanol to the dregs. The extract is filtered to obtain filtrate II. The macerate was mixed until homogeneous and pure, then filtered to separate the filtrate from the remaining powder. Furthermore, the extraction results were concentrated using a rotary vacuum evaporator at 50 °C.

2.3. Scratch Wound Healing Assay

Cell concentration required for the scratch wound healing method is $7.5x10^4$ cells/well-distributed into 24-well (500 µL/well). Cells were incubated for 24 hours in a CO_2 incubator at 37 °C. Then, scratches are made on the bottom surface of the well using a sterile yellow tip. Media was discarded, and the cell culture was washed with 500 µL PBS (Phosphate Buffer Saline) each well. It is necessary to ensure that the cells are washed completely clean so that there are no cells attached to the scratches and no floating cells. The cells were then given EMDW solution with a concentration of 162.5, 325, and 650 µg/mL as much as 500 µL/well, then incubated in a CO_2 incubator. Control media used empty wells without cells with the addition of DMSO (Dimethyl Sulfoxide) solvent and culture media. Observations were made at 0, 18, 24, and 42 hours after treatment. The search results were documented every observation time with the same magnification microscope and camera. Image-J software measured the initial scratch area with the empty area (CCRC, 2015).

2.4. Data Analysis

The area of cell closure in each treatment group was analyzed using the Microsoft Excel 2013 program to obtain data on the percentage closure ratio for each treatment. The formula for calculating percent closure:

$$\%\ Closure : \frac{(\text{Area t0} - \text{Area tn})}{\text{Area t0}} \times 100\% \quad (1)$$

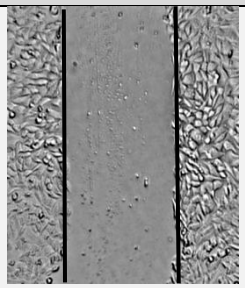
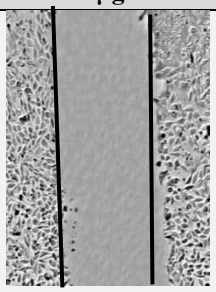
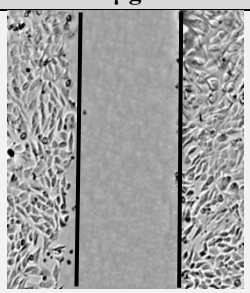
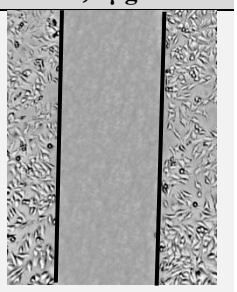
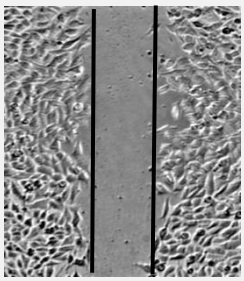
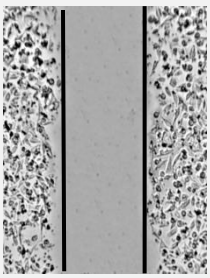
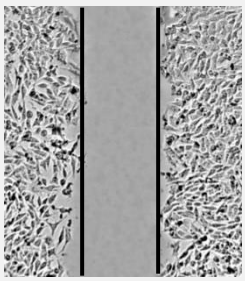
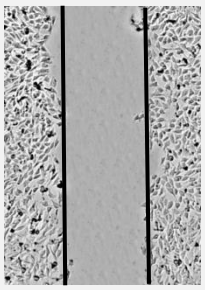
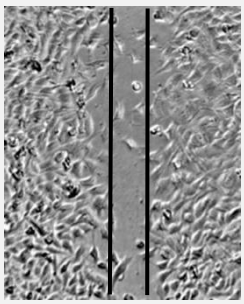
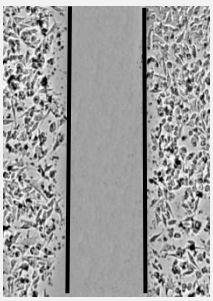
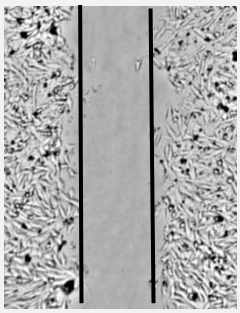
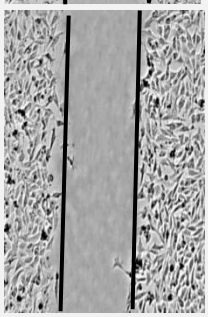
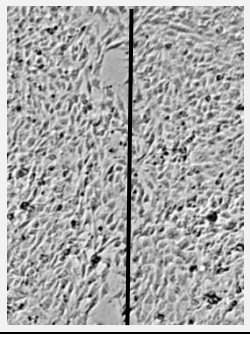
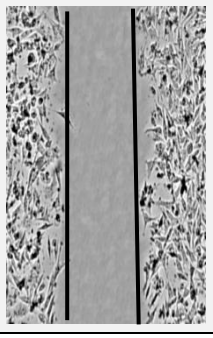
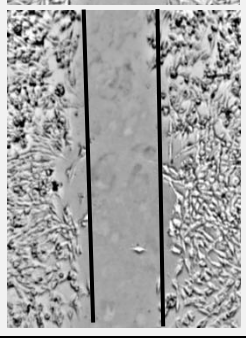
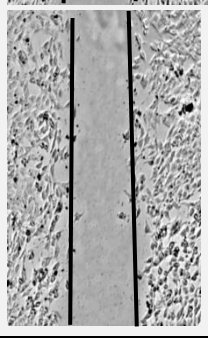
Data on the results of % closure at the 0, 18, 24, and 42 hours were analyzed using the Anova Repeated Measure test. Percent closure is said to have a difference at each observation

time across groups if the value is ($p < 0.05$) (Costantini et al., 2022). The smaller the percentage of cell closure (% closure), the methanol extract of waru leaves has better 4T1 breast cancer cell migration inhibition activity (Seppatria, 2019; Zulharini et al., 2018).

3. RESULTS AND DISCUSSION

Extraction of the active substance from war leaves Simplicia powder using the cold method of extraction, namely maceration. The choice of the maceration method aims to reduce the risk of damage to the active compound content, especially flavonoid compounds, because these compounds are not resistant to high temperatures. Methanol extract of waru leaves (MEWL) obtained a yield of 12.98%. Research on hibiscus leaves extracted using 96% ethanol solvent showed a yield of 10.2% (Hidayati et al., 2022). This shows that a greater yield was obtained using methanol solvent. The concentration of MEWL used for research on inhibiting cell migration with the scratch wound healing method was 162.5, 325, and 650 µg/mL. The observation time was repeated at 0, 18, 24, and 42 hours after treatment. Treatment of 4T1 breast cancer cells with MEWL showed activity in inhibiting cell migration (**Table 1**).

Table 1. Microscopic activity of inhibition cell migration after treatment with MEWL

Time	Control cell	MEWL 650 µg/mL	MEWL 325 µg/mL	MEWL 162,5 µg/mL
0 hours				
18 hours				
24 hours				
42 hours				

Based on the results of migration inhibition (**Table 1**) it can be seen that MEWL had better 4T1 cancer cell migration inhibition activity than control cells at 18, 24 and 42 hours of observation. The activity of inhibiting MEWL cell migration is thought to be due to the presence of flavonoids, alkaloids, tannins, and terpenoids contained in MEWL. Another study stated that the methanol extract of red betel leaves containing flavonoid compounds effectively inhibited the migration of 4T1 cells with 58% cell closure results and no increase in the percentage of cell closure at 18, 24, and 42 hours (Zulharini et al., 2018).

Flavonoid compounds can inhibit cell migration and invasion through the mechanism of significantly suppressing the activity of Matrix Metalloproteinase 9 (MMP-9) by blocking the signaling pathways Protein Kinase C (PKC-α), Extracellular Signal Regulated Kinase (ERK), and Mitogen-Activated Protein Kinase (MAPK), as well as reducing the expression of RhoA, Rac1, and Cdc42 which play an important role in regulating migration activity in cancer cells (Uddin et al., 2011). Other studies have shown that hibiscus leaves contain a class of flavonoid compounds (Vuletic et al., 2015). The quercetin compound is able to suppress cell migration by reducing the expression of Focal Adhesion Kinase (FAK) which is mediated by quercetin compounds (Huang et al., 2018). Quercetin also inhibits TNF-α-induced apoptosis (Chen et al., 2020). Based on the results of calculating the average % closure of the treatment and control groups, there was a significant difference (**Figure 1**).

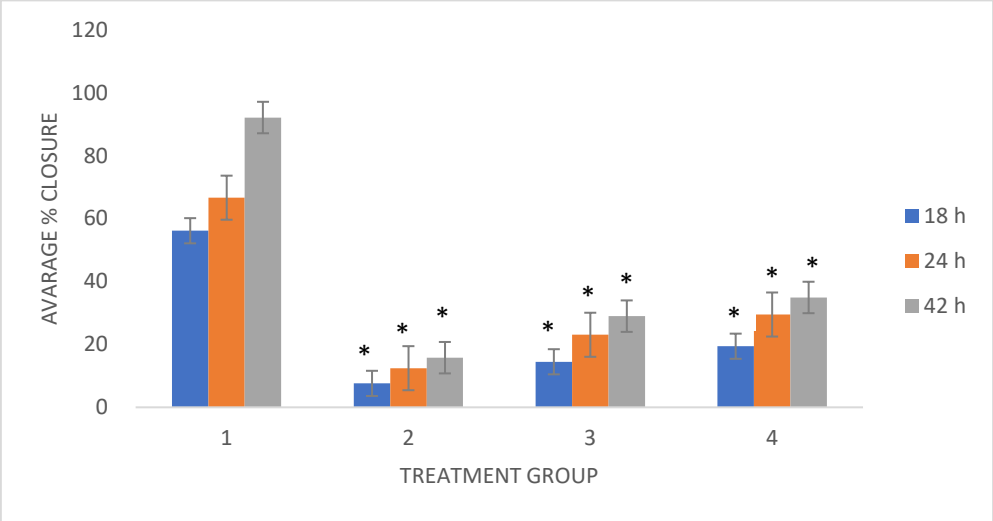


Figure 1. % closure average of migratory cells every after treatment using repeated measures ANOVA statistical test (*p<0,05 has as significant difference to control cells).

The statistical test results for each treatment group using the repeated measurement ANOVA method showed significant differences ($p<0.05$) in the treatment group in inhibiting cell migration compared to control cells at each observation time. While the data on the percentage of cell closure at the 18, 24, and 42 observations of all treatment groups, there was a significant difference ($p <0.05$). So it can be said that MEWL has good cell migration inhibition activity, which is indicated by a small percentage of closure. The smallest percentage of closure was shown at a concentration of 650 µg/mL, which at 42 hours was still able to inhibit cell migration as indicated by the percentage of closure of 16%, while the percentage of closure in the control group was 92%. Similar research regarding antimigration from hibiscus leaf extract using ethanol as a solvent showed that it was able to inhibit the migration of 4T1 cancer cells. Percentage cell closure at 18, 24, and 42 hours with a concentration of 892 µg/mL (Hidayati et al., 2022). These results show that the use of methanol solvent has a smaller concentration in inhibiting 4T1 cell migration compared to the use of ethanol solvent. So, the use of methanol solvent is recommended. In this study, only cell migration was tested, then western blot testing can be carried out to determine the

specific proteins involved in cell migration. But, the IC_{50} in this study still has a fairly large concentration, it may be necessary to isolate the compound from waru leaves to produce maximum effects.

4. CONCLUSION

Methanol extract of waru leaves at concentrations 162,5; 325 and 650 µg/mL showed activity in inhibiting the migration of 4T1 cancer cells so that it could be further developed as an anti-migratory agent.

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6. AUTHOR DECLARATION

Authors' Contributions and Responsibilities

The authors made substantial contributions to the conception and design of the study. The authors took responsibility for data analysis, interpretation, and discussion of results. The authors read and approved the final manuscript.

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Availability of Data and Materials

All data are available from the authors.

Competing Interests

The authors declare no competing interest.

Additional Information

No additional information from the authors.

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USE D-OPTIMAL MIXTURE DESIGN IN FORMULATION OF ONCHIDIID SLUG (ONCHIDIUM TYPHAE) INSTANT POWDER AS FUNCTIONAL FOODS

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ABSTRACT
Onchidiid slug (*Onchidium typhae*) is an animal with bioactive compounds with high nutritional value and the potential to be used as a functional food product. The study aimed to optimize the onchidiid slug instant powder formula, analyze its proximate content, and determine its hedonic level. The formula of instant powder was made with a comparison of the composition of maltodextrin and dextrose based on the run in the D-Optimal Mixture Design (DMD) program, namely run 1 (20%:10%), run 2 (10%:20%), run 3 (13.33%: 16.67%), run 4 (16.67%:13.33%), run 5 (15%:15%), run 6 (12.5%:17.5%), run 7 (10%: 20%), run 8 (20%:10%). The powder is tested for water content and dissolution time, and the optimal formula is analyzed using One Sample T-Test in SPSS. The results showed that the composition of maltodextrin and dextrose significantly affected the characteristics of instant powder. The optimal instant powder formula combines 18.690% maltodextrin and 11.310% dextrose with a water content of 4.892% and a soluble time of 118.052 seconds. The results of statistical analysis in verifying the optimal formula show a p-value > 0.05 (not significant). The results of the proximate content test were 8.21% water content, 0.42% ash content, 0.85% crude fiber, 3.54% protein, and 1.54%. They had a preference level in the like and acceptable in terms of color, taste, texture, and scent.

Keywords: *Onchidium typhae*; Functional food; D-Optimal Mixture Design; Hedonic Assay

1. INTRODUCTION

West Kalimantan is a province that boasts diverse biological resources with potential for use as food sources and bioactive compounds with significant economic value. However, research on marine biological resources as food ingredients has been minimal compared to terrestrial biological resources, even though marine biological resources have been proven to contain various potential active ingredients (Faulkner, 2002; Lordan et al., 2011). Aquatic organisms are a rich source of secondary metabolites that exhibit pharmacological activity and can be used to develop healthy food products (Karthikeyan et al., 2022; Pringgenies, 2010). One such organism is the onchidiid slug from the genus Onchidium.

The Onchidium genus is a marine biota that contains bioactive compounds with high nutritional value. One species of this genus is the onchidid slug (*Onchidium typhae*) (Wang et al., 2021). Previous research shows that onchidid slug chloroform extract contains alkaloids, steroids, saponins, and free amino acids. Proximate analysis shows that onchidiid slug meat simplicia powder is protein-rich (67.88%) (Wijianto, et al., 2022). This high protein value makes onchidiid slugs have the potential to be used as health food products that function well as a source of energy, building blocks in the body, and forming antibodies (Budiyanto, 2015; Hayes & Mora, 2021).

Nowadays, food products widely consumed by the public are practical health drinks that do not require much time to prepare but can have a good effect on health. The method used to extend the shelf life related to product storage is processing it into an instant powdered functional drink (Ariska & Utomo, 2020). Instant powdered drinks are food products in the form of practical powders to serve and dissolve easily in water.

Instant powder has problems, namely the loss of several vital substances due to inappropriate drying techniques (Utomo & Ariska, 2020). Foam mat drying is a technique for drying liquid and heat-resistant materials through foaming techniques with the addition of foaming agents. The foam mat drying method is relatively simple and cheap and is carried out at low temperatures ranging from 50-80 °C to retain essential substances. Fillers and foaming agents influence the quality of instant powder using the foam mat drying method (Purbasari, 2019). Fillers and foaming materials commonly used in the foam mat drying method are maltodextrin and tween 80. Another filler that is also useful as a sweetener is dextrose. Apart from playing a role in adding taste, dextrose sweetener also plays a role in creating the texture of a food product (Rahmawati & Sutrisno, 2015).

Research on the use of onchidid slugs as a raw material in the formulation of functional drinks has never been carried out, thus encouraging this research to find the optimal formula for preparations in the form of instant powdered functional drinks that utilize fine flesh filtrate of onchidiid slugs using the foam mat drying method. The formulation of the onchidiid slug instant powder functional drink was optimized using the Design Expert 13 software and the D-Optimal Mixture Design method to obtain the optimal formula. The Design Expert program, the D-Optimal Mixture Design method, was chosen because of its high flexibility and high accuracy in determining a suitable mathematical model for optimization (Usman et al., 2023). The optimized factor is a filler consisting of a combination of maltodextrin and dextrose based on the physical characteristics of the powder, including water content and dissolution time. Maltodextrin and dextrose in this study are combined because they have the largest proportions in the formulation that can affect the characteristics and quality of the functional drinks. Maltodextrin and dextrose, besides serving as fillers, also function as sweetening agents, thus affecting the hedonic acceptance and testing by respondents. In order to determine the nutritional value of a product, a proximate analysis is conducted to measure the content of water, ash, crude fiber, protein, fat, and carbohydrates. A liking level (hedonic test) is also performed to assess the level of acceptability and preference for the instant powder. This test aims to create instant powder functional drink products that are both popular and high-quality, with good nutritional value and instant powder characteristics that meet the required standards.

2. METHODS

2.1. Material

Onchidiid slug fresh meat (*Onchidium typhae*), Design Expert 13.0.0 Trial software, ginger, lime, maltodextrin (LIHUA food grade brand, batch no. 20171015), dextrose (LIHUA food grade brand, batch no. 20220409), tween 80 (Chemical Nitra, COA, batch no. 38133). Another supporting instrument in this study consisted of a computer Intel Core i-5, blender (Mitochiba CH-200), mixer (Philips HR1530), food dehydrator (PAPALOLO), analytical balance (Ohaus Pioneer PX224/e), 60 mesh sieve, glassware (iwaki), pH meter (HANNA), Kern Moisture Balance DLB 160-30A.

2.2. Research Stages

2.2.1. Sample Preparation

Onchidid slug samples were washed and cleaned of dirt and mud until clean. Onchidid slugs are separated from the flesh and innards; then, the flesh is rewashed until it contains no mucus. Next, the clean onchidiid slug meat is mashed using a blender for 10-30 minutes to a

smaller size by adding water in a ratio of 1:1 (w/v), then boiled at 90 °C for 8-10 minutes and filtered to take the filtrate (Wijianto, et al., 2022) .

2.2.2. Instant Powder Formulation Stage

In order to create instant powder, the essence of the ingredients must be extracted through a multi-stage process involving drying or evaporation to remove any water content. For this research, foam or mat drying is the preferred method for instant powder formulation. Mix primary and additional ingredients, and dry using a foam mat at 70-80 °C for 15-20 mins per formula. The mixture is then refined using a blender and sifted through a 60 mesh, resulting in the ideal instant powder. Packaging is the final step in the process (Purbasari, 2019).

2.2.3. Physical Characteristics Evaluation Stage

Water Content Test - The Kern Moisture Balance DLB 160-30A was used to determine the percentage of water content in the powder as part of this study's water content testing.

Soluble Time Test - To conduct the dissolution time test, we start by weighing 5 grams of powder and dissolving it in 100 ml of cold water (25-28 °C) while continuously stirring. Using a stopwatch, we record the time for the sample to dissolve fully. An ideal completion time for this process is less than 5 minutes. This methodology was established by Husni et al., 2020.

2.2.4. Proximate Analysis

A proximate analysis was carried out to determine the water and ash content through the gravimetric method. The fat content was determined using the Weibull-modified Soxhlet method, while the protein content was tested using the Kjeldahl method. The carbohydrate content was calculated by difference, and the crude fiber content was tested according to SNI 01-2891-1992, which is related to food and drink testing (Wijianto, et al., 2022).

2.2.5. Hedonic Assay

The hedonic assay is a method used to evaluate product acceptance or liking. It considers various factors, including color, taste, texture, and aroma. In a recent study, 20 panelists were selected for the assay. Although trained, the panelists required further expertise in identifying the organoleptic characteristics of the products. They were provided with preparations formulated with the ideal ingredients and asked to rate them on a questionnaire using a hedonic scale from 1 to 4, which included options like "immensely dislike," "do not like," "like," and "really like" (Apandi & Restuhadi, 2016).

3. RESULTS AND DISCUSSION

This research involved the development of an instant powder preparation formula using the Design Expert 13 software program. The study aimed to find the optimal combination of maltodextrin, dextrose, fillers, and sweeteners to produce a high-quality powder. Eight formulations were created with varying amounts of maltodextrin and dextrose. The fillers and sweeteners used in the formulations included various options to produce a sound and favorable powder for consumption. The correct number of fillers and sweeteners was determined based on the instant powder's water content and dissolution time. The instant powder was produced using the Foam Mat Drying method, which uses a foaming agent to shorten the drying process. This method proved to be effective in reducing the drying time and resulted in a high-quality instant powder. Overall, this research provides valuable insights into the development of instant powder preparation formulas that are both effective and easy to produce. The results of 8 runs of D-Optimal Mixture Design are shown in **Figure 1**.

3.1. Evaluation of Instant Powder Characteristics

3.1.1. DMD Analysis Water Content Test

The tests that were carried out revealed the moisture content of the instant powder to range between 5.2% and 10.51%. A low percentage of water content is critical in producing high-quality

powder that can be stored for a long duration without compromising quality. Upon evaluation, the results indicated that the water content in each formula was below 10%, which is in line with the standard requirements of the Regulation of the Head of the Food and Drug Supervisory Agency. The analysis of the water content response is expressed in equation 1, providing a clear understanding of the moisture level present in the instant powder. ANOVA Cubic Model Water Content Response and the equations can be seen in **Table 1** and Eq. (1).

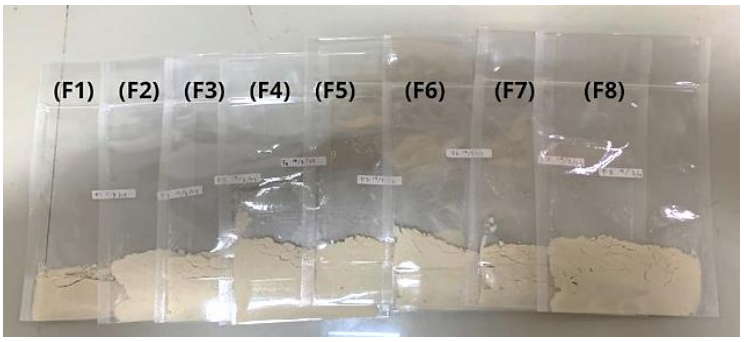

Figure 1. Onchidiid slug instant powder from 8 runs of D-Optimal Mixture Design: F1 combination Maltodextrin:Dextrose (20%:10%); F2 Maltodextrin:Dextrose (10%:20%); F3 Maltodextrin:Dextrose (13.33%:16.67%); F4 Maltodextrin:Dextrose (16.67%:13.33%); F5 Maltodextrin:Dextrose (15%:15%); F6 Maltodextrin:Dextrose (12.5%:17.5%); F7 Maltodextrin:Dextrose (10%:20%); F8 Maltodextrin:Dextrose (20%:10%)

Table 1. ANOVA cubic model water content response

No	Source	P value	Result
1	*Model Cubic*	0.0452	Sig.
2	*Lack of Fit*	0.1160	Not Sig.

The model equation or polynomial for water content response analysis is contained in the following Eq. (1).

$$Y = 3.21825\ A - 3.46023\ B + 0.0501421\ AB \quad (1)$$

Where: Y = Water content (%); A = Maltodextrin; B = Dextrose; AB = Combination of Maltodextrin and Dextrose Proportions

According to Eq. (1), the component that affects the water content in instant powder is the interaction coefficient A, which represents maltodextrin and has a positive value. This means that the more maltodextrin is added during production, the higher the water content will be. On the other hand, the coefficient B, which represents dextrose, has a negative value, indicating that it has a more negligible impact on increasing water content than maltodextrin. The AB coefficient value is positive, meaning that the combination of maltodextrin and dextrose has a negligible effect on increasing water content. A graph showing the results of the normality of the water content test from research conducted using the D-Optimal Mixture Design method can be found in **Figure 2**.

When added to a substance, maltodextrin can significantly impact the water content. This is due to the unique hygroscopic properties of maltodextrin, which allow it to absorb water and increase the overall water content of the substance. Research has shown that the ability of maltodextrin to bind OH groups from water makes it such an effective water-absorbing agent (Hui, 1992).

In order to provide more insight into this matter, numerous studies have been conducted on various powders that contain varying levels of maltodextrin. For example, research by Corie et al. (2023) found that powders with 20% maltodextrin had a higher water content of 3.31%,

compared to powders with 10% and 15% maltodextrin, which had lower water contents of 3.13% and 3.33%, respectively. This suggests that the amount of maltodextrin added can significantly impact a substance's overall water content.

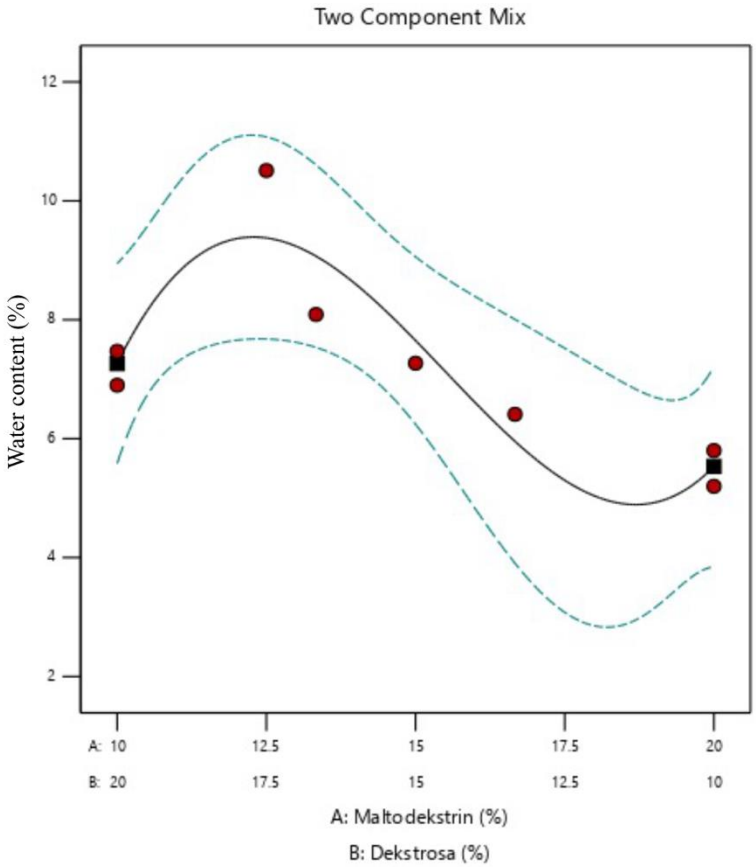

Figure 2. Two-component mix maltodextrin and dextrose on water content

3.1.2. DMD Analysis of Soluble Time Test

The results of the tests revealed that the instant powder for onchidiid slug's dissolves quickly, within a range of 120.22 – 138.45 seconds, making it easy to consume. The evaluation results concluded that all formulas dissolve entirely within five minutes, meeting the instant dissolving time requirements. The analysis of the dissolving time response recommended using a cubic polynomial model for the dissolving time test, according to the Design Expert 13 program. This means that the dissolving time of the instant powder for onchidiid slugs is in line with the recommended industry standards for the instant dissolving time. The response time of the ANOVA cubic model is listed in **Table 2.**

Table 2. ANOVA soluble time response cubic model

No	Source	P value	Result
1	*Model Cubic*	0,0060	Sig.
2	*Lack of Fit*	0,4826	Not Sig.

The mathematical model equation or polynomial for soluble time response analysis is contained in the following Eq. (2).

$$Y = 16.9565\ A - 9.9703\ B + 0.109326\ AB \quad (2)$$

Where: Y = Soluble Time (seconds); A = Maltodextrin; B = Dextrose; AB = Combination of Maltodextrin and Dextrose Proportions

Based on the equation obtained above, the component that significantly influences the dissolving time is the interaction coefficient A because its value is positive (+) 16.9565. The higher the addition of maltodextrin in making instant powder, the more the powder's dissolution time increases. The coefficient B value has a negative value (-) 9.9703, which means that the ability of dextrose to increase the speed of dissolution time is not greater than maltodextrin. The AB coefficient value is positive (+) 0.109326, which states that the combination of maltodextrin and dextrose affects the dissolution time of instant powder. Result of component Mix between Maltodextrin and Dextrose on Soluble Time Test are shown in **Figure 3**.

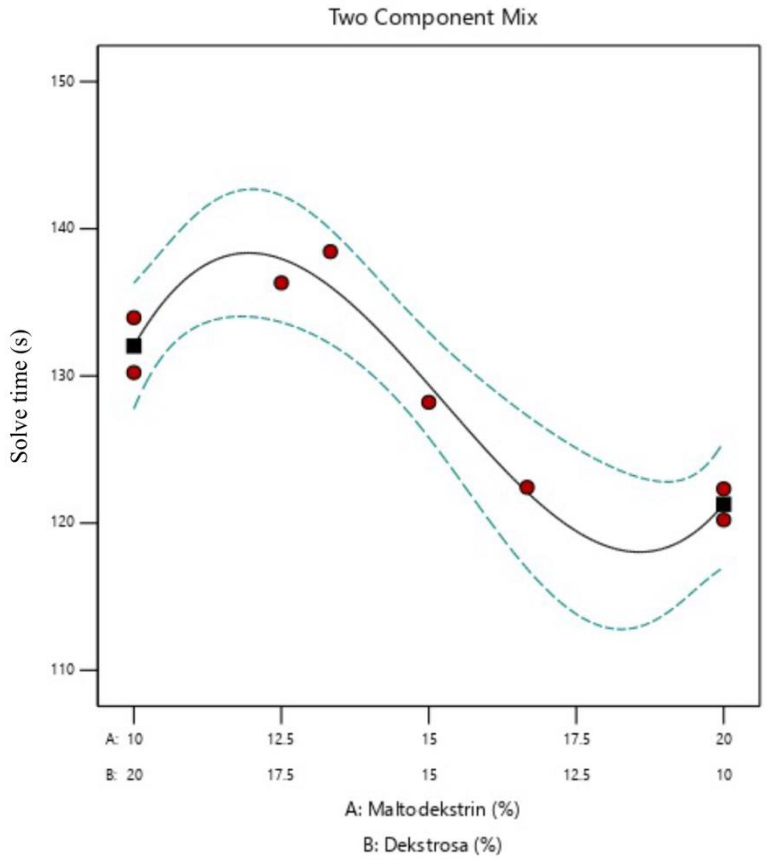

Figure 3. Two-component mix maltodextrin and dextrose on soluble time test

According to the results of a recent study, the addition of maltodextrin can have a significant impact on the dissolution time of instant powder drinks. As pointed out by Corie et al., 2023 the water content of a material is a crucial factor that influences the dissolution time. Instant powdered drinks with a higher water content take longer to dissolve due to the formation of lumps, which require more time to dissolve. This statement is further supported by the research conducted by Kaljannah et al. (2018), who found that noni-powder drinks with high water content take longer to dissolve than powders with low water content. Therefore, the water content of a material is a crucial factor that must be considered when determining the dissolution time of instant powder drinks.

3.1.3. D-optimal Mixture Design (DMD) Analysis

As per the findings of Adhayanti & Ahmad (2021), the most effective blend to predict outcomes involves a mixture of 18.690% Maltodextrin and 11.310% Dextrose, resulting in a desirable outcome score of 0.932. This formula is expected to maintain a water content of 4.892% and dissolve within 118.052 seconds. A desirability score closer to one indicates that the formula performs optimally according to the test variable. Graph of desirability can be seen in **Figure 4**.

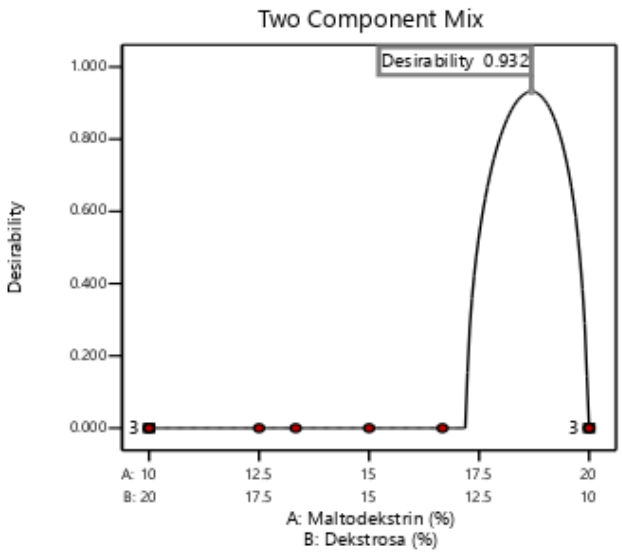

Figure 4. Optimal instant powder desirability graph

3.2. Proximate Analysis of Optimal Formula Instant Powder

The optimal formula of instant onchidiid slug powder was subjected to a proximate analysis test at the Food and Nutrition Center Study Laboratory of Universitas Gajahmada (UGM). The protein content in the powder was found to be 3.54%, which is relatively low compared to previous research on onchidiid slug simplicia powder, which had a protein content of 67.88% (Wijianto et al., 2022). The reduced protein content in the instant onchidiid slug powder is attributed to the cooking process of the filtrate of fresh ground onchidiid slug meat at a temperature of 90 ℃ for 10 minutes, which causes protein denaturation and amino acid damage. Research by Putri & Amrizal (2020) suggests that minimizing the drying process during powder production can help maintain higher protein levels. Widyanti et al. (2019) also found that protein levels in egg powder can be well maintained at 12.8-13.4% with low drying at 44 ℃. The results of the approximate test analysis based on the test parameters can be seen in **Table 3**.

Table 3. Results of proximate analysis

Sample	Result (%)					
	Water	**Ash**	**Fat**	**Protein**	**Carbohydrate**	**fiber**
Powder Instant	8,21	0,42	1,54	3,54	86,3	0,85

3.3. Hedonic Test (Level of Likeability)

The study conducted to evaluate the preference level of the onchidiid slug instant powder functional drink sample for various parameters such as color, aroma, taste, and texture has yielded significant results. The results indicate that the sample has a high preference level for each hedonic test parameter. Upon analyzing the results in detail, it can be concluded that the functional drink sample containing onchidiid slug instant powder, ginger, and lime has an excellent acceptance value and is suitable for consumption. The unique combination of these ingredients offers a distinctive flavor and texture that satisfies the taste buds and provides functional benefits. The results of the hedonic test can be seen in **Figure 5**.

The extent of the research is confined to developing and evaluating its efficacy as a functional food for wound healing support therapy. Additional tests are necessary to assess the stability of the preparation, followed by clinical trials on human subjects.

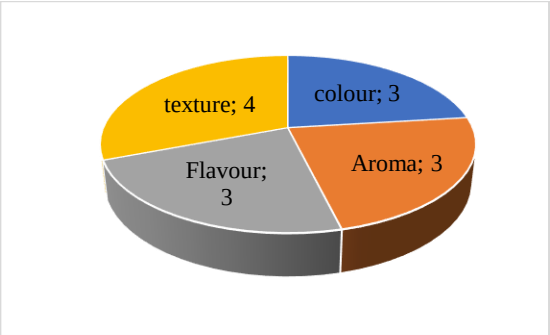

Figure 5. Graph of likeability level for hedonic test factors

4. CONCLUSION

The optimal DMD formula for instant powder is 18.690% maltodextrin and 11.310% dextrose with a water content of 4.892% and a dissolving time of 118.052 seconds. Onchidid slug instant powder contains 8.21% water, 0.42% ash, 0.85% crude fiber, 3.54% protein, 1.54% fat, and 86.3% carbohydrates. Optimization of maltodextrin and dextrose in the onchidiid slug instant powder formula can produce good characteristics of instant powder, with increased dissolution time and more exciting color. Hedonic test result Onchidid slug instant powder is shown as an attractive, functional drink with a great taste, texture, aroma, and color that is widely liked.

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6. AUTHOR DECLARATION

Authors' Contributions and Responsibilities

The authors made substantial contributions to the conception and design of the study. The authors took responsibility for data analysis, interpretation, and discussion of results. The authors read and approved the final manuscript.

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Availability of Data and Materials

All data are available from the authors.

Competing Interests

The authors declare no competing interest.

Additional Information

No additional information from the authors.

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COMPARISON OF FORECASTING DRUG NEEDS USING TIME SERIES METHODS IN HEALTHCARE FACILITIES: A SYSTEMATIC REVIEW

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ABSTRACT
Drug planning is essential to ensure the fulfillment of the right type, amount, and time criteria. Forecasting can be utilized during the planning stage to predict future drug needs. Perfect forecasting is impossible due to uncertainties in various factors, necessitating selecting the best method. This study aimed to identify the optimal forecasting method for healthcare facilities based on the smallest Mean Absolute Deviation (MAD), Mean Square Error (MSE), and Mean Absolute Percent Error (MAPE) values obtained from forecasting results using time series methods like Single Moving Average (SMA), Weight Moving Average (WMA), (Single Exponential Smoothing) SES, Double Exponential Smoothing (DES), and Triple Exponential Smoothing (TES). This research involved a descriptive observational study with retrospective data and adhered to PRISMA guidelines. PubMed, Google Scholar, and Garuda served as the data sources. Nine articles meeting the eligibility criteria were employed. The findings revealed that the SES, DES, and TES methods produced forecasts with MAPE values below 10%, indicating highly accurate forecasting. The MAPE values for the SMA and WMA methods were less than 50%, which is still acceptable. Therefore, the ES methods, particularly SES, are highly recommended for accurate drug planning. Forecasting accuracy factors include data stability, pattern consistency, and smoothing constants. The SES method emerged as the best forecasting method, generating the smallest MAD, MSE, and MAPE values compared to other methods, falling below 10%, reflecting highly accurate forecasting.

Keywords: Exponential smoothing; Forecasting; Healthcare facilities; Moving average

1. INTRODUCTION

The pharmaceutical service provider must ensure that the availability of pharmaceutical preparations, medical devices, and consumable medical materials are safe, of good quality, practical, and affordable. Planning drug needs and controlling drug supplies is a chain of drug governance that includes selection, planning of drug needs, procurement, receipt, distribution, destruction, withdrawal, administration, monitoring, and evaluation (Kemenkes, 2019). Drug planning is essential to fulfill the criteria of the right type, amount, and time (Laurensia et al., 2020). The planning stage can use forecasting to predict future drug needs. Forecasting is an attempt to predict future drug needs by considering and considering events or data from the past (Safitri et al., 2017).

Forecasting methods in healthcare facilities are critical, especially in analyzing drug management. Performing drug management using forecasting methods is essential in decision-

making, especially in estimating the number of drug needs in the future. In addition, it can avoid the occurrence of excess or shortage of drug stocks so that it can manage drug supplies more efficiently. Forecasting analysis is carried out using historical data on previous drug use so that it can project drug needs more accurately. Accurate forecasting can make better decisions regarding drug procurement to improve the quality of health services to patients (Aji et al., 2022). There are many forecasting methods, but the time series method is commonly used, which is based on a sequence of equally spaced data points in time (weekly, monthly, quarterly, and others) (Hernadewita et al., 2020). The time series method is a forecasting method using pattern analysis of the relationship between the variable to be estimated and the time variable. This method assumes that past data is a good indicator of the future. Commonly used time series methods are Single Moving Average (SMA), Weight Moving Average (WMA), Single Exponential Smoothing (SES), Double Exponential Smoothing (DES), and Triple Exponential Smoothing (TES). Some factors contain uncertainty, so it is impossible to do perfect forecasting, so it is necessary to find the best forecasting method. Various forecasting methods require criteria that can be used to compare and select models (Santiari & Rahayuda, 2021).

Model accuracy can be determined using several forecasting error measures, namely Mean Absolute Deviation (MAD), Mean Square Error (MSE), and Mean Absolute Percent Error (MAPE). Forecasting results are calculated using Excel to obtain error measures, namely MAD, MSE, and MAPE. A small error value means that forecasting with the method used has a small error compared to the actual data. The smaller the error value, the smaller the deviation between accurate data and forecasting (Satibi, 2014). MAD is a method to evaluate forecasting by measuring the forecast accuracy through the absolute value of each error in the same unit as the original series. MSE is an approximation forecasting method that manages significant forecasting errors because the errors are squared. MAPE is calculated using the absolute error in each period divided by the real observed value and then averaging the absolute percentage error. This approach is functional when the size of the forecast variable is essential in evaluating forecast accuracy. MAPE indicates how much the error in forecasting is compared to the actual value (Ginantra & Anandita, 2019). The interpretation of the MAPE value is <10%, including very accurate forecasting; 10-20%, including good forecasting; 20-50%, including reasonable forecasting; and >50%, including inaccurate forecasting (Fajrul et al., 2022). The novelty of this systematic review is that there is no need to discuss further the best forecasting method that can be used to determine drug planning in healthcare facilities. Therefore, this systematic review aims to determine the best forecasting method based on the smallest MAD, MSE, and MAPE values from forecasting results using time series methods, namely SMA, WMA, SES, DES, and TES.

2. METHODS

This research is a descriptive systematic review with retrospective data, and the guidelines used to review this article are the PRISMA guidelines. In September 2023, a systematic review was conducted on forecasting drug planning using the time series method in healthcare facilities.

2.1. Search Strategy

A search was conducted across three databases: PubMed, Google Scholar, and Garuda. The search employed the keywords "exponential smoothing", "forecasting," “hospital”, and "moving average". Boolean operators "AND" and "OR" were used to combine keywords strategically.

2.2. Selection Criteria

Articles were selected based on the following search criteria: Indonesian and English, published between 2014-2023, available in full-text, and health care facilities in Indonesia. After applying the search based on the established criteria, the retrieved articles were evaluated for valid and relevant studies that met the inclusion criteria. The inclusion criteria in this systematic review were: a) discussing forecasting of drug demand planning in various health care facilities in

Indonesia; b) using the time series method; c) there are indicators of forecasting accuracy; and d) quantitative research journals. Exclusion criteria in this systematic review are: a) discussing forecasting outside the health sector; b) the year of publication of the article is not listed in the research article; c) proceedings; and d) theses and theses.

2.3. Quality Assessment

The quality assessment in this systematic review was conducted using an evaluation tool provided by the Joanna Briggs Institute (JBI). The quality assessment included analysis of various aspects, including research methodology, clarity of research questions, selection of participants, research design, data analysis, and interpretation and conclusion of results. This evaluation process aims to ensure that the research methodology used in this journal meets the quality standards JBI sets so that the research findings are trustworthy, reliable, and relevant. The assessment result is 6/8, indicating that the research meets most of the quality standards JBI sets. This score indicates that of the eight aspects assessed, six have been well met, while the other two aspects still need to meet the set criteria fully.

2.4. Data Analysis

Articles satisfying the inclusion criteria, particularly those about forecasting drug needs in healthcare facilities, were further analyzed to align with the objectives of this systematic review. Employing the PRISMA application approach, the review eventually included nine articles.

3. RESULTS AND DISCUSSION

3.1. Selection Study

A search was performed across three databases: PubMed (yielding 114 articles), Google Scholar (yielding 353 articles), and Garuda (yielding 253 articles). Duplicate studies were removed during the initial screening. Subsequently, the remaining articles were assessed based on the inclusion criteria, resulting in 35 articles. Further screening of abstracts narrowed the selection down to fourteen (14) potentially relevant articles. Finally, nine (9) articles met all the eligibility criteria for inclusion in the systematic review. PRISMA Flowchart Diagram can be seen in Figure 1.

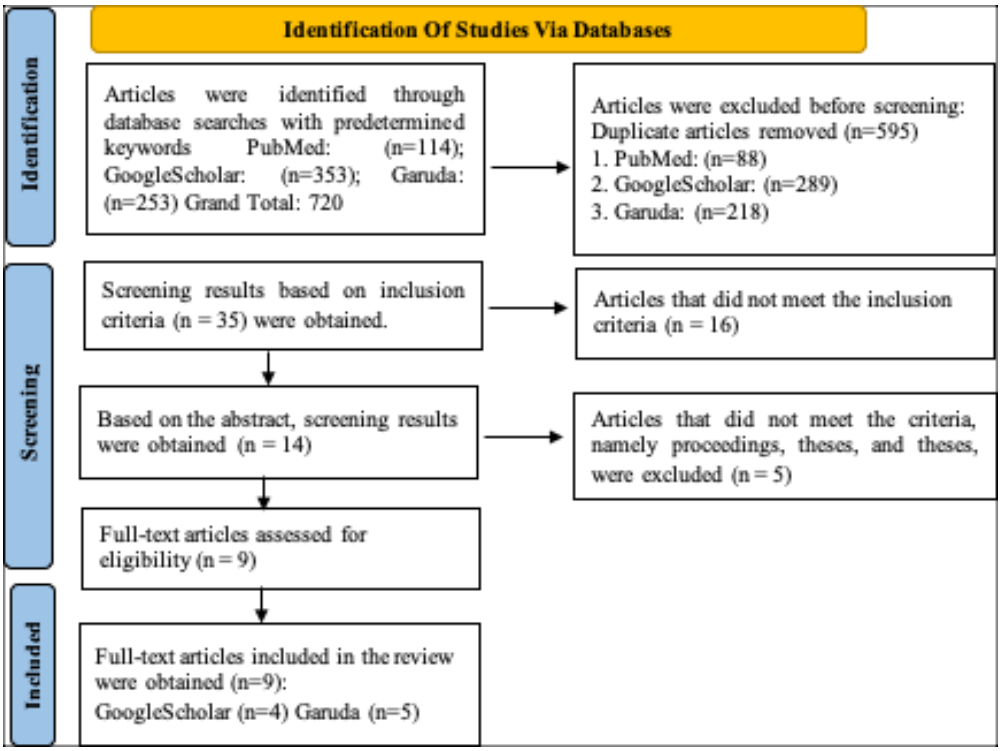

Figure 1. PRISMA flowchart diagram

3.2. Study Characteristics

Nine articles were identified in this study for quantitative assessment to determine the optimal forecasting method for drug demand planning in healthcare facilities. The publication years of the study used were between 2016-2022. The data used was retrospective data on previous drug usage. The study characteristics of each study can be seen in Table 1.

Table 1. Characteristics of forecasting methods

No	Author Name and Year	Place	Data Type	Data Period
1	(Sesario et al., 2022)	Pharmacy	Retrospective	September 2021-August 2022
2	(Sari et al., 2023)	Pharmacy	Retrospective	February 2020 -October 2022
3	(Aji et al., 2022)	Clinic	Retrospective	January 2020-March 2022
4	(Sabarina et al., 2021)	Hospital	Retrospective	Data for the 2018-2019 period
5	(Sophia et al., 2021)	Clinic	Retrospective	January 2018-December 2018
6	(Nangi et al., 2018)	Hospital	Retrospective	January 2016-December 2016
7	(Vimala & Nugroho, 2022)	Pharmacy	Retrospective	January 2019-December 2021
8	(Puspitasari et al., 2022)	Hospital	Retrospective	January 2018-December 2020
9	(Hendriani et al., 2016)	Community Health Center	Retrospective	October 2015-March 2016

3.3. Drug Planning Forecasting against Accuracy Indicators

The results of this systematic review are based on research at Sida Waras Farma Pharmacy and Simpang F Pharmacy; forecasting was carried out using the SES method, which resulted in highly accurate forecasting, namely a MAPE value of <10%. The MAPE value <10% is considered highly accurate forecasting because the lower the MAPE value produced, the less the forecasting error (Sari et al., 2023; Sesario et al., 2022). Sesario et al. (2022) conducted forecasting using the SES method with an alpha constant (α) of 0.1. At the same time, the forecasting conducted by Sari et al. (2023) used a constant alpha (α) of 0.22. The alpha constant value determined using a genetic algorithm (application) produces more accurate forecasting results than conventionally set values (Falani, 2018). The alpha constant is used as a smoothing parameter value or reduces the burden of forecasting error. The provisions for an excellent constant value for forecasting are $0 < \alpha < 1$ (Hudaningsih et al., 2020). The magnitude of the alpha value generated for each drug item does not indicate the forecasting quality but instead reflects the analyzed data pattern. α values close to 1 are used for random data, while α values close to zero are used for more stable data. A higher α value is given to more recent data so that the appropriate α parameter value will provide an optimal forecast with the smallest error value (Fachrurrazi, 2015).

Based on research at the Healthy Prayer Clinic by Aji et al. (2022), they conducted forecasting using the SMA, WMA, and ES methods. The SMA method aims to reduce random variations in demand over time and use the average value to forecast demand for the coming period (Satibi, 2014). The three forecasting methods used produce a MAPE value of <10%, which includes highly accurate forecasting. However, the ES method produces the smallest MAPE value of 0.90%, so it can be concluded that it is the best forecasting method (Aji et al., 2022). Research at Condong Catur Hospital by Sabrina et al. (2021) and Sofia Medika Clinic by Sophia et al. (2021) conducted forecasting using the DES method. DES is a forecaster whose data is not seasonal and patterned with up-and-down trends. The alpha constant used is 0.1. The forecasting results obtained are the MAPE value <10%, including highly accurate forecasting (Sabarina et al., 2021; Sophia et al., 2021). TES forecasting conducted by Nangi et al. (2018) at RSUD Kab. Muna and Apotek Mandiri Medika by Vimala and Nugroho (2022), with an alpha constant of 0.1, resulted in very accurate forecasting, namely the MAPE value <10%. The Triple Exponential Smoothing method is suitable for forecasting seasonal data with trends or fluctuating data (Kristianto et al., 2017). In addition, Vimala and Nugroho also conducted forecasting using the SES and DES methods. In this method, the MAPE values> 10% are 10.46% and 11.39%, which is included in good forecasting because they are in the range of 10-20% (Vimala & Nugroho, 2022). Forecasting drug demand planning using the SMA method at UGM Academic Hospital by

Puspitasari et al. (2022) resulted in a MAPE value < 50%, which ranged from 8-32%. MAPE values in the 20-50% range include reasonable forecasting. Based on research by Hendriani et al. (2016), drug planning forecasting is carried out using the Weighted Moving Average (WMA) method, resulting in a MAPE value = 15.298%, which is included in the excellent forecasting category because it is in the 10-20% range. Forecasting using the WMA method is intended for forecasting with horizontal or stationary data patterns that fluctuate around a constant and consistent average value over time. This method is used with a moving average model using several recent actual demand data to increase the value of demand forecasting in the future (Rizqi et al., 2021). Thus, the ES method, especially SES, produces the most accurate forecasting, as evidenced by the smallest MAPE value.

The effect of the SES method on the MAD value depends on the smoothing constant used. A smaller smoothing constant will give greater weight to the most recent data and make the forecast more responsive to recent changes in the data. This can lead to more significant fluctuations in the forecast and, in turn, increase the MAD value. If a larger smoothing constant is used, the weight given to the most recent data will be smaller, and the forecast will be smoother and less responsive to recent changes. This can reduce forecast fluctuations and result in a lower MAD value. The selection of an appropriate smoothing constant should be based on analysis of historical data and an understanding of the patterns and trends in the data. An optimal smoothing constant can result in a lower MAD value and more accurate forecasting, resulting in a small error value (Nu et al., 2024). The effect of the SES method on the MSE (Mean Squared Error) value is that the smaller the α value, the slower the method reacts to trends and fluctuations in historical data. Thus, if the α value is meager, the SES method gives almost equal weight to all historical data, which can lead to forecasting that is less responsive to actual changes in the data. If the α value is very high, the method will give a higher weight to the most recent data, so the forecast will react more quickly to trend changes or new fluctuations. However, using too high a value of α can also lead to instability in forecasting and overreaction to random fluctuations. Thus, if the resulting forecast is very close to the actual value, the MSE value will be lower, indicating better forecasting quality (Box et al., 2015). Forecasting interpretation results based on accuracy indicators can be seen in Table 2.

3.4. Advantages and Disadvantages of Forecasting Methods

The Single Moving Average (SMA) method is a forecast of future periods with historical data over a certain period. SMA is a forecasting method that is done by taking a group of observation values and finding the average value as a forecast for the coming period. The Weight Moving Average (WMA) method is a weighted moving average method that predicts by giving weight to the data of the previous n periods and then dividing it by the number of weights. The WMA method gives greater weight to the most recent data, making it more responsive to changes in recent drug demand trends (Aji et al., 2022). The Single Exponential Smoothing (SES) method is short-term forecasting with the assumption that the data fluctuates near a fixed mean value without trend data and fixed growth patterns. The SES method is a development of the simple moving average model. In the exponential smoothing method, the value of 1/n is replaced with α (Sesario et al., 2022). In this method, forecasting is done by repeating the calculation continuously using the latest data. Each data is given a weight, newer data is given a greater weight. This method is more suitable for forecasting things that fluctuate randomly or irregularly (Yuniarti, 2021). The Double Exponential Smoothing (DES) method is forecasting in the presence of a trend like simple smoothing except that two components must be updated each period level and trend. Level is a smoothed estimate of the data value at the end of each period. The trend is a smoothed estimate of the average growth at the end of each period (Sophia et al., 2021). The Triple Exponential Smoothing (TES) method is forecasting that uses trend and seasonal data forms that can be performed simultaneously (Vimala & Nugroho, 2022). Based on the review of these

results, the advantages and disadvantages of each forecasting method used in health facilities can be seen in Table 3.

Table 2. Forecasting interpretation results based on accuracy indicators

No	Author Name and Year	Methods	Accuracy Indicator			Interpretation
			MAD	MSE	MAPE	
1	(Sesario et al., 2022)	*SES*	21,4	710,4	7%	Highly accurate forecasting
2	(Sari et al., 2023)	*SES*	-	-	4.61%	Highly accurate forecasting
3	(Aji et al., 2022)	*SMA*	1.040	1.749.587	2.63%	Highly accurate forecasting. The ES method produces the smallest MAPE value so that the forecasting performed is the most accurate.
		WMA	1.028	1.628.449	2.27%	
		ES	883	1.341.137	0.90%	
4	(Sabarina et al., 2021)	*DES*	-	-	9.89%	Highly accurate forecasting
5	(Sophia et al., 2021)	*DES*	221.0	324.84	7.26%	Highly accurate forecasting
6	(Nangi et al., 2018)	*TES*	-	48.21	4.25%	Highly accurate forecasting
7	(Vimala & Nugroho, 2022)	*SES*	-	109.58	10.46%	Good Forecasting
		DES	-	129.82	11.39%	
		TES	-	91.84	9.58%	Highly accurate forecasting
8	(Puspitasari et al., 2022)	*SMA*	-	-	<50% which is between 8-32%.	Reasonable Forecasting
9	(Hendriani et al., 2016)	*WMA*	-	-	15.29%	Good Forecasting

**Single Moving Average* (SMA), *Weight Moving Average* (WMA), *Single Exponential Smoothing* (SES), *Double Exponential Smoothing* (DES), *Triple Exponential Smoothing* (TES), *Mean Absolute Deviation* (MAD), *Mean Square Error* (MSE), and *Mean Absolute Percent Error* (MAPE).

Table 3. Advantages and disadvantages of forecasting methods

No	Methods	Advantages	Disadvantages
1	*SES*	Short-term forecasting, usually only one month ahead, considers the weight of previous data by assigning weights to each data period to distinguish priorities-better handling of outliers, more flexible parameters, and better data smoothing mechanisms (Lusiana & Yuliarty, 2020).	Not capable of long-term forecasting as this method is more suitable for short- or medium-term forecasting. This method tends to be less accurate for long-term forecasting, especially if there is a solid long-term trend (Lusiana & Yuliarty, 2020).
2	*DES*	This method can be used to solve trend-patterned data. Uses relatively little data. Fewer parameters and more accessible data management (no data transformation is required if the data is non-stationary and no autoregression analysis is used) in forecasting (Junita & Primandari, 2023).	Periodic maintenance and regular checks must be carried out by checking whether the data entered is correct. To get an accurate stock forecast, you must have a lot of sales data per month (Setyawan et al., 2021).
3	*TES*	It can use relatively little data when compared to other methods. The linear exponential and seasonal smoothing method is used for trend-and seasonal-patterned data. This method uses three parameters: α, β, and γ (Febriyanti & Rifai, 2022).	Dependence on historical data, i.e., if historical data varies significantly or suddenly changes, this method does not produce good forecasts. It is difficult for irregular or varying seasonal periods, so this method must be more accurate (Febriyanti & Rifai, 2022).
4	*SMA*	It is easy to calculate and simple. Each year's data is given equal weight, which means that both the earlier and more recent data are considered equal in their influence. This method is effective, simple and efficient (Dewi & Chamid, 2019).	This method requires a lot of historical data. It cannot follow drastic changes and is unsuitable for forecasting data with trend symptoms because the resulting forecast will be too late to follow the changes. Last year's data, which should be too small or too large, is just averaged to forecast the coming year (Dewi & Chamid, 2019).
5	*WMA*	Forecasting is more accurate because the data used is relevant and given a high weight. Each historical data set is given a different weight, and the latest data sets are given a higher weight, so forecasting will be more accurate (Sudarthio et al., 2020).	It requires historical data, needs to catch up with drastic changes, and is appropriate when forecasting data with a trend (Eris et al., 2014).

*SMA (*Single Moving Average*), WMA (*Weight Moving Average*), SES (*Single Exponential Smoothing*), DES (*Double Exponential Smoothing*), TES (*Triple Exponential Smoothing*)

3.5. Relationship between Forecasting and Drug Planning

Forecasting is an essential tool in effective and efficient planning. A forecast is a prediction of what will happen in the future, while a plan is a determination of what will be done. There is a difference between a forecast and a plan. Forecasting is forecasting what will happen but is not necessarily implemented by the company (Saepulloh & Handoko, 2018). Forecasting shows an estimate of what will occur in a particular situation. In contrast, planning uses the forecast to help decision-makers choose the best alternative. In other words, forecasting is an approach to quantitatively estimating what will happen in the future based on related and relevant historical data. At the same time, planning is an effort by decision-makers to influence the results that will occur through various strategies based on actual information obtained from the past (Ahmad, 2020). Forecasting is essential in all aspects of business, but forecasting is only an estimate of demand until actual demand becomes known. Forecasting drives decisions in many areas. Demand forecasting will impact three activities: supply chain management, human resources, and capacity (Heizer & Render, 2016).

Based on the results of the systematic review, the forecasting method that produces highly accurate forecasts is the Single Exponential Smoothing (SES) method. Planning drug needs in healthcare facilities can be done using the Single Exponential Smoothing forecasting method because, in this method, short-term forecasting is carried out with data properties that fluctuate near a fixed mean value without trends and seasonality (Yuniarti, 2021). The SES method is a procedure that continuously improves estimates by averaging the past values of data in a decreasing (exponential) manner. This forecasting method is suitable for forecasting things that have random (irregular) fluctuations. Thus, this SES method can help plan and manage drugs in each health service facility to predict the amount of drugs needed in the future (Gustriansyah, 2017).

3.6. Factors that Affect Forecasting

Based on research conducted by Sesario et al. (2022) and Sari et al. (2023), they have resulted in highly accurate forecasting with a MAPE value of <10%. Some factors affect the accuracy of Single Exponential Smoothing forecasting with MAPE <10%, namely data stability, pattern consistency, and alpha (α) weighting strength. Forecasting tends to give accurate results if the historical data is relatively stable and has no significant fluctuations. If the historical data pattern shows a constant trend or regular seasonal pattern, then the Exponential Smoothing method can produce accurate forecasts (Romaita et al., 2019). The factors that cause forecasting to fall into a reasonable category, namely those carried out by Vimala and Nugorho (2022), are complex patterns, unexpected trends or patterns, unexpected seasonal changes, and the influence of extreme data. The resulting reasonable forecasting is with a MAPE value of 10.46% (Vimala & Nugroho, 2022). Meanwhile, the SMA method must effectively handle more complex data patterns, such as emerging trends or significant seasonal fluctuations. If the historical data has a clear trend or seasonal pattern, this method cannot capture it accurately, which can lead to a slightly higher MAPE value. If the historical data has irregular or unstable patterns, SMA forecasting cannot accurately process the data against changes. If the historical data is irregular and tends to have fluctuations or unexpected changes in the pattern, the SMA method cannot keep up well with the changes (Montgomery et al., 2015). Writing a systematic review has limitations. The literature on forecasting in each health service facility is minimal, so not much can be included in this systematic review in both Indonesian and English journals.

4. CONCLUSION

A systematic review based on the discussion that has been made regarding the comparison of forecasting drug needs using time series methods in healthcare facilities can conclude that the best forecasting method is the exponential smoothing method, especially single exponential smoothing. The MAD, MSE, and MAPE values of the Exponential Smoothing method produce

the smallest values when compared to other methods. The MAPE value in the Single Exponential Smoothing method is <10%, which is included in the highly accurate forecasting category. Furthermore, it is recommended that research be conducted in healthcare facilities related to forecasting drug demand planning using the Single Exponential Smoothing method.

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Authors' Contributions and Responsibilities

The authors made substantial contributions to the conception and design of the study. The authors took responsibility for data analysis, interpretation, and discussion of results. The authors read and approved the final manuscript.

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Competing Interests

The authors declare no competing interest.

Additional Information

No additional information from the authors.

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STUDENT OF PHARMACY, NURSE, PUBLIC HEALTH, NUTRITIONIST AND PHYSICAL EDUCATION READINESS TOWARD INTERPROFESSIONAL EDUCATION (IPE)

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ABSTRACT
The implementation of interprofessional education (IPE) into higher education curricula is a significant method for creating professionals with the skills necessary for interprofessional collaboration (IPC), including those in the health sciences, nursing, pharmacy, and nutrition. Due to the changes in health services, which are becoming more integrated, it is vital to have the capacity for interprofessional collaboration. The readiness of the pupils for IPE must be assessed before to its implementation in order to identify the subjects that should be highlighted at that time. This research is a cross-sectional study using descriptive analysis method. The distribution of respondents was as all in 4th semester students, consist of department of Pharmacy (n=92), department of Nursing (n=88), department of Public Health (n=95), department of Nutrition (n=66) and department of Physical Education (n=42) in Faculty of Health Sciences. Questionnaire data collection was carried out online. The independent variable that is measured is the student's readiness for Interprofessional Education. The results findings there is a significant difference between the readiness of students in Nurse department and students of all department. The nurse department received the highest score (score = 68.47) and the Health Education received the lowest score (score = 65.59). But all department receive score > 80% of the total score. There is still room for improvement in the areas related to the value of learning together in practical situations.

Keywords: Readiness pharmacy; Health sciences; Interprofesional education

1. INTRODUCTION

Interprofessional collaboration practice (IPC) in health-care happen when multiple health-care professionals from various professional backgrounds deliver comprehensive services by collaborating with patients, their families, careers, and communities to provide the highest quality of care throughout settings (WHO, 2010). The establishment of interprofessional partnerships between health practitioners and students, patients, families, and communities with the ultimate goal of attaining improved health outcomes is what is known as interprofessional collaboration (The Canadian Interprofessional Health Collaborative, 2010). Compared to a team of a single discipline, a multidisciplinary healthcare team can give better treatment outcomes and higher-quality care. The members of an interprofessional team each bring their special role in order to improve the safety and quality of life for their patients. According to the World Health Organization (WHO), Interdisciplinary collaborative practice in healthcare settings, increases patient management frameworks (Maharajan et al., 2017).

Interprofessional Collaboration bring a lot of potential benefits. Therefore, IPC needs to be implemented in delivering services to the patients. Health workers need to have the skills or ability

to collaborate with other health workers, or work as a team with other health worker professions (WHO, 2010). To gain this collaboration skills, health workers need to implement Interprofessional Education (IPE). IPE (Interprofessional Education) is the key factor for achieving successful teamwork in healthcare settings (Bridges et al., 2011; WHO, 2010).

The World Health Organization (WHO) defines interprofessional education (IPE) as the process by which two or more healthcare professionals learn from, with, and about one another in order to enhance collaboration and the standard of care. WHO also promotes a viable strategy for creating a workforce that is prepared for workplace collaboration (WHO, 2010). Interprofessional Education (IPE) is a concept that emphasizes the growth of effective teamwork and communication across all healthcare professionals in order to deliver high-quality patient care (Alruwaili et al., 2020; Gilbert et al., 2010). IPE enables students to become familiar with the primary functions in their particular profession as well as the roles of their team members in related fields of employment (Maharajan et al., 2017).

Several studies concluded that IPE plays a significant role in creating an effective collaborating environment in a health-care setting (Guraya & Barr, 2018; O'Donoghue & Cusack, 2012), leading to a strong recommendation to include IPE as an integral part of the curriculum of undergraduate medical and health-related professions (Gilbert et al., 2010). Several schools have incorporated IPE standards into their medical curriculum (Alruwaili et al., 2020). The major objective of interprofessional education (IPE), which combines students from at least two distinct health care professions, is to improve patient outcomes by fostering collaborative practice and communication skills (Gilbert et al., 2010; Huebner et al., 2020). Researchers have discovered that IPE at the prelicensure stage improves patient satisfaction, fosters teamwork, and lowers clinical error (Nelson et al., 2017; Reeves et al., 2013). IPE is one of the ten recommendations for future education in the professions and is anticipated to be the primary element affecting education in the health care and medical areas in the twenty-first century (The Canadian Interprofessional Health Collaborative, 2010; Yune et al., 2020).

IPE outcomes are more likely to be favorable when students have positive views regarding it. A thorough understanding of the students' readiness for future interprofessional collaboration can be gained by evaluating their perspectives of IPE (Alruwaili et al., 2020). It is vital to investigate the variations in students' interpretations of IPE in order to establish and administer IPE programs in different major as medicine, nursing, and pharmacy. IPE difficulties may arise from these variations. In order to better construct on IPE program, it is crucial to investigate how they view IPE (Yune et al., 2020). In the health education, IPE is commonly implemented to medical doctor and nurse students. But in the health care services, patients receive services not only from doctors and nurses, but also from other health workers, such as pharmacists, public health, physiotherapy, etc (D'Costa et al., 2022; WHO, 2010). Therefore, IPE also should be carried out in in this health profession education and assessing student readiness is the first step to initiate IPE. Jenderal Soedirman University has four department in health profession, consist of department of Pharmacy, Nurse, Public Health, Nutritionist, and Health Education in one faculty as Faculty of Health Sciences. As having those department in one faculty, allows their students to interact from the beginning of academic activities, thus will provide different readiness from health professional students from separate faculties. assessing its readiness for IPE will give important finding about students' readiness and wether they are differences of student's readiness based on each department. This study's objectives were to explore and to know which department has the most readiness for IPE.

2. METHODS

This research is a cross-sectional study using descriptive analysis method. In this study, the respondents were students in departments of Pharmacy, Nursing, Public Health, Nutrition and Physical Education, Faculty of Health Sciences (FIKES). The respondents used were all in 4th

semester students with a total of 383 student respondents. The distribution of respondents was as follows, department of Pharmacy (n=92), department of Nursing (n=88), department of Public Health (n=95), department of Nutrition (n=66) and department of Physical Education (n=42). Questionnaire data collection was carried out online. The independent variable that is measured is the student's readiness for Interprofessional Education and the dependent variable is the student's department. This research has received Ethical Clearance from KEPK FIKES Unsoed, No: 567/EC/KEPK/XI/2021.

Data collection on student readiness for Interprofessional Education was carried out using the Readiness for Interprofessional Learning Scale (RIPLS) questionnaire by (Tyastuti et al., 2014). Prior to use, the questionnaire's reliability and validity were assessed using the Alpha Cronbach test and Pearson correlation test, respectively. The score for each question item obtained from the questionnaire were then analyzed descriptively. The difference in student readiness for IPE between majors was assessed using the Kruskal Wallis test on the overall score. The greater the total score obtained on each questionnaire, the better the student's readiness for IPE.

3. RESULTS AND DISCUSSION

This research was conducted to get an overview of the readiness for IPE of FIKES students, as well as whether there are differences in these variables based on each department. This information is used to be able to build IPE materials that are more targeted to student needs. All of the RIPLS questionnaire's questions were valid, according to the findings of the Pearson Correlation validity test (p 0.001), and the Cronbach's Alpha reliability test also supported this finding (Cronbach's Alpha value of 0.898). **Table 1** displays the results of the average score for each question item. Students from different departments scored differently on the RIPLS questionnaire, according to the results of the overall score.

The highest scores were in the nursing department (score = 68.47), then pharmacy (score = 66.61). All majors scored > 80% of the total score. The greater the total score obtained; the more prepared students are for IPE. This shows that all students have good readiness for IPE. The score results for each question item show that the majority of students are ready for IPE by giving a score of 4 out of 5. Some of the questions that still score below 4 are questions about about collaboration learning activities to achieve IPE (item number 10, 11, and 12). In the other hand, learning activity that is carried out collaboratively with other professions, mutual interaction between students from different professions is the essence of IPE (International Pharmaceutical Federation (FIP), 2015; WHO, 2010). Therefore, learning activities are carried out collaboratively the same as students from other professions is the main requirement of IPE.

Based on these results, students need to emphasize the importance of collaboratively learning, communication and collaboration (The Canadian Interprofessional Health Collaborative, 2010) as well as designing learning methods that make students actively involved in collaborating and communicating directly between students and other department. This can be accomplished by giving students exposure to different professions that are directly involved in teaching and learning activities, in addition to learning. Students are intended to feel as though they are genuinely interacting and working with students from other professions through this direct exposure. This hands-on engagement and teamwork experience can come in handy when collaborating with other professions in the real world.

Table 2 shows the outcomes of the Kruskal Wallis test. Based on the overall result, this test show whether one department is more prepared than another. According to the test's results, there is a considerable gap between the readiness of students in the nursing department and that of the other departments. The Nurse department outperformed all other departments, receiving the highest score (score=68.47). With a score of 65.59, the Department of Health Education received the lowest rating.

Table 1. Student readiness for IPE based on department

Readiness for Interprofessional Learning Scale items	Skor / Department				
	Pharmacy	Nurse	Public Health	Nutritionist	Health Education
Learning with other students / professionals will make me a more effective member of a health and social care team	4.32	4.3	4.2	4	4.12
Patients would ultimately benefit if health and social care students / professionals work together	4.68	4.72	4.54	4.56	4.54
Shared learning with other health and social care students / professionals will increase my ability to understand clinical problems	4.51	4.48	4.41	4.3	4.44
Communications skills should be learned with other health and social care students / professionals	4.27	4.41	4.16	4.06	4.27
Team-working skills are vital for all health and social care students / professionals to learn	4.48	4.5	4.35	4.35	4.44
Shared learning will help me to understand my own professional limitations	4.34	4.36	4.29	4.21	4.24
Learning between health and social care students before qualification and for professionals after qualification will improve working relationships after qualification / collaborative practice.	4.4	4.43	4.29	4.15	4.29
Shared learning will help me think positively about other health and social care professionals	4.33	4.41	4.28	4.15	4.44
For small-groups who are learning to work, students / professionals need to respect and trust each other	4.62	4.65	4.54	4.42	4.51
I don't want to waste time learning with other health and social care students / professionals	3.02	3.31	3.37	4	2.73
It is not necessary for undergraduate / postgraduate health and social care students / professionals to learn together	3.37	3.86	3.85	3.98	3.46
Clinical problem solving can only be learnt effectively with students / professionals from my own school / organisation	3.1	3.6	3.51	3.73	2.93
Shared learning with other health and social care professionals will help me to communicate better with patients and other professionals	4.32	4.42	4.23	4.14	4.37
I would welcome the opportunity to work on small group projects with other health and social care students / professionals	4.29	4.36	4.16	4.05	4.32
I would welcome the opportunity to share some generic lectures, tutorials or workshops with other health and social care students / professionals	4.26	4.35	4.16	4	4.22
Shared learning and practice will help me clarify the nature of patients' or clients' problems	4.3	4.31	4.13	4.03	4.27
Total Score	66.61	68.47	66.47	66.13	65.59

*Note: The RIPLS questionnaire uses Likert scale of 1-5; Unfavorable question scores (numbers 10, 11 and 12) have been adjusted

Table 2. Student readiness significance value based on between department

No.	Department	Significance Value
1.	Health Education-Nutritionist	0.415
2.	Health Education-Public Health	0.336
3.	Health Education-Pharmacy	0.296
4.	Health Education-Nurse	0.004*
5.	Nutritionist-Public Health	0.911
6.	Nutritionist-Pharmacy	0.832
7.	Nutritionist-Nurse	0.017*
8.	Public Health-Pharmacy	0.912
9.	Public Health-Nurse	0.012*
10.	Pharmacy-Nurse	0.017*

*Level of significance: 0.05.

In comparison to others department, the Nurse department is the readiest for IPE, according to this score. This is due to the fact that the nursing department included the idea of interprofessional collaboration in one of its lectures, making it more well known than in any other department. The other department does not yet offer any lectures on working with other professions (Fikes, 2021). Therefore, it can be hypothesized that students' reactions to IPE teaching and learning activities varied during the actual implementation of IPE. However, the departments of pharmacy, public health, nutrition, and health education also achieved scores that were more than 80% of the possible total. This demonstrates the high level of departmental readiness for group learning. Rasmita conducted research on the readiness of Nurse, Public Health and Pharmacy students and reported that 80% of the respondents were ready for IPE (Rasmita et al., 2018). Comparable findings were also made by Febriana, who stated that 85% of nursing students were prepared (Febriana, 2019). Moreover, Mobalen, 2021 reported that students specializing in nursing, nutrition, and midwifery also received high score on the IPE readiness test (Mobalen et al., 2021). This finding describes the readiness of students in forth semester or in the second year only and cannot be generally use to describe the readiness from first year student or third year student. Their readiness maybe different because differences in understanding about profession and collaboration due to amout of knowledge that has been studied along the length of study. Also, asessing readiness in first year and third year students will give wider understanding about readiness toward IPE.

4. CONCLUSION

According to the outcomes, in comparison to others department, the Nurse department is the readiest department for IPE, nevertheless, every department in FIKES was ready for the IPE. As a result, implementing IPE lectures or programs will be easier. Based on these findings, topics about communication, collaboration and the the importance of collaboratively learning among the students are several topics recommended to be emphasized more when implementing IPE program for all department.

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6. AUTHOR DECLARATION

Authors' Contributions and Responsibilities

The authors made substantial contributions to the conception and design of the study. The authors took responsibility for data analysis, interpretation, and discussion of results. The authors read and approved the final manuscript.

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Availability of Data and Materials

All data are available from the authors.

Competing Interests

The authors declare no competing interest.

Additional Information

No additional information from the authors.

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MOLECULAR DOCKING STUDIES OF FLAVONOIDS FROM SECANG WOOD (*Caesalpinia sappan* L.) AGAINST GLUCOKINASE ENZYME AS ANTIDIABETIC CANDIDATES

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ABSTRACT

Diabetes mellitus (DM) is a metabolic disease caused by a deficiency of insulin secretion, insulin resistance, and increased hepatic glucose production. Secang wood (*Caesalpinia sappan* L.) is known to have antihyperglycemic activity. However, these compounds are not yet known. In silico studies are needed to determine the compounds that act as antidiabetics. This study performed molecular docking of flavonoid compounds in sappan wood against the 1V4S glucokinase receptor. The results showed that all flavonoid compounds of sappan wood were predicted to have antidiabetic activity because they had a lower docking score than metformin, the first-line therapy of type 2 diabetes mellitus. Butein is expected to have the best activity. It has the lowest docking score (-94.4836). Visualization of the docking results shows that butein interacts with the identical amino acid residues as metformin, namely ARG 63 and THR 65, through the formation of hydrogen bonds and Van der Waals interactions. SWISS-ADME web tool predicted that butein has good oral absorption and excretion. The toxicity prediction tool showed a slight contradiction in the mutagenic effect. Based on this research, molecular docking may be able to design new drugs, especially from butein in sappan wood (*Caesalpinia sappan* L.), as antidiabetic candidates.

Keywords: Diabetes mellitus; Caesalpinia sappan; Glucokinase; Molecular docking; Flavonoid

1. INTRODUCTION

Diabetes mellitus (DM) is a metabolic disease characterized by an increase in blood glucose levels (≥ 200 mg/dL) (Soelistijo et al., 2019). Diabetes mellitus can be caused by a deficiency of insulin secretion, insulin resistance, and increased hepatic glucose production, which can cause abnormalities in the carbohydrate, lipid, and protein metabolism system. Total or partial insulin deficiency during diabetes can impair carbohydrate metabolism resulting in inadequate glucose utilization and increased hepatic glucose production (Mahendran et al., 2014). According to the International Diabetes Federation (IDF), patients with DM in Indonesia are seventh-ranked worldwide. They are predicted to increase from 10.7 million in 2019 to 16.6 million in 2045 (IDF, 2019). From these data, it can be seen that DM is a problem that must be addressed immediately. One of them is developing new drugs from natural substances that are efficient in treating diabetes and have few adverse effects.

Secang (*Caesalpinia sappan* L.) is known to have activity in reducing blood sugar levels. Secang wood infusion with concentrations of 10% w/v, 15% w/v, and 20% w/v reduced blood sugar levels in male mice (*Mus musculus*) (Yusuf & Wati, 2019). The secang drink is reported to have an antihyperglycemic effect in adult women with prediabetes. Secang plant boiled with water can reduce fasting blood glucose levels by 14.36 ± 19.19 mg/dL (Sa'pang, 2015). In

addition, the isolation of secang wood extract is known to have antihyperglycemic activity by inhibiting alpha-glucosidase and alpha-amylase enzymes (Arsiningtyas, 2015).

Secang wood (*Caesalpinia sappan* L.) has flavonoid compounds with antidiabetic activity (Al-Ishaq et al., 2019). Flavonoid compounds contained in the secang wood are brazilin, brazilein, brazilide a, butein, (E)-3-(3,4-dihydroxybenzylidene)-7-hydroxychroman-4-one, 3-deoxysappanon b, protosappanin a, protosappanin b, protosappanin c, protosappanin d, protosappanin e, sappanchalcone, sappanone b, 3,8,9-trihydroxy-6H-benzo[c]chromen-6-one, and 3-deoxysappanchalcone (Nirmal et al., 2015).

Molecular docking is a computational method that can be used as the basis for drug discovery (Setiawan & Irawan, 2017). This method can predict the potential compound of the antidiabetic candidate. Molecular docking studies of flavonoid compounds from *Justica gendarussa* Burm.f. identified an antidiabetic activity by forming hydrogen bonds with glucokinase enzymes (Adelina, 2020). There is no information regarding the interaction of flavonoid compounds from the secang wood with the glucokinase enzyme (1V4S), so it is necessary to conduct an in silico study to determine the docking score and the interactions that occur.

2. METHODS

2.1. Tools and Materials

The docking molecule in this research used a laptop ASUS (X41C series) with specification Intel(R) Core(TM) i3-3217U CPU @1.8GHz processor, 2GB RAM, and Windows 10 Pro 64-bit operating system. The docking analysis used PLANTS (Protein-Ligand ANT-System) (http://www.tcd.uni-konstanz.de), YASARA (Yet Another Scientific Artificial Reality Application) (http://www.yasara.org), MarvinSketch (https://chemaxon.com), and Discovery Studio Visualizer (https://discover.3ds.com).

This research used the crystal structure of the human glucokinase enzyme (PDB CODE: 1V4S) as the target protein, which was downloaded from Protein Data Bank (PDB) (https://www.rcsb.org). Flavonoid compounds from secang wood (*Caesalpinia sappan* L.) and metformin as an antidiabetic drug were downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov). A total of fifteen compounds contained in secang wood were selected in this study, including Brazilin, Brazilein, Brazilide A, Butein, (E)-3-(3,4-dihydroxybenzylidene)-7-hydroxychroman-4-one, 3-Deoxysappanone B, Protosappanin A, Protosappanin B, Protosappanin C, Protosappanin D, Protosappanin E, Sappanchalcone, Sappanone B, 3,8,9-trihydroxy-6H-benzo[c]chromen-6-one, and 3-Deoxysappanchalcone.

2.2. Lipinski's Rule of Five Tests

The Lipinski rule of five tests was conducted on http://www.scfbio-iitd.res.in to determine the physicochemical properties such as molecular weight, Log P, Hydrogen Bond Donor, Hydrogen Bond Acceptor, and Molar Refractivity of flavonoid compounds from secang wood (*Caesalpinia sappan* L.).

2.3. Protein and ref_ligand Preparation

The glucokinase enzyme was prepared by downloading the protein structure from the Protein Data Bank (PDB CODE: 1V4S). Enzyme separation, with native ligands and other molecules, and the addition of hydrogen molecules were conducted using YASARA. The separated protein and native ligand structures were stored in the file. mol2.

2.4. Ligands Preparation

The ligand preparation was done by downloading the flavonoid compounds from the sappan wood (*Caesalpinia sappan* L.) as the test ligand and metformin as the comparison ligand from PubChem. The conformational search was performed using MarvinSketch and then saved as the file. mol2.

2.5. Docking Protocol Validation

The docking protocol was validated by redocking the native ligand of the glucokinase enzyme (1V4S) using YASARA to obtain the Root Mean Square Deviation (RMSD) value.

2.6. Molecular Docking

Molecular docking was carried out by docking the test ligand (flavonoid compounds from secang) and the comparative ligand (metformin) with the glucokinase enzyme (1V4S) using PLANTS through the Command Prompt (CMD) to obtain docking scores.

2.7. Docking Result Visualization

The docking results of each ligand are visualized using Discovery Studio Visualizer to see the interactions that occur.

2.8. ADME Prediction

ADME (Adsorption, Distribution, Metabolism, and Excretion) of the compound was predicted using the SWISS-ADME web tool predictor (http://www.swissadme.ch) by its canonical SMILES structure from PubChem (https://pubchem.ncbi.nlm.nih.gov).

2.9. Toxicity Prediction

Toxicity prediction of the compound was performed using Toxtree v3.1.0 software (https://toxtree.sourceforge.net/), OSIRIS tool (https://www.organic-chemistry.org/prog/peo/), and pkCSM web tool (https://biosig.lab.uq.edu.au/pkcsm/prediction) by inserting the canonical SMILES structure from PubChem (https://pubchem.ncbi.nlm.nih.gov).

3. RESULTS AND DISCUSSION

3.1. Lipinski Rule of Five

Lipinski's rule of five (Ro5) analyzes drug-like compounds or drug-likeness by looking at a ligand's physical and chemical properties (Chen et al., 2020). Parameters or criteria in the Lipinski rule of five (Ro5) can be the basis that a drug-like compound has good absorption and permeability. Based on **Table 1**, the physicochemical properties of the Secang flavonoid compounds, except protosappanin D and protosappanin E, correspond to the Ro5 rule. It can be concluded that the compounds have similarities to drugs and can be used as test ligands for molecular docking processes.

Table 1. Lipinski rule of five test results for flavonoid compounds

Ligand	Lipinski Rule Of Five Parameters				
	MW	Log P	HBD	HBA	MR
MRK (2-Amino-4-Fluoro-5-[(1-Methyl-1H-Imidazol-2-Yl)Sulfanyl]-N-(1,3-Thiazol-2-Yl)Benzamide)	349	3.001400	3	5	88.210594
Metformin	129	-1.243830	5	5	37.223495
Brazilin	286	1.382200	4	5	72.937180
Brazilein	284	0.958900	3	5	72.594383
Brazilide A	318	0.374400	2	7	72.789581
Butein	272	2.405099	4	5	72.907677
Protosappanin A	272	1.974399	3	5	71.078377
Protosappanin B	304	1.128700	5	6	78.106972
Protosappanin C	302	1.335300	4	6	77.085175
Protosappanin D	604	3.532302	8	12	151.678467
Protosappanin E	586	3.024500	7	11	146.634644
Sappanchalcone	286	2.708099	3	5	77.794876
Sappanone B	302	1.352300	4	6	76.357674
(e)-3-(3,4-Dihydroxy benzylidene)-7-hydroxychroman-4-one	284	2.462099	3	5	75.680878
3,8,9-Trihydroxy-6H-benzo[c]chromen-6-one	244	2.002999	3	5	61.830894
3-Deoxysappanchalcone	270	3.002499	2	4	76.130081
3-Deoxysappanon B	286	1.646700	3	5	74.692879

Molecular weight (MW) indicates the rate of diffusion of a molecule. Compounds with a molecular weight exceeding 500 daltons (Da) will cause the molecule to be unable to diffuse through the cell membrane. Most Secang flavonoid compounds have a molecular weight under 500 Da, and it can be predicted that they can diffuse through cell membranes readily. The Log P value indicates the bioavailability of compounds. All of the Secang flavonoid compounds have Log P lower than 5. It suggests that flavonoid compounds should be well absorbed orally. Most flavonoid compounds have Hydrogen Bond Donor (HBD) lower than five and Hydrogen Bond Acceptor (HBA) lower than 10, except protosappanin d and protosappanin e, which indicates there is not required much energy for the absorption process. Molar Refractivity (MR) most flavonoid compounds have a value between 40-130. It shows good absorption and adequate oral bioavailability (Kilo et al., 2019; Ibrahim et al., 2021).

Protosappanin D has a molecular weight of 604 Da, 8 hydrogen bond donors, 12 hydrogen bond acceptors, and 151.678467 molar refractivities, with 4 violations of the Lipinski rule of five. Protosappanin E has a molecular weight of 586 Da, 7 hydrogen bond donors, 11 hydrogen bond acceptors, and 146.634644 molar refractivities, with 4 violations of the Lipinski rule of five. Protosappanin D and E likely present absorption or permeability issues because they have more than 3 Lipinski's violations (Al Mogren et al., 2020). The native ligand (MRK) has a molecular weight of 349 Da, log P 3.001400, 3 hydrogen bond donors, 5 hydrogen bond acceptors, and 88.210594 molar refractivities. It means that the native ligand follows the Lipinski rule of five. Metformin has a violation in the Lipinski rule of five. The molar refractivity of metformin (37.2223495) is lower than 40. Based on the results of the Lipinski rule of five, native ligand (MRK) and butein have better physicochemical properties than metformin. Hence, metformin still has good bioavailability issues because it only has 1 violation of Lipinski's rule of five (Al Mogren et al., 2020).

3.2. Results of ligand-receptor Molecular Docking with PLANTS

3.2.1. Docking Protocol Validation

The docking protocol was validated by re-docking the native ligand on the target protein (1V4S). The smaller RMSD value indicates that the predicted has a suitable ligand pose because it is closer to the conformational shape of the native ligand. In comparison, the more considerable RMSD value (> 2 Å) results in a substantial difference in conformation between the predicted ligand pose and the native ligand, which can contribute to a high prediction error rate of ligand-protein interaction (Putri *et al*., 2019). The results of the RMSD analysis obtained an RMSD value of 0.6114 from the fifth conformation, which had the lowest score of -102.972 (**Figure 1**). Based on the RMSD value, it can be seen that the validation of the docking protocol is acceptable because it meets the criteria of < 2.0 Å and can be continued for further research processes.

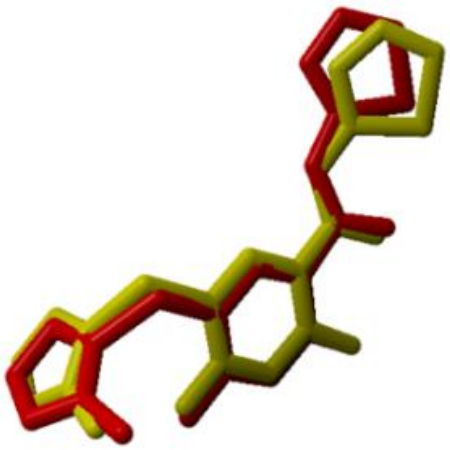

Figure 1. The pose of the native glucokinase ligand (red) and the pose of the re-docking ligand (yellow) with RMSD value = 0.6114

3.2.2. Docking Results

Molecular docking was performed using PLANTS (Protein-Ligand ANT-System) on flavonoid compounds from Secang wood (*Caesalpinia sappan* L.) and glucokinase receptors (PDB CODE: 1V4S).

The docking results between glucokinase protein (1V4S) with flavonoid ligands from Secang wood (*Caesalpinia sappan* L.) are shown in **Table 2**. Based on the docking scores, it showed that all ligands could interact with glucokinase protein. The butein compound has the lowest docking score (-94.4836) compared to the comparison ligand metformin (-59.7875). The lowest docking score indicates that butein has a good affinity and is more stable than metformin. The negative value indicates that the ligand and receptor interaction runs spontaneously (Nitami & Febriansah, 2019; Adriani, 2018). The docking score shows that butein is more stable than metformin when interacting with glucokinase receptors. It can be seen that butein compounds are potential candidates for antidiabetic drugs.

Table 2. The value of the docking ligand score from the molecular docking results

No	Ligand	Score docking	
		Average	Bestranking
1.	MRK (2-Amino-4-Fluoro-5-[(1-Methyl-1H-Imidazol-2-Yl)Sulfanyl]-N-(1,3-Thiazol-2-Yl)Benzamide)	-101.44084	-102.9720
2.	Metformin	-59.14353	-59.7875
3.	(e)-3-(3,4-Dihydroxybenzylidene)-7-hydroxychroman-4-one	-88.67528	-90.4340
4.	3-Deoxysappanchalcone	-80.73401	-81.5542
5.	3-Deoxysappanon B	-81.66752	-84.8542
6.	3,8,9-Trihydroxy-6H-benzo[c]chromen-6-one	-77.70257	-79.8999
7.	Brazilin	-77.17793	-78.2738
8.	Brazilein	-82.74491	-85.0942
9.	Brazilide A	-69.91013	-73.2438
10.	Butein	-93.65594	-94.4836
11.	Protosappanin A	-77.98330	-81.5760
12.	Protosappanin B	-67.55506	-68.4040
13.	Protosappanin C	-66.28681	-67.2410
14.	Sappanchalcone	-86.08326	-86.9112
15.	Sappanone B	-84.65582	-86.4820

3.2.3. Molecular Interactions

The molecular docking results were analyzed using the Discovery Studio Visualizer to see the interactions and the amino acid residues on the active side of the interacting protein. The interaction between the flavonoid compounds of the Secang wood and the glucokinase receptor (1V4S) resulted in hydrogen bonds, van der Waals interactions, and hydrophobic interactions (**Figure 2-Figure 4**).

The visualization analysis (**Table 3**) shows that the hydrogen bonding in butein, the best ligand with the lowest docking score, interacts with the same amino acid residues as the comparison ligand metformin, Arginine 63 (ARG 63). These hydrogen bonds provide a stable interaction between the test ligand and the glucokinase receptor (Sari et al., 2020). In addition, the hydrogen bond distance (**Table 4**) shows that MRK has more hydrogen bonds than butein and metformin. However, butein has the smallest hydrogen bond distance compared to MRK and metformin. The greater the distance of the hydrogen bonds will cause the bonds to break easily; conversely, the smaller the bond distance, the stronger the bond will be (Rachmania et al., 2015).

Butein formed one hydrogen bond with ARG 63 through the oxygen atom, one with TYR 61 through the hydroxyl group, and two with TYR 215 through the oxygen atom and carbonyl oxygen atom. Metformin only formed two hydrogen bonds with ARG 63 and LEU 451 through hydrogen atoms in amino groups. The number of hydrogen bonds formed when the protein

interacts with the ligand will contribute to the stability of the complex structure (Rachmania et al., 2015). It could explain the lower butein binding score compared to metformin.

Table 3. Interaction of native ligand (MRK), test ligand (butein), and comparison ligand (metformin) against glucokinase receptors (1V4S)

Ligand	Amino Acid Residues Involved		
	Hydrogen Bond	**van der Waals interactions**	**Hydrophobic Interaction**
MRK (Native ligand)	ARG 63, SER 64, THR 65	GLN 98, MET 210, GLU 221, HIS 218, ARG 250	TYR 214, VAL 62, ILE 159, VAL 455, PRO 66, ALA 456, ILE 211, MET 235
Metformin (Comparison ligand)	ARG 63, LEU 451	THR 65, ILE 211, GLN 98, PRO 66, VAL 62, VAL 455, ALA 456, ILE 159	-
Butein (Test ligand)	TYR 61, ARG 63, TYR 215	THR 65, MET 210, SER 64, LEU 451, ALA 201	TYR 214, PRO 66, VAL 452, ILE 159, ALA 456, VAL 62, VAL 455, ILE 211

Table 4. Hydrogen bonding distance of MRK, butein, and metformin

Bonding type	Ligand	Amino Acid Residue	Bonding Distance (Å)
Hydrogen Bond	MRK	ARG 63	4.92; 5.97; 7.40
		SER 64	3.52
		THR 65	3.75
	Metformin	ARG 63	5.35; 6.61
		LEU 451	4.46
	Butein	ARG 63	3.87
		TYR 61	5.85
		TYR 215	5.18; 6.63

Electrostatic interactions play a role in the stability of the ligand-receptor complex. Electrostatic interactions are interactions between atoms due to the differences in polarity. These interactions include weak and non-covalent interactions so that they are easily separated. However, many electrostatic interactions can significantly contribute to the conformational formation of protein. One of the electrostatic interactions is the van der Waals interaction which is a relatively weak electric attraction because of the permanent or induced polarity of the molecule. This interaction can occur in charged and uncharged residues (Arwansyah et al., 2014). The interaction between the comparison ligand metformin and the test ligand butein on the glucokinase receptor forms a van der Waals interaction with the same amino acid residue, namely Threonine 65 (THR 65).

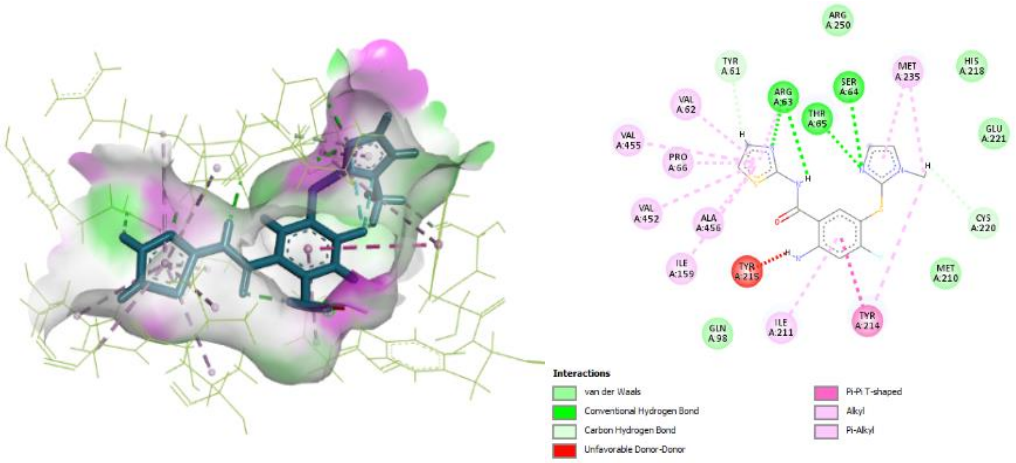

Figure 2. Pose and interaction results of MRK ligands with glucokinase receptors (1V4S)

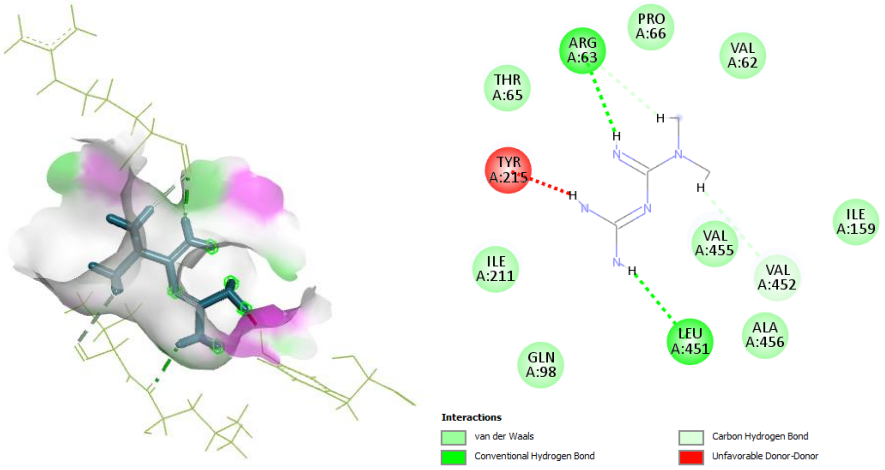

Figure 3. Pose and interaction results of metformin ligands with glucokinase receptors (1V4S)

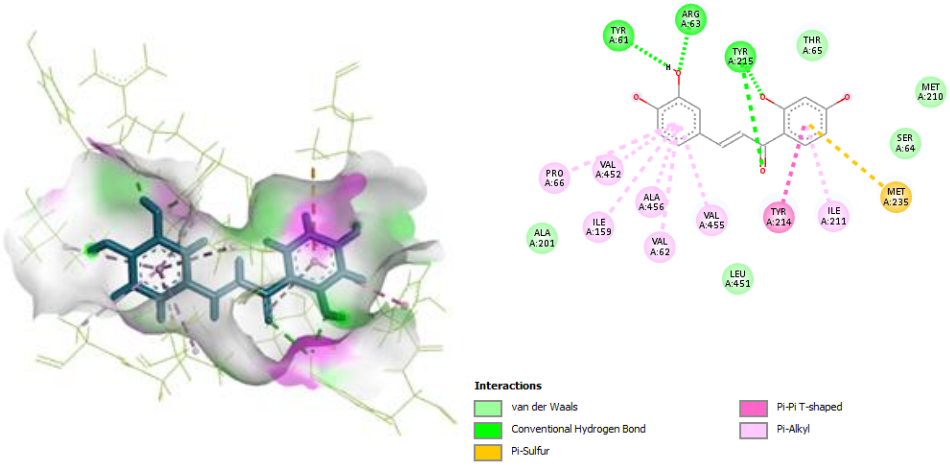

Figure 4. Pose and interaction results of butein ligands with glucokinase receptors (1V4S)

Hydrophobic interactions also play a role in the stability of the complex. These interactions avoid a liquid environment (Arwansyah et al., 2014). The hydrophobic interaction of butein with glucokinase receptors occurs with amino acid residues of Tyrosine 214 (TYR 214), Proline 66 (PRO 66), Valine 452 (VAL 452), Isoleucine 159 (ILE 159), Alanine 456 (ALA 456), Valine 62 (VAL 62), Valine 455 (VAL 455), and Isoleucine 211 (ILE 211). Metformin, as a comparison ligand, did not form a hydrophobic interaction because it does not have Pi-orbitals of the aromatic ring system (Khan et al., 2022).

Butein also forms interactions with the same amino acid residues as the native ligand (MRK) on the amino acid residue of Arginine 63 (ARG 63) via hydrogen bond; Methionine 210 (MET 210) via van der Waals interaction; Tyrosine 214 (TYR 214) (Pi-Pi T-Shaped); Valine 62 (VAL 62), Valine 452 (VAL 452), Valine 455 (VAL 455), Isoleucine 159 (ILE 159), Proline 66 (PRO 66), Alanine 456 (ALA 456), and Isoleucine 211 (ILE 211) via Pi-Alkyl interactions. It is similar to previous studies where MRK forms hydrogen bonds with Arginine 63 (ARG 63) amino acid residue. In addition, van der Waals interactions occur with Leucine 451 (LEU 451) and Serine 64 (SER 64) residue; and Pi-Alkyl interactions occur with Proline 66 (PRO 66), Valine 452 (VAL 452), and Valine 455 (VAL 455) amino acid residue (Astuty & Komari, 2022). The number of similarities between the amino acid residues that interact with MRK and butein allows the docking scores to be similar.

This research showed that butein has a potential antidiabetic drug by inhibiting glucokinase enzyme (1V4S). It is based on the docking score of butein, which is lower than the other test ligands and metformin. In addition, the interaction between butein and glucokinase through

hydrogen bonds and van der Waals interactions with the same amino acid residues as metformin binds, Arginine 63 (ARG 63) and Threonine 65 (THR 65).

3.3. ADME Properties

The results of pharmacokinetics properties for butein, metformin, and native ligand are compared in **Table 5**.

Table 5. Physicochemical and Pharmacokinetic properties of MRK (native ligand), butein, and metformin using SWISS-ADME

Parameters	Compounds		
	MRK (native ligand)	Butein	Metformin
TPSA	139.37	97.99	91.49
log Kp (cm/s)	-6.59	-5.96	-7.99
Consensus Log P	2.29	1.96	-0.89
BBB permeant	No	No	No
GI absorption	Low	High	High
CYP1A2 inhibitor	Yes	Yes	No
CYP2C19 inhibitor	Yes	No	No
CYP2C9 inhibitor	Yes	Yes	No
CYP2D6 inhibitor	Yes	No	No
CYP3A4 inhibitor	Yes	Yes	No
Pgp substrate	No	No	No

MRK's topological surface is (TPSA), butein, and metformin are less than 150 Å, indicating strong polarity. As a consequence, all compounds have good oral absorption and membrane permeation. Log Kp value (< –2.5 cm/s) shows all compounds have high skin permeability (Rajalakshmi et al., 2021). The consensus Log P value is the arithmetic mean of the five Log P prediction values (iLOGP, XLOGP3, WLOGP, MLOGP, and SILICOS-IT) (Daina et al., 2017). Based on the consensus Log P value, MRK and butein are more lipophilic than metformin (Al Mogren et al., 2020).

Pharmacokinetic properties are evaluated by brain penetration (BBB permeant) and passive gastrointestinal absorption (GI absorption). These correlations can be seen in the boiled egg model (**Figure 5**). The position of each compound is a function of WLOGP versus TPSA. Butein and metformin are in a white region, indicating a high gastrointestinal absorption probability and no BBB permeability (Ram et al., 2022). MRK is outside of the egg, predicted as not absorbed in the gastrointestinal and not brain penetrant. All compounds are displayed in a red dot, indicating non-Pgp substrate (Daina et al., 2017). It means there is no issue with the excretion process (Shweta & Rashmi, 2019).

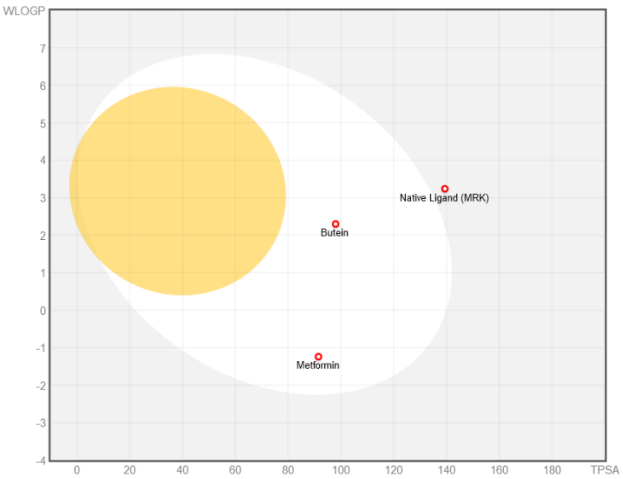

Figure 5. Boiled egg model of MRK, butein, and metformin

The interaction between compounds and cytochromes (CYP) 450 enzyme plays a vital role in liver metabolism. The CYP isoenzymes in SWISS-ADME prediction are CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4. Metformin did not inhibit any CYP isoenzymes, indicating that it is well metabolized in the liver and readily eliminated from the body. However, MRK showed as an inhibitor of all CYP isoenzymes, which means that MRK has poor elimination. Butein is predicted as a CYP1A2 inhibitor, CYP2C9 inhibitor, and CYP3A4 inhibitor. It might accumulate in the body and cause toxicity (Ononamadu & Ibrahim, 2021).

3.4. Toxicity Prediction

Toxicity prediction is critical to detect whether a compound is toxic or non-toxic. In this study, Toxtree, OSIRIS, and pkCSM were used to predict the toxicity of compounds. Toxtree prediction test showed that MRK, butein, and metformin are classified as high-class toxicity compounds based on Crammer's rule. MRK and butein have no risk based on Kroes's TTC decision tree. The structure of MRK and butein has a potential genotoxic carcinogenicity. The test compound's structures showed no significant alert for nongenotoxic carcinogenicity and potential carcinogen based on QSAR (**Table 6**).

Table 6. Toxicity prediction of MRK (native ligand), butein, and metformin using Toxtree, OSIRIS, and pkCMS

Parameters	Compounds		
	MRK (native ligand)	**Butein**	**Metformin**
Toxtree			
Crammer's rule	High Class	High Class	High Class
Kroes TTC decision tree	Negligible risk	Negligible risk	Substance would not be expected to be a safety concern
Negative for genotoxic carcinogenicity	No	No	Yes
Negative for nongenotoxic carcinogenicity	Yes	Yes	Yes
Skin irritation	Unknown	Yes	Unknown
Eye irritation and corrosion	Unknown	Unknown	Unknown
Potential carcinogen based on QSAR	No	No	No
OSIRIS			
Mutagenic	Low risk	High risk	Low risk
Tumorigenic	Low risk	Low risk	Low risk
Reproductive effect	Low risk	Low risk	Low risk
Irritant	Low risk	Medium risk	Low risk
pkCSM			
AMES toxicity	Yes	No	Yes
Hepatotoxicity	Yes	No	No
Pgp substrate	No	No	No

MRK and metformin were classified as having a low risk of mutagenic, carcinogenic, teratogenic, and irritant based on the OSIRIS tool's toxicological prediction. In contrast, butein has a high mutagenic risk and a moderate irritant risk. The pkCSM web application predicted that MRK and metformin are mutagenic. Butein, in contrast, is neither mutagenic nor hepatotoxic. Before butein can be used as a drug, it is necessary to conduct in vivo testing to ascertain its toxicity based on the three toxicity prediction tools.

4. CONCLUSION

All flavonoid compounds from the secang wood (*Caesalpinia sappan* L.) were predicted to have activity against the glucokinase enzyme as indicated by a lower docking score (-67.2410 to -94.4836) than the comparison compound metformin (-59.7875). Butein was predicted to have

the best antidiabetic activity, with a docking score of -94.4836. The interaction occurs between the flavonoid compounds of the secang wood and the glucokinase (1V4S) enzyme through the formation of hydrogen bonds, van der Waals interactions, and hydrophobic interactions. Butein showed good oral absorption and excretion. The toxicity of butein requires further study.

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6. AUTHOR DECLARATION

Authors' Contributions and Responsibilities

The authors made substantial contributions to the conception and design of the study. The authors took responsibility for data analysis, interpretation, and discussion of results. The authors read and approved the final manuscript.

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Availability of Data and Materials

All data are available from the authors.

Competing Interests

The authors declare no competing interest.

Additional Information

No additional information from the authors.

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POTENTIAL INHIBITION OF AKT1 AND P53 PROTEIN IN COLON CANCER BY GALLIC ACID DERIVATIVES COMPOUND WITH MOLECULAR DOCKING APPROACH

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ABSTRACT
Colon cancer is a degenerative disease that attacks the large intestine through a process of initiation, promotion and progression. Related research reports that overexpression of the AKT1 protein was found to be 60-70% and p53 at 50%. This research analyses the affinity, stability and interaction of gallic acid derivative compounds and reference ligands with the target proteins AKT1 and p53 by molecular docking. The study stages carried out include preparation and optimization of target proteins and ligand compounds, file creation and simulation processes, and analysis and visualization of docking results. The docking simulation results show that four gallic acid derivative compounds provide potential inhibitory activity against the AKT1 and p53 proteins based on binding energy values. BG and 2HBG compounds have strong inhibitory power against target proteins, thus enabling the formation of strong interactions and complexity towards the active site of amino acids with a bond distance of <3.0 Å. Thus, gallic acid derivative compounds have potential as inhibitors and are expected to activate other proteins, causing cancer cell apoptosis.

Keywords: AKT1 and p53; Colon cancer; Gallic acid and its derivatives; Molecular docking

1. INTRODUCTION

Colon cancer is one of the most diagnosed and third-deadly diseases in the world and kills many every year (Nelson et al., 2020). methods generally use histopathological tissue analysis. APC (adenomatous polyposis cancer) gene mutations, delete in colon cancer (DCC), AKT, K-Ras, p53, BRAF serine/threonine proto-oncogenes, and damaged genes are markers of colon cancer development (Ahmed Monjur., 2020). Protein kinase AKT is a proto-oncogene from the serine/threonine kinase family that regulates several metabolic processes, cell mediators and controls cell survival (Revathidevi & Munirajan., 2019). In addition, it exerts influence on the promotion of cell cycle progression and inhibits apoptosis. AKT activity in highly active colon cancer ranges from 60 to 70% (Pandurangan., 2013) and is involved in the proliferation, metabolism, and survival of cancer cells (Zhu & Thompson., 2019). The compound MK-2206 showed inhibitory activity on the AKT protein in human colorectal cancer pre-clinical trials (Al-Saffar et al., 2018). AKT protein is a crucial and rational target in the development of anticancer drugs (Song et al., 2019). Research by Li et al, 2018 showed that the compound resveratrol provides excellent chemotherapeutic effects for AKT1 and AKT2 proteins in colon cancer (Li et al., 2019).

The p53 gene is a regulator of several biological functions of cells including proliferation, oncogene signaling, ribosomal stress, DNA damage, metabolism, DNA repair, differentiation,

and apoptosis, which also affects the process of carcinogenesis (Boutelle & Attardi., 2021). The expression of p53 in normal cells is at a low level because it is degraded by the ubiquitin ligase MDM2, Pirh2, and COP1 (Pant & Lozano., 2014). The p53 gene mutation in colon cancer is around 50%. The mutation occurs due to dysfunction of the p53 gene in tumors in the DNA-binding domain by hetero-oligomerization with evil-type p53 (Shah et al., 2020). This causes the cells to be oncogenic, leading to the invasion, proliferation, metastasis, and survival of cancer cells (Bergers & Fendt., 2021).

Gallic acid (GA) is a bioactive compound of the polyphenol group which is widely contained in various plants, vegetables, nuts, and fruit (Li et al., 2017; Bai et al., 2021). GA has several pharmacological activities including anticancer, antibacterial, anti-inflammatory, and antioxidant (Nouri et al., 2020; Kahkeshani et al., 2019). The activity of GA as an anticancer was reported by Wang et al, 2016 namely the inhibition of ROS-specific N-acetyl cysteine (NAC), further stimulating the apoptotic pathway via mitochondria in H446 SCLC cells (Wang et al., 2016). In addition, AG can inhibit the growth of several cell lines, namely HCT116 and HT-29 for colon cancer (Lin et al., 2021), as well as MCF-7 for breast cancer (Rezaei-Seresht et al., 2019).

The structure-activity relationship of gallic acid derivative compounds shows that hydroxyl group substituents, alkyl esters and the number of aromatic rings contribute to cytotoxic activity, solubility, lipophilicity and inhibition against cancer cells (AL Zahrani et al, 2020). This study became the basis for modifying the structure of the lead compound GA by adding hydroxy, alkyl groups and -OH substitution to methoxy. Thus, it is hoped that it can increase its lipophilicity and inhibitory activity against cancer target cells

The research approach is using the molecular docking method by analyzing the affinity, stability, and binding activity with molecular targets of protein. In addition, predictions of pharmacokinetic profiles for these compounds were also carried out.

2. METHODS

2.1. Tools and Material

The docking molecular study was carried out using an Intel Core i3-1115G4 computer with 8 GB of RAM. The software used is Chimera 1.10.2, MarvinSketch 15.5.11, PyMol 2.3.3, Autodock 4.2, passonline (way2drug) and Discovery Studio V21.1.0.20298. The material used is a gallic acid derivative which is designed and modified based on the structure and comparison compound gossypol (**Figure 1**) AKT1, and p53 target proteins downloaded from the Protein Data Bank (http://www.rscb.org/pdb/).

2.2. Method

The structure of the target protein in the form of AKT1 and p53 was traced through the website https://www.rcsb.id with PDB IDs 3MVH and 1XQH, respectively. The structure of the gallic acid-derived ligand was designed and fabricated using Marvinsketch software.

The first is the preparation of the target protein which includes tracing, downloading, optimizing, and separating from non-standard residues. The structure of the target protein was traced and downloaded from the PDB web with PDB-ID 3MVH (AKT1) and 1XQH (p53). The second is the preparation of the ligands which includes structure-based design and the manufacture of two-dimensional structures which are then converted into three dimensions, as well as the addition of hydrogen atoms and Gasteiger energy. The third is tethering the coordinates of Gridbox 40x40x40 with Grid center size x=17,485, y=-1,885, and z=27,403 for AKT1 and Gridbox 40x40x40 with Grid center x=8,217, y=-15.898 and z=11.33 for p53. Each ligand is in a flexible state that will interact with the target protein in a rigid state. Next, create a Grid Parameter File (GPF) and a Docking Parameter File (DPF) of the target protein complex with ligands. The last is the process of simulation, analysis, and visualization of docking results.

Pharmacokinetic studies were carried out on gallic acid derivatives and comparison ligands using the preADMET software via the website address https://preadmet.bmdrc.kr. The parameters measured were the permeability of Caco-2 cells to predict absorption in the intestine, the percentage of Human Intestinal Absorption (%HIA), and binding to plasma proteins to predict the distribution of these compounds.

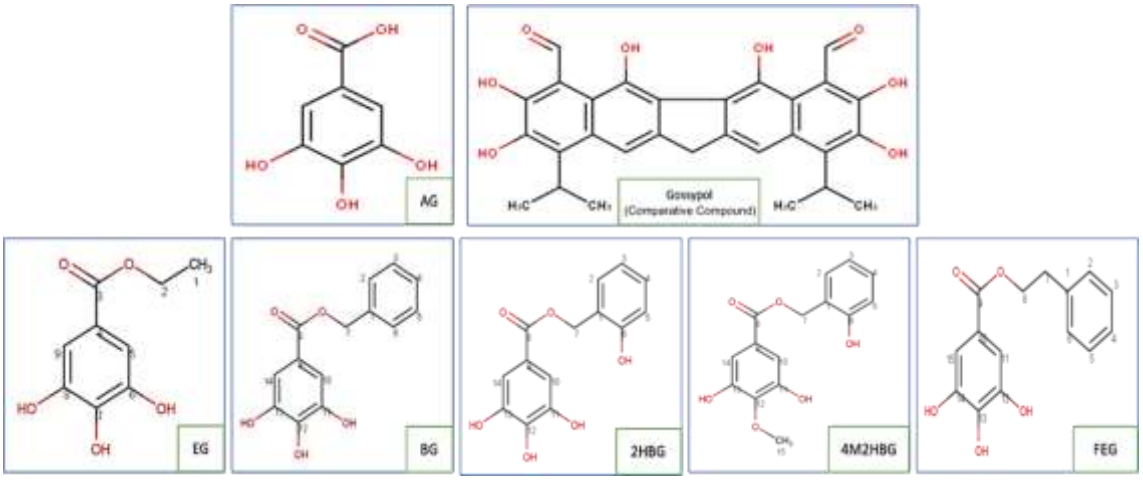

Figure 1. Structure Design of Gallic Acid Derivative Compounds (GA: Gallic acid; EG: Ethyl gallate; BG: Benzyl gallate; 2HBG: 2 hydroxy benzyl gallate; 4M2HBG: 4 methoxy 2 hydroxy benzyl gallate; FEG: phenylethyl gallate and Gossypol as ligand comparison)

3. RESULTS AND DISCUSSION

The study of molecular docking is performed on gallic acid derivative compounds with proteins that stimulate apoptosis, namely AKT1 and p53. The results of docking analysis are sorted by Affinity, stability, and bonding activity to the target protein and visualized to identify the bonding distance that occurs.

The relationship between the structural activity of gallic acid derivatives compound to both AKT1 and p53 target proteins gives varying binding energy values (**Figure 2** and **Figure 3**). Modification of the chemical structure by adding hydroxyl and methoxy groups greatly affects the inhibitory activity. In addition, gallic acid parent compounds consisting of benzene and hydroxyl groups play an important role in the activity of inhibition (Badhani et al., 2015). The number of hydroxyl groups affects the magnitude of inhibition effectiveness (Anantharaju et al., 2016). In addition, alkyl groups in the form of methoxy give the activity of the binding and inhibition are quite strong (Humaedi & Ernie., 2021).

Gallic acid derivatives compound used in the docking simulation for target proteins AKT1 and p53 met Lipinski's criteria, namely molecular weight <500 gram/mol, hydrogen bond proton acceptor group <10, proton donor group bonded hydrogen < 5, the logarithm of partition coefficient in water and 1-octanol < 5 (Chagas, Moss & Alisaraie, 2018). Compounds that meet these criteria are considered to have the potential to enter cell membranes and be absorbed by the body. Apart from that, based on way2drug.com analysis, it shows that this compound has anti-carcinogenesis activity with a Probability of Activines (pa) value > 0.3.

In silico studies regarding the activity of gallic acid derivatives against a certain target, proteins were reported by Kahkeshani et al (2019) and Variya et al (2012) that aryl-3,4,5-trimethyl gallate inhibits COX-1 as an anti-inflammatory and other gallic acid derivative compound as a strong inhibitor of PPAR-Y receptor as anti-diabetic. Gallic acid derivative compound, namely 2-hydroxy benzyl gallate, has inhibitory activity against BRAF protein in colon cancer (Humaedi et al., 2017). Research by Raghi et al, 2018 reported that the combined compound between gallic acid and 1,3,4-oxadiazole inhibited the activity of the ABL kinase receptor (Raghi et al., 2018).

Furthermore, 3,4,5 trimethoxy-phenylmethyl gallate compounds have the potential as inhibitors in prostate cancer by inhibiting the expression of androgen receptors (Humaedi & Ernie., 2021).

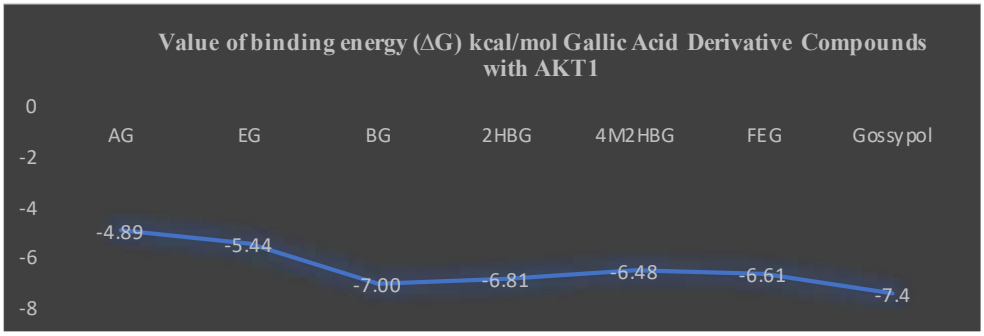

Figure 2. Value of binding energy (ΔG) kcal/mol of gallic acid derivatives compound with AKT1 (AG: Gallic acid; EG: Ethyl gallate; BG: Benzyl gallate; 2HBG: 2 hydroxy benzyl gallate; 4M2HBG: 4 methoxy 2 hydroxy benzyl gallate and FEG: phenylethyl gallate)

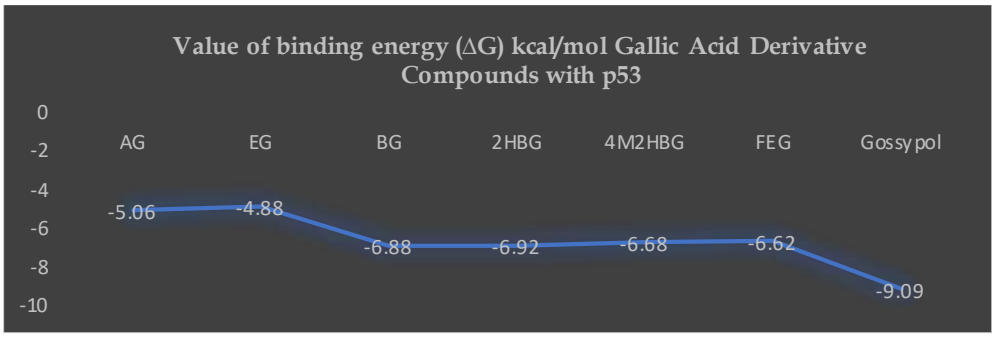

Figure 3. Value of binding energy (ΔG) kcal/mol of gallic acid derivatives compound with p53 (AG: Gallic acid; EG: Ethyl gallate; BG: Benzyl gallate; 2HBG: 2 hydroxy benzyl gallate; 4M2HBG: 4 methoxy 2 hydroxy benzyl gallate and FEG: phenylethyl gallate)

3.1. Docking Result Analysis with AKT1

The docking simulation data in **Figure 2** shows that four gallic acid derivative compounds provide the lowest binding energy values and have the potential as inhibitors for AKT1 protein, namely BG, 2HBG, 4M2HBG, and FEG. It has been validated before docking is carried out, with an Rmsd value < 2, namely akt1 is 1.77 and p53 is 1.154. The gallic acid derivative which has the best (ΔG) value is BG (-7.00 kcal/mol). Meanwhile, the comparison ligand, gossypol, gave a relatively higher inhibitory activity of -7.74 kcal/mol. This is possible because the molecular weight factor is large enough to affect its activity.

In addition to binding energy, other indicators that support the results of the docking simulation are the inhibition constant (μM), amino acids bound by hydrogen bonds, and bond distances. The smaller the value (Ki), the lower the binding energy so the greater the effect of inhibition. The interaction between the amino acids of the protein AKT1 with gallic acid derivative compounds formed a strong enough hydrogen bond with an average bond distance of 2.35 Å, which allows apoptosis through the activation of caspase 3 and 9. The stable hydrogen bond interaction analysis has criteria as hydrogen donor and acceptor with bond distance < 3.4 Å (Juhas & Zitko, 2020).

The Interaction Model between the four gallic acid derivatives compound and the comparison ligand with the AKT1 protein showed a favorable binding of hydrogen bonds and Van der Walls bonds. The compound predominantly binds to the amino acids Leu156, Ala230, and Glu228 to form hydrogen bonds (**Figure 4**). Chuang et al research, 2015 reported that A46 and a48 compounds based on in vitro activity test results provide excellent activity potential, followed by docking simulation of AKT proteins and hydrogen bonds with amino acids Ala230 and Asp292 as well as Thr211 and Ala230 (Chuang et al., 2015). Furthermore, imidazopyridine

derivative compounds exert inhibitory activity against the AKT1 protein and form interactions with the amino acids Thr211, Tyr272, and Asn53 for compound 44 as well as Thr211 and Tyr272 for compound 5 (Gu et al., 2019). Research by Rehan et al, 2014 describes the compound MK-2206 strongly binds to protein AKT1 on amino acids Asn53, Gln59, Leu78, Trp80, Val201, Tyr272 (Rehan et al., 2014).

3.2. Docking Result Analysis With p53

Figure 3 shows that four gallic acid derivative compounds provide the lowest binding energy values, namely 2HBG, BG, 4M2HBG, and FEG against the p53 target protein. The 2HBG compound gave a fairly good inhibitory effect of -6.92 kcal/mol. While the comparison ligand gossypol has a very strong inhibitory effect with a fairly high binding energy value of -9.90 kcal/mol. In addition to the molecular weight factor, the large number of hydroxyl and benzene groups in the Gossypol structure chain causes a large inhibitory effect.

Other criteria are the inhibition constant (μM) which is directly proportional to the binding energy value, amino acids are bound by hydrogen bonds and the bond distance shows a strong interaction (**Table 1** and **Table 2**) with an average bond distance of 2.32 Å. These interactions occur with the amino acids Glu, His, Glu, and Asn with different codes with strong bonds, both gallic acid derivatives and their comparison ligands (**Figure 5**). Research by Abbasi et al., 2015 reported that the interaction between the ligand PK7242 and p53 formed hydrogen bonds in the amino acids Leu145 and Thr230 with an average bond distance of 3.25 (Abbasi et al., 2015). Furthermore, Shah et al, 2020 explained that there was a strong interaction between the GK02723 ligand and p53 protein on His, Thr, Leu, Ser, Tyr, and Cys amino acids with varying codes (Shah et al., 2020).

Table 1. Molecular weights, inhibition constants, hydrogen-bonded amino acids. and average bond distances for AKT1 target proteins

Compounds	BM	Ki (μM)	Amino acids for AKT1	Average bond distance
AG	170.12	259.58	LYS_{179},GLU_{198},THR_{211}, GLU_{228},ASP_{292}	2.46 Å
EG	198.05	102.32	LYS_{179},GLU_{198}, THR_{211},ASP_{292}	2.59 Å
BG	260.06	7.87	LYS_{179},GLU_{198},ASP_{292}	2.37 Å
2HBG	276.23	10.17	LEU_{156},GLU_{228},ALA_{230},	2.24 Å
4M2HBG	290.07	17.67	Leu_{156}	2.19 Å
FEG	274.08	14.39	GLU_{228},ALA_{230}	2.37 Å
Gossypol	502.519	3.76	GLY_{159},ALA_{230}	2.08 Å

Table 2. Molecular weights, inhibition constants, hydrogen bonded amino acids and average bond distances for p53 target proteins

Compounds	BM	Ki (μM)	Amino acids for p53	Average bond distance
AG	170.12	196.53	SER_{225},HIS_{297},GLU_{356}	2.26 Å
EG	198.05	102.31	GLU_{356}	1.99 Å
BG	260.06	9.10	GLU_{228},HIS_{293}	2.05 Å
2HBG	276.23	8.87	GLU_{228},HIS_{293}, ASN_{296},HIS_{297},TYR_{335}	2.96 Å
4M2HBG	290.07	12.79	ASN_{296},TYR_{335},GLU_{356}	2.32 Å
FEG	274.08	14.09	GLU_{356}	1.94 Å
Gossypol	502.519	215.43	ASN_{282}, LYS_{294}, ASN_{296}, GLU_{356}	2.13 Å

3.3. Pharmacokinetic Studies

The results of pharmacokinetic tests explain that the four compounds with the lowest Gibbs energy meet the required parameters. These parameters, namely the ability of absorption with classification (1) poorly absorbed (0-20%), (2) moderately absorbed (20-70%), and (3) well absorbed (70-100%). Permeability ability with classification (1) low (<4 nM Sec-1), moderate (4-70 nM Sec-1), and high (>70 nM Sec-1). Protein-binding plasma with classification (1) is strongly

bound (>90%) and weakly bound (<90%) (Tabeshpour et al, 2018). The first parameter is Human Intestinal Absorption according to the criteria range of good absorption properties, namely 70-100%. Furthermore, the permeability of the compounds indicated was in the moderate category between 4-70 nM Sec-1. Finally, the measured plasma protein binding was very strong, namely three compounds with a value >90% and one compound approaching a value of 90% (**Table 3**).

Table 3. Predicted results of pharmacokinetic studies of gallic acid compounds. and their derivatives as well as comparative ligands

Compounds	Absorption		Distribution
	HIA (%)	Cell Permeability Caco-2 (nM Sec^{-1})	Plasma Protein Binding (%)
AG	53.70	13.85	65.38
EG	72.04	0.18	96.03
BG	86.46	17.24	95.83
2HBG	75.31	13.44	96.05
4M2HBG	86.12	14.00	87.82
FEG	87.15	19.00	95.19
Gossypol	84.40	20.86	100.00

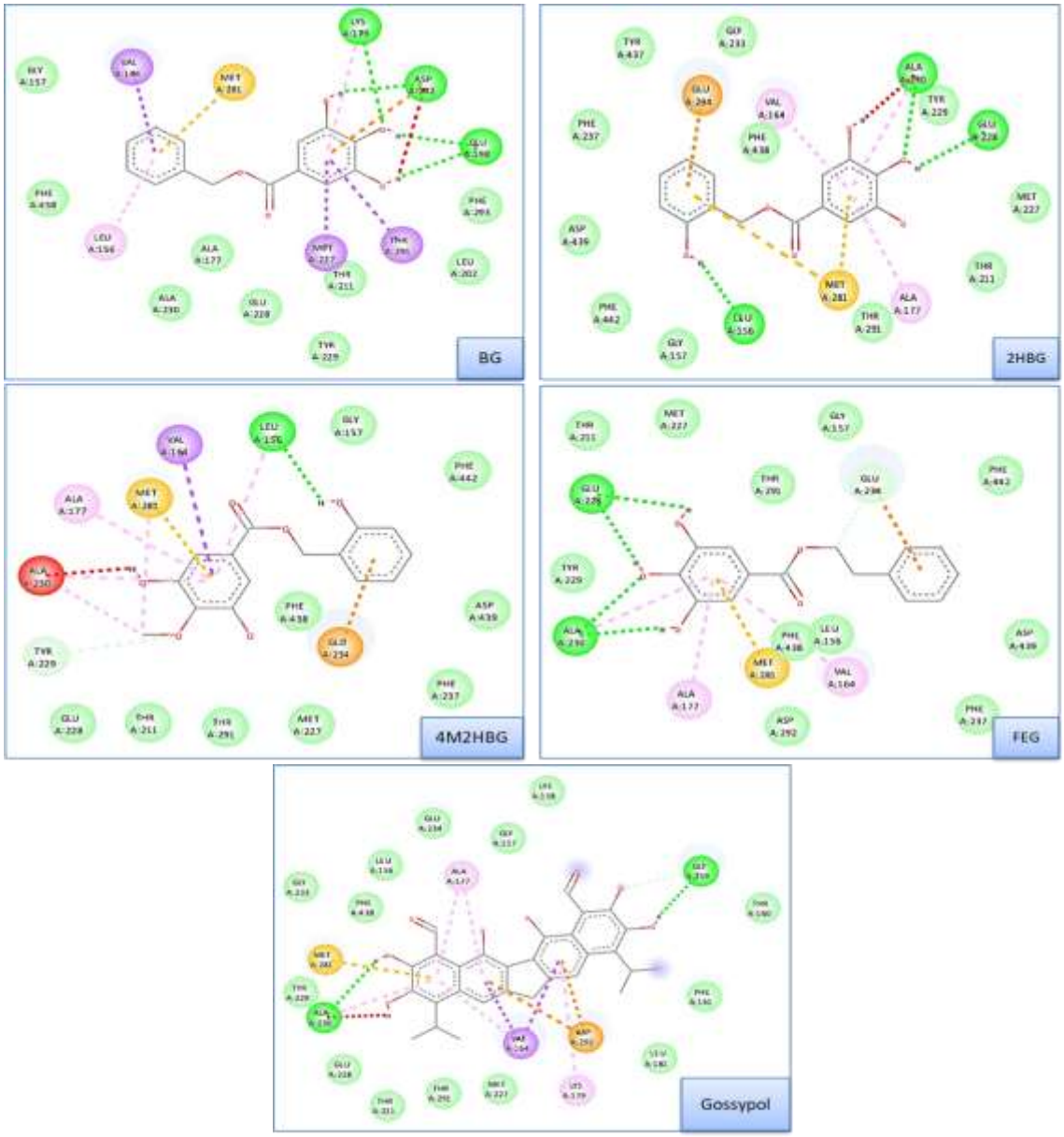

Figure 4. Interaction of AKT1 Protein with Gallic Acid Derivatives Compound (BG: Benzyl gallate, 2HBG: 2-hydroxy benzyl gallate, 4M2HBG: 4 methoxy 2 hydroxy benzyl gallate, FEG: phenylethyl gallate Comparative ligand: Gossypol)

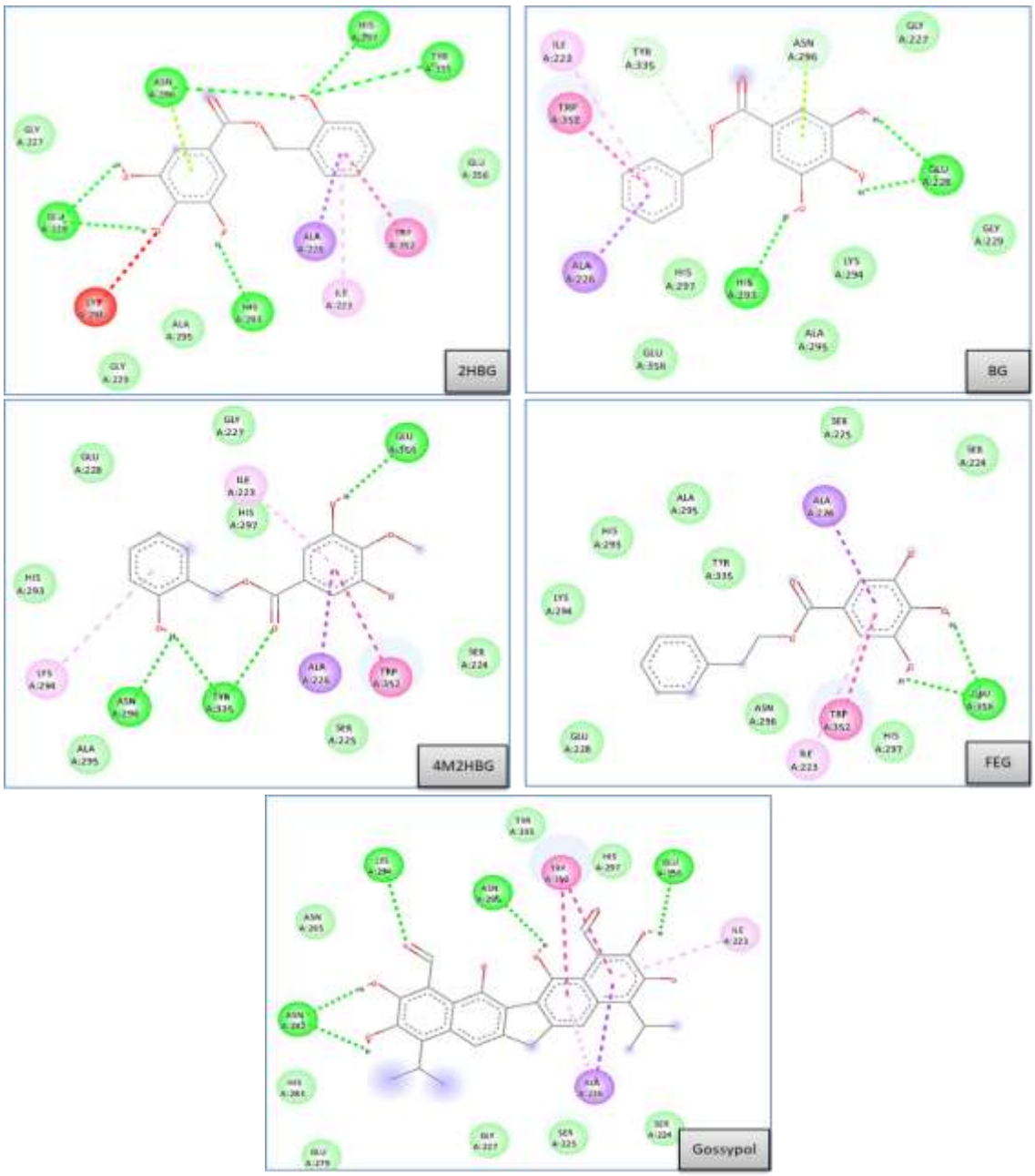

Figure 5. Interaction of p53 Protein with Gallic Acid Derivatives Compound (BG: Benzyl gallate, 2HBG: 2-hydroxy benzyl gallate, 4M2HBG: 4 methoxy 2 hydroxy benzyl gallate, FEG: phenylethyl gallate Comparative ligand: Gossypol)

4. CONCLUSION

This study shows that gallic acid derivative compounds have potential as colon cancer inhibitors by inhibiting the activity of AKT1 and p53 proteins with a docking simulation approach. Further research needs to be carried out using molecular dynamics to see the stability of the compound. Next, the synthesis and cytotoxic tests were carried out on this compound so that it has the potential to become a drug candidate for colon cancer.

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6. AUTHOR DECLARATION

Authors' Contributions and Responsibilities

The authors made substantial contributions to the conception and design of the study. The authors took responsibility for data analysis, interpretation, and discussion of results. The authors read and approved the final manuscript.

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Availability of Data and Materials

All data are available from the authors.

Competing Interests

The authors declare no competing interest.

Additional Information

No additional information from the authors.

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THE EFFECTIVENESS OF CORN SILK EXTRACT AGAINST DENTAL CARIES-CAUSING BACTERIA AND ITS FORMULATION IN MOUTHWASH PREPARATION

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ABSTRACT

Indonesia is undergoing a dental caries emergency with a prevalence of 51.1%. This may cause tooth decay due to dental plaque bacteria such as *Streptococcus mutans*. Mouthwash can be a solution because it has antibacterial properties and reaches interspaces on the teeth. Unfortunately, the active ingredient of mouthwash, chlorhexidine, can cause cancer-related mutations if used continuously. Therefore, it requires alternatives to natural ingredients, such as corn silk. The research aims to determine the corn silk phytochemical profile and minimum inhibitory concentration (MIC) against *Streptococcus mutans*. The research started by macerating corn silk simplicia using 70% ethanol. Afterwards, thin layer chromatography (TLC) was conducted to determine its phytochemical profile. Disk-diffusion and broth microdilution methods were conducted using various concentrations of corn silk extract to determine its antibacterial activity and minimum inhibitory concentration (MIC) against *Streptococcus mutans*. Then, the MIC_{50} was used as the minimum dose of corn silk extract concentration in mouthwash formulation which qualities controlled by pH and organoleptic tests. The yield of corn silk extracted was 16.17%w/w. The phytochemical profile from TLC showed that flavonoids, tannins, and terpenoids were present. Corn silk extract has antibacterial activities against *Streptococcus mutans* with MIC_{50} of 7.2%w/v. Corn silk extract, tween 80, sorbitol, sodium benzoate, sodium metabisulfite, oleum menthae piperitae, and distilled water were used in four mouthwash formulas (F1-F4). The pH of all formulas was 5 and the organoleptic test showed that from 30 panelists, the majority chose F2 as the best in terms of taste, color, and smell.

Keywords: Corn silk; Caries; *Streptococcus mutans*; Mouthwash

1. INTRODUCTION

Oral and dental diseases have become a serious issue in Indonesia. Data from the Basic Health Research (2018) indicates that the prevalence of oral and dental problems in the Indonesian population is 57.6%, with 88.8% of that being dental caries. This is primarily due to the lack of attention to oral health, leading to plaque formation on teeth. One of the primary bacteria responsible for plaque and tooth decay is *Streptococcus mutans*, which produces acid that demineralizes teeth, making them mineral-deficient and prone to damage (Ambarawati et al., 2020).

Mouthwash can offer a solution to the problem of dental plaque, as it can kill bacteria, eliminate bad breath, prevent plaque formation, and reach small interdental spaces (Yuniarsih, 2017). Unfortunately, many of the mouthwashes used today still contain synthetic ingredients such as chlorhexidine, which, if used continuously, can have mutagenic (cancer-causing) effects

in the oral cavity (Rahayu et al., 2022). Therefore, the need for safer alternatives, such as natural ingredients, is evident.

Indonesia is a significant corn producer, with a production of 29.02 million tons of corn in 2020 (Komalasari, 2021). Regrettably, a substantial amount of corn silk is often discarded (Rohmadianto et al., 2018). However, corn is known to contain compounds like flavonoids, tannins, saponins, and other phenolic compounds, which possess antibacterial and antioxidant properties (Nurani et al., 2022). This enhances the potential of corn silk as a candidate active ingredient for an antibacterial mouthwash.

Several methods are required to test the activity of corn silk extract as mouthwash. First, corn silk is extracted using a maceration method to obtain the active compounds present in corn silk. Subsequently, the chemical compound profile is analyzed using Thin-Layer Chromatography (TLC), and the antibacterial activity is confirmed using solid diffusion methods. Afterward, microdilution antibacterial tests are conducted to determine the Minimum Inhibitory Concentration (MIC) of corn silk extract in inhibiting *S. mutans*. This MIC value serves as a reference for determining the extract dosage in mouthwash formulation. No previous research has explored the potential of corn silk extract as a mouthwash formulation, which is why we are interested in further investigating this topic.

2. METHODS

2.1. The Location and Time of The Research

The research was conducted for 4 months (July-October 2023) at the Pharmacognosy and Phytochemistry Laboratory, the Cellular Biology and Microbiology Laboratory, the Formulation Technology Laboratory of Semi-Condensate and Liquid Preparation of Faculty of Pharmacy, Gadjah Mada University.

2.2. Materials and Tools

Erlenmeyer, gauze, filter paper, blender, water bath, shaker, Buchner funnel, UV lamp, Petri dish, measuring cylinder, tweezers, micropipette, yellow tip, blue tip, white tip, Vernier caliper, 96-well microplate, microplate reader, vortex, magnetic stirrer, stirrer bar, ultra turrax homogenizer, pH meter, centrifuge, analytical balance, viscometer, beaker glass, ethanol 96% (Brataco), citroborate reagent, silica gel 60 F254 (Merck), $FeCl_3$ reagent, Dragendorff reagent, anisaldehyde reagent, toluene, ethyl acetate, diethylamine acid, glacial acetic acid, formic acid, hexane, Luria Bertani (LB) media, agar, *Streptococcus mutans* ATCC 25175, tween 80, oleum menthae piperitae, sodium metabisulfite, sodium benzoate, sorbitol (Brataco), distilled water, and corn silk simplicia (age of 50 days) from Gemahan, Ringinharjo, Bantul, Special Region of Yogyakarta, Indonesia.

2.3. Data Collection Method

2.3.1. Preparation and Maceration of Corn Silk Extract

A total of 301.55 grams of corn silk was dried in an oven at 50°C and ground into a powder. Then, the powder was macerated in 70% ethanol at room temperature for 3 days with a powder-to-solvent ratio of 1:5. It was then separated into supernatant and residue through filtration. The residue was macerated again with the same ratio for 2 days and separated from the supernatant through filtration. The filtrate was evaporated in a water bath to obtain a concentrated extract (Kurnia et al., 2021).

2.3.2. Thin-Layer Chromatography (TLC) Test

The TLC plate, used as the stationary phase, was heated in an oven at 105°C for 5 minutes. Approximately 100 mg of corn silk extract was dissolved in 300 µL of ethanol and 200 µL of distilled water, and then spotted on the plate. Reference substances (rutin and quercetin for flavonoids, quinine for alkaloids, gallic acid for tannins, and thymol for terpenoids) were also

spotted on the plate. The plate was then placed in the mobile phase. The mobile phase used for testing the rutin group of flavonoids (Yadnya-Putra et al., 2019) was a mixture of ethyl acetate: formic acid: glacial acetic acid: water (100:11:11:27), while testing quercetin flavonoids (Yanti et al., 2021) used a mixture of hexane: ethyl acetate: formic acid (6:4:0.2). The alkaloid test (Fahturroji and Riza, 2020) used a mixture of toluene: ethyl acetate: diethylamine (7:2:1). The tannin test (Rakasiwi and Soegiharjo, 2014) used a mixture of ethyl acetate: formic acid: toluene: water (6:1.5:2:0.5), and the terpenoid test (Fahturroji and Riza, 2020) used hexane: ethyl acetate (93:7). Subsequently, the plate was sprayed with various reagents to visualize its chemical content. Spray reagents used included sitroborate for flavonoids, $FeCl_3$ for tannins, Dragendorff for alkaloids, and anisaldehyde for terpenoids (Kurnia et al., 2021).

2.3.3. Solid Diffusion Antibacterial Test

Corn silk extract was dissolved in 1% DMSO. Positive control used 1% ampicillin, and the negative control was 1% DMSO. Next, 10 µL of the sample, positive control, and negative control were placed on paper disks. Each paper disk, with the liquid applied, was placed on LB (Luria-Bertani) media in a petri dish containing *S. mutans*. Incubation was carried out for 24 hours at 37 °C, and then the inhibitory zones formed were observed (Fajrina et al., 2021).

2.3.4. Microdilution Antibacterial Test

S. mutans bacterial inoculum was prepared in liquid LB media with an OD600 value of 0.264-0.30. Then, in a 96-well microplate, 100 µL of sterile LB media, 50 µL of bacteria, and 50 µL of extract (2.5%, 5%, and 10% w/v) with 1% DMSO as a solvent were added. Each concentration was replicated three times. The negative control was filled with 200 µL of media, while the positive control contained 150 µL of media plus 50 µL of bacteria. The microplate containing negative control, positive control, and extracts was then incubated for 24 hours at 37 °C. Subsequently, it was analyzed using a microplate reader at a wavelength of 600 nm to obtain the percentage of inhibition and determine the minimum inhibitory concentration (MIC) value (Septiani et al., 2017).

2.3.5. Mouthwash Formulation

The preparation of the mouthwash formulation was made in a quantity of 100 mL with various concentrations of corn silk extract in the formula. The formulation started by mixing tween 80, sorbitol, sodium metabisulfite, sodium benzoate, and peppermint oil in a glass beaker. Then, the corn silk extract obtained from the maceration step was added according to its minimum inhibitory concentration. All ingredients were mixed using an ultra turrax homogenizer until homogenous, and distilled water was added to the specified calibration limit. The formulation was then transferred to plastic bottles, and an evaluation of the mouthwash formulation was conducted, including pH testing and organoleptic evaluation (Thomas et al., 2022).

2.4. Analytical Methods

2.4.1. Maceration of Corn Hair Ethanol Extract

The percentage yield of corn hair extract obtained is calculated using the formula:

$$\%Extract\ Yield = \frac{The\ weight\ of\ the\ evaporated\ extract}{The\ weight\ of\ the\ dried\ corn\ silk\ powder} x\ 100\% \quad (1)$$

2.4.2. Thin-Layer Chromatography Test

The data analysis of the thin-layer chromatography test results in a chemical content profile in the extract, which is represented by the color changes (chromatogram) on the thin-layer chromatography plate. The color changes indicate the presence or absence of the analyzed compounds.

2.4.3. Solid Diffusion Antibacterial Test

The diameter of the inhibition zones, represented as clear areas around the paper discs, indicates whether the corn hair extract possesses antibacterial properties or not. The measurements are taken with a ruler, and the data is graphed in a bar chart using Excel 2019 for comparison.

2.4.4. Microdilution Antibacterial Test

The absorbance data obtained from the ELISA reader is used to calculate the percentage of inhibition. The formula for calculating the percentage of inhibition is as follows:

$$\%Bacterial\ inhibition = \frac{Negative\ control - (treatment\ extract - control\ extract)}{Negative\ control} x\ 100\% \quad (2)$$

The percentage of inhibition from three replicates is averaged, and a linear regression is performed using Microsoft Excel 2019 to obtain the equation y = bx + A, where "y" represents the percentage of inhibition (%) and "x" represents the concentration of corn silk extract (% v/v). The minimum inhibitory concentration (MIC) can be calculated by inputting 50 for the "y" variable and obtaining the value of "x" as the MIC, which represents the concentration at which 50% inhibition is achieved.

2.4.5. Quality Control Testing of Dosage form

The pH measurement of mouthwash is carried out using pH paper, which changes color upon contact. The resulting color can be matched with the pH paper container's instructions. The pH data from three replicates is averaged and a bar graph is created using Microsoft Excel 2019, allowing for a comparison of pH values against each other to determine the quality criteria for mouthwash pH.

For organoleptic evaluation, responses from the public are collected through a Google Form filled out by a panel of individuals after tasting, smelling, and visually examining the formulated mouthwash. Evaluation is based on a scale of 1-5, with each person allowed to provide a single rating. The total scores are then compared to determine which formulation receives the best response from the public.

3. RESULTS AND DISCUSSION

3.1. Sample Preparation

Samples were obtained with an age of 50 days from Gemahan, Ringinharjo, Bantul, Special Region of Yogyakarta, Indonesia. The Laboratory of Microbiology and Cell Biology of the Faculty of Pharmacy UGM determined that the sample is a species of Zea mays L. of the Poaceae family. Subsequently, the samples were dried at 50 °C for 2 days to prevent fungal growth before the extraction process to longer storage time. The dried samples were powdered, and 285.49 grams powder was obtained.

3.2. Extraction

Simplicia powder was soaked with ethanol 70% for 72 hours with a powder-ethanol ratio of 1:5. Subsequently, the result was filtered using the Buchner funnel and re-macerated with the same ratio for 24 hours and then re-filtered. The resulting filter applied onto the water bath, then a condensed extract of 46.19 grams was obtained with 16.17%b/b yield.

Extraction was done by maceration method. Maceration was used because it is easy, simple, and does not damage the active compounds during extraction. The cell walls and cell membranes will break down due to the pressure difference between the outside and the inside of the cell so that the secondary metabolites present in the cytoplasm will break and dissolve in the organic solvent used (Novitasari & Putri, 2016). Therefore, a suitable solvent is required to dissolve the active compound of simplicia.

The solvent used is 70% ethanol. Ethanol has a low boiling point of 79 °C, which requires less heat for evaporation. Ethanol is also one of the solvents that is safe or non-toxic when consumed due to its low level of toxicity. Another reason for choosing ethanol 70% solvent is because the flavonoid compound that is the majority of compounds in corn silk is generally in the form of a polar glycoside, so it has to be dissolved with a polar solvent.

3.3. Thin-Layer Chromatography (TLC)

Thin-layer chromatography (TLC) was observed through the color changes that occurred after the addition of visualization reagents. Flavonoids appeared yellow after the addition of sitroborate due to the reaction between sitroborate and the ortho-hydroxy groups in flavonoids. Tannins changed to a brown color when sprayed with $FeCl_3$, resulting from the formation of a colored complex compound, trisianoferitric potassium ferric (III). Terpenoids turned purplish Panelistse after the addition of anisaldehyde and sulfuric acid due to the ability of terpenoids to form color in the presence of H_2SO_4 in the solvent. Additionally, alkaloids could undergo a color change to red when Dragendorff reagent was added, leading to the formation of reddish-colored $[Bil4]^-$ (Fahrurroji & Riza, 2020).

Table 1. Results of Thin-Layer Chromatography

Chemical Compound	Reagent	Before	After	Result
Alkaloid	Dragendorff	Colorless	Colorless	negative
Flavonoid	Sitroborate	Colorless	Yellow	positive
Tannin	$FeCl_3$	Colorless	Brown	positive
Terpenoid	Anisaldehyde-sulfuric acid	Colorless	purplish Panelistse	positive

Based on **Table 1**, it is evident that the corn silk extract contains compounds from the flavonoid, tannin, and terpenoid groups, while alkaloids were not detected. This conclusion is drawn based on the color changes that occurred after applying the visualization reagents, as depicted in the supplementary figures. In the flavonoid test, both the sample and control (either rutin or quercetin) turned from colorless to bright yellow upon spraying with sitroborate, and this change was observable under 366 nm light. Similarly, the tannin test showed color changes in both the sample and control (gallic acid) after reacting with $FeCl_3$, transitioning from a vague or unclear color to a visible brown hue under visible light. Furthermore, the terpenoid test resulted in color changes in both the sample and control (thymol) after adding anisaldehyde, transforming from colorless to purplish Panelistse. However, in the alkaloid test, only the control (quinine) exhibited a color change from colorless to reddish Panelistse after the addition of Dragendorff reagent, while the sample showed no color change.

3.4. Agar Diffusion Antibacterial Test

The solid diffusion antibacterial test is based on the ability of extract compounds to diffuse, with the sample placed on paper discs that diffuse into a medium containing *Streptococcus mutans* at an OD600 (optical density or turbidity level indicating the bacterial population) of 0.25. This OD600 value is chosen because it corresponds to a phase of rapid bacterial growth, making it suitable for the test. Compounds that diffuse create an area, and if these compounds have antibacterial properties, bacteria cannot grow in that area, resulting in a visible difference in color or what is commonly referred to as an inhibition zone (Balouiri et al., 2015).

As shown in **Figure 1**, it is evident that corn silk extract possesses antibacterial properties. The diameter of the inhibition zone increases with the rising concentration of the extract. The respective lengths of the inhibition zone diameters for the 5%, 10%, and 20% extracts are 0.73, 0.93, and 1.3 cm. Furthermore, in the positive control (1% ampicillin), the diameter of the inhibition zone is 1.3 cm, while there is no inhibition zone in the negative control. The positive control serves as an indicator of bacterial growth inhibition, which is visually observable through the formation of inhibition zones. Additionally, the negative control demonstrates that the solvent

used does not possess antibacterial properties. Thus, it is highly likely that the inhibition zones observed in both the extract and the positive control are indeed due to the compounds themselves, rather than the solvent.

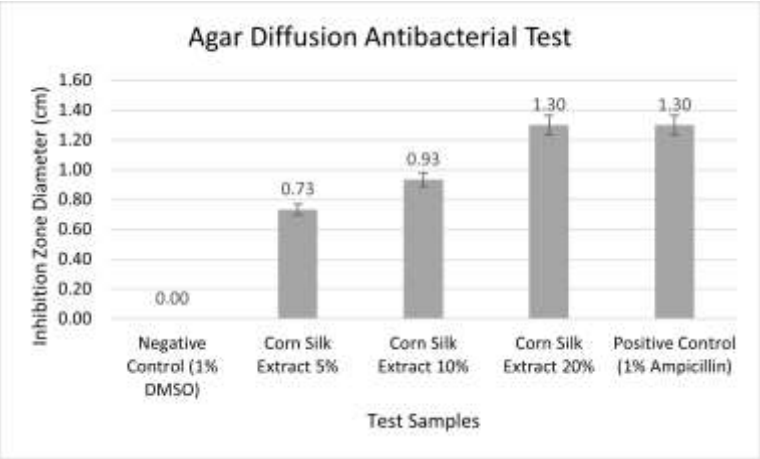

Figure 1. Graph of Solid Diffusion Antibacterial Inhibition Zones

The antibacterial properties of the corn silk extract are likely attributed to the presence of flavonoids, tannins, and terpenoids. Flavonoids can inhibit nucleic acid synthesis, disrupt cell membrane function, and interfere with energy metabolism. Tannins can form hydrophobic complexes with bacterial proteins, inactivate enzymes and transport proteins in the cell wall, and cause the cell wall to contract, disrupting its permeability. Additionally, terpenoids can damage porins (transmembrane proteins in bacteria), disrupting their permeability and causing bacterial nutrient deprivation (Xie et al., 2015).

3.5. Microdilution Antibacterial Test

The microdilution antibacterial test relies on the turbidity level of bacteria. The more turbid, the more bacteria have grown. In a 96-well microplate, compounds are mixed with bacteria, and their turbidity is assessed. If a compound exhibits antibacterial properties, its turbidity will not differ significantly from the control without bacteria. Concentration series of the test compound are used for the microdilution test, allowing the creation of a linear regression equation to calculate the minimum inhibitory concentration at 50% (MIC_{50}), indicating the concentration of the test compound required to inhibit 50% of the bacteria (Balouiri et al., 2015).

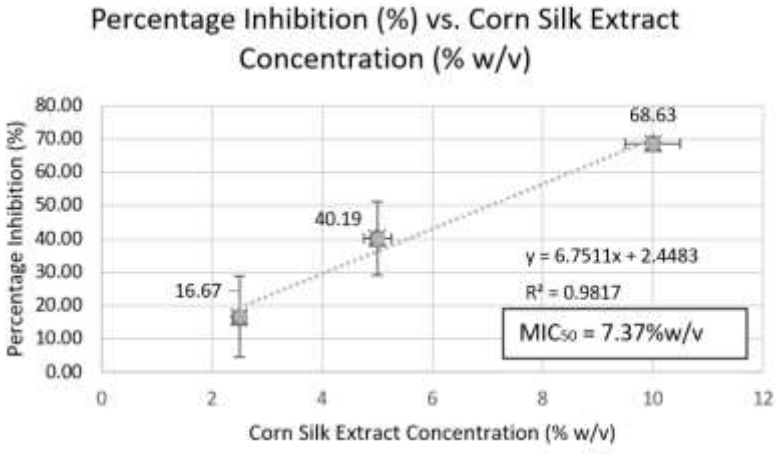

Figure 2. Graph of Percentage Inhibition of *S. mutans* vs. Corn Hair Extract Concentration

Based on **Figure 2**, it is evident that the percentage inhibition or growth inhibition of *S. mutans* bacteria increases with the rising concentration of the extract. The percentage inhibition of *S. mutans* from concentrations of 2.5%, 5%, and 10% is 14.73%, 38.17%, and 68.09%, respectively. This data is plotted in the form of a graph, and a linear regression equation is derived, y = 6.9531x - 0.23, where "y" represents the percentage inhibition of *S. mutans*, and "x" represents the concentration of corn silk extract (% w/v). To find the MIC_{50}, the value of 50 is input into the variable "y," resulting in a value of "x" of 7.22. Therefore, the MIC_{50} of corn silk extract against *S. mutans* is 7.37%, and this concentration serves as a reference for calculating the dosage in the formulation of corn silk extract mouthwash.

3.6. Mouthwash Formulation

The mouthwash formulation is prepared with a final weight of 100 grams using two concentrations of corn silk extract, namely 8% and 10%, in several formulations with ingredient ratios as shown in **Table 2**. The dosage used should be equal to or greater than the MIC_{50} value to ensure optimal efficacy of the mouthwash. Tween 80 is used as a surface tension reducer and surfactant, allowing the mouthwash to penetrate small crevices in the teeth. Sorbitol serves as a sweetener with low-calorie content, making it less likely for any remaining bacteria on the teeth to break down sorbitol into smaller sugar molecules compared to other sweeteners like aspartame and saccharin. Sodium metabisulfite is utilized as an antioxidant, while sodium benzoate acts as a preservative. Water is employed as the solvent. To maintain consistency and ensure that differences in antimicrobial activity between formulations are attributed solely to other variables, all formulations in this study contained the same concentrations of sodium metabisulfite and sodium benzoate. This uniform level of preservatives was selected to effectively prevent microbial contamination and ensure product stability, without affecting the antimicrobial activity of the corn silk extract or other components. The corn silk extract and these additional ingredients are varied in four different formulations (F1, F2, F3, and F4) to determine which one receives the best response from the public.

Table 2. Mouthwash formula

Ingredients	F1 (%w/w)	F2 (%w/w)	F3 (%w/w)	F4 (%w/w)
Corn silk extract	8	8	8	8
Tween 80	1	1	1	1
Sorbitol	10	12	10	12
Sodium metabisulfite	0,1	0,1	0,1	0,1
Sodium Benzoate	0,1	0,1	0,1	0,1
Oleum menthae pip.	0,2	0,18	0,2	0,18
water	Ad 100	Ad 100	Ad 100	Ad 100

3.7. Quality Control Test

Based on **Figure 3**, all four formulations have a pH of 5 from three replicates, indicating that these formulations meet the criteria for a good mouthwash, with a pH range of 5-6. This is because bacteria typically thrive at a pH range of 6-7, so having a different pH can make it challenging for bacteria to survive (Hidayanto et al., 2017).

Table 3 indicates that Formula F2 has the best public response with a total score of 142. Formula F3 and F4 have the second-best response with equal scores, both totaling 120. Formula F1 ranks last with a total score of 118.

Formula F2 has a slightly sweet taste, a reddish-brown color, and a sweet and fresh minty aroma. Formula F1 has a darker color than F2 with the same taste but a stronger aroma. Formula F3 has a darker color than F1 with a slightly more bitter taste. Formula F4 is not significantly different from F2 in terms of taste but has a brighter color than F1 and F4, with an aroma similar to F2.

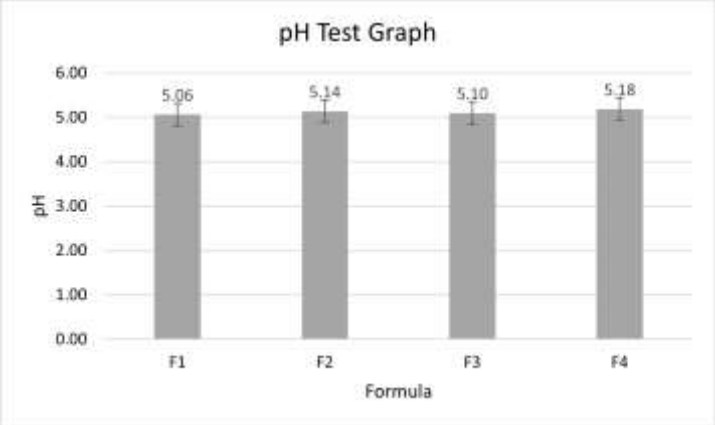

Figure 3. pH Test Graph

Table 3. Public response scores

Formula	Score 5	Score 4	Score 3	Score 2	Score 1	Total Score
F1	2 Panelists	24 Panelists	4 Panelists	0 Panelists	0 Panelists	118
F2	22 Panelists	8 Panelists	0 Panelists	0 Panelists	0 Panelists	142
F3	4 Panelists	22 Panelists	4 Panelists	0 Panelists	0 Panelists	120
F4	5 Panelists	20 Panelists	5 Panelists	0 Panelists	0 Panelists	120

4. CONCLUSION

Our research findings indicate that corn silk extract contains flavonoids, tannins, and terpenoids, while alkaloids are not present. The minimum inhibitory concentration (MIC_{50}) of corn silk extract against *Streptococcus mutans* is determined to be 7.37% w/v. Among the various formulations, Formula F2 garnered the most favorable response from the public, characterized by a reddish-brown color, a minty aroma, a slightly sweet taste, and consisting of 8% corn silk extract, 1% Tween 80, 10% sorbitol, 0.1% sodium metabisulfite, 0.1% sodium benzoate, 0.18% peppermint oil, and water to reach 100% w/w.

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6. AUTHOR DECLARATION

Authors' Contributions and Responsibilities

The authors made substantial contributions to the conception and design of the study. The authors took responsibility for data analysis, interpretation, and discussion of results. The authors read and approved the final manuscript.

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