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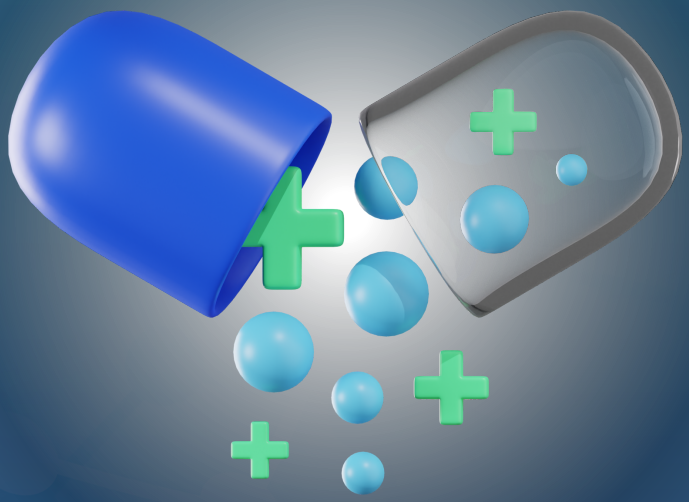
The 3rd Mandala Waluya International Conference on Pharmaceutical Science and Practice

"Pharmaceutical and Biotechnology on Natural Medicines"



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Pharmacy Study Program
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PROCEEDING BOOK

The 3rd Mandala Waluya – International Conference on Pharmaceutical Science and Practice (3rd MW-ICPSP)

"Pharmaceutical and Biotechnology on Natural Medicines"

Kendari, January 25th, 2024

Pharmacy Departement

Faculty of Science and Tehcnology

Mandala Waluya University

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PREFACE

This preceeding contains articles with studies on the latest advances in drug discovery and development. This proceeding comes from the International Conference entitled The 3rd Mandala Waluya – International Conference on Pharmaceutical Science and Practice (3rd MW-ICPSP), which was held by the Department of Pharmacy, University of Mandala Waluya in Kendari in 2023. Various issues and topics in various fields of pharmacy from The conference was brought into the proceedings, including in the field of pharmaceutical science, including Pharmaceutical Technology and Pharmaceutics, Pharmaceutical Analysis, Medicinal Chemistry, Pharmacology and Toxicology, Pharmaceutical Biology, Community Pharmacy, and Clinical Pharmacy.

The success of The 3rd Mandala Waluya – International Conference on Pharmaceutical Science and Practice (3rd MW-ICPSP) led us to make the studies at the conference to be recorded in this proceeding, with the hope that it can be an additional reference for pharmaceutical researchers out there.

Kendari, January, 2024

Dr. apt. Mus Ifaya, S.Farm., M.Farm.
Chairman of The 3rd Mandala Waluya
International Conference on Pharmaceutical Science and Practice

CONTENT

Ethnopharmacy Of Medicinal Plants Originating From Flores And Makassar Tribes For Anticancer Treatment

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ABSTRACT

Indonesian medicinal plants make up a large part of the world's drug production. As many as 45 of the most important medicinal species in the United States come from tropical medicinal plants, 14 of which are native to Indonesia. So people utilize medicinal plants as a treatment. Some tribes in Indonesia have their own experiences in overcoming health problems. This study aims to determine the types of plants used as traditional medicine, how to process, how to use and the types of plants that have the potential to be further studied for bioactivity, especially anticancer bioactivity, Informant Consensus Factor (ICF) and Utility Value (UV) as parameters used to determine the significant value of each plant used in a location. The plants used by the Makassar Tribe (Bollangi Hamlet, Pattallassang District, Gowa Regency) as breast cancer drugs are Benalu Tree (Lorantus sp), Bidara Leaf (*Ziziphus mauritiana* Lam.), Benalu Batu (*Begonia medicinalis*), Gedi Leaf (*Abelmoschus manihot* (L.)) with the value of use value (ICF).) Medik with a use value (UV) of 0.3 and an ICF value of 0.5 respectively, while from the Flores Tribe (Satarpunda Village, North Lambaleda District, Manggarai Regency, NTT) are Ndusuk (*Melastoma malabathricum* L). Nggeduk (*Equisetum hyemale* L), Soursop (*Annona muricata*), Batang Anggur Hitam (*Imperata cylindrica*). With Use Value (UV) of 0.3 and Informant Consensus Factor (ICF) value of 0.3, respectively.

Keywords: Ethnopharmacy, Cancer Medicinal Plants, Flores and Makassar Tribe

INTRODUCTION

Indonesian medicinal plants make up a large part of the world's medicinal production. Of the 45 important medicinal species in the United States, come from tropical medicinal plants, 14 of which come from Indonesia. Medicinal plants can be utilized in various ways, namely drinking, sticking and inhaling, so that their efficacy fulfills the concept of cell receptor work in receiving chemical compounds or stimuli. (Siregar et al., 2020).

Public interest in the use of traditional medicine in Indonesia is still very high. So that people utilize medicinal plants. Some tribes in Indonesia have their own experiences in overcoming health problems. It is also known that 22.3% of Indonesians prefer traditional medicine that uses medicinal plants (herbs). In the future there will be adaptations, maybe we will return to natural medicine (back to nature), so the state and similar institutions must pay attention and regulate their facilities. (Anonymous, 2018).

The utilization of medicinal plants is one way to discover new medicines, traditional medicine is often the pioneer in the creation of new drugs. Ethnopharmacy is a branch of medicine that involves the understanding of medicinal herbs used and the study of the medicinal use of special ethnic groups or communities. The field of ethnopharmacy is the medicinal plants used and how they are used. Regional ethnic communities have unique cultures and local wisdom due to regional differences. This study aims to determine the types of plants that are used as traditional medicine, how to process, how to use and the types of plants that have the potential to be further studied for bioactivity.

METHODS

In this study, the utilization of medicinal plants in traditional medicine uses a Bioactivity Test testing system to determine the biological activity of a sample, especially anticancer bioactivity, Informant Consensus Factor (ICF) for a value that states the consistency of information between informants who are research samples based on disease categories, Utility Value (UV) as a parameter used to determine the significant value of each plant used in a location. This research was conducted in the area of North Lambaleda District, one of the sub-districts in East Manggarai Regency, where the Flores tribe lives, Satarteu Village, one of the villages in Langke Rembong District and Bollangi, a village in Gowa Regency, precisely in Pattallassang District, Timbuseng Village. About 17 km from the capital of Gowa Regency, Sungguminasa.

How to process and use plants as traditional medicine.

Makassar Tribe

a. Leaves of Tree Benalu *(Lorantus sp),* 3 benalu leaves and 2 cups of water boiled until 1 cup remains, taken once a day.
b. Bidara Leaf *(Ziziphus mauritiana Lam.),* 3 bidara leaves and 2 cups of water boiled until 1 cup remains, boiled and drunk 1x a day.
c. Benalu Batu Leaf *(Begonia medicinalis),* 3 benalu batu leaves and 2 cups of water boiled until 1 cup remains, boiled and drunk 1x a day.
d. Red Gedi Leaf *(MalvaceaeAbelmo schus manihot (L.) Medik.),* 7 leaves and 2 cups of water boiled until 1 cup remains, boiled and drunk 1x a day.

Flores Tribe

a. Ndusuk *(Melastoma malabathric um L.*), 7 pieces of flowers/leaves boiled with 500ml of water until 200 ml is drunk, drink twice a day and young leaves are pounded, then applied to the breast, Apply every day.
b. Nggeduk *(Equisetum hyemale L.)*, roots and stems cut into small pieces, 1 handful boiled + enough water, boil until boiling, drink twice a day and flower/leaf sheet boiled with 500ml of water until 200ml is drunk, Apply every day.
c. Forest Vine Leaf (*Graptophyllum pictum),* Stems are cut small, 1 handful boiled + enough water, boil until boiling, drink twice a day and Stems are pulverized and added with rice flour, Apply every day.
d. Soursop *(Annona muricata)*, Flower/leaf sheet boiled with 500ml of water until 200ml is drunk, drink twice a day and young leaves are pounded, then applied to the breast, Apply every day.

RESULTS AND DISCUSSION

Research Results (Makassar Tribe)

Ethnopharmacological research conducted in Bollangi Hamlet, Patalassang Subdistrict, Gowa Regency. about the use of medicinal plants as herbal medicines for diseases, The results showed that, in 2023 in Gowa Regency found 4 types of medicinal plants for breast cancer and 6 people who had suffered from breast cancer.

Table 1. The Use Value (UV) of Makassar Tribe

No.	Lokal Name	Scientific Name	U	n	Uv
1	Tree Benalu	Lorantus sp.	6	24	0,3
2	Bidara Leaf	Ziziphus mauriliana Lam.	6	24	0,3
3	Benalu stone	Begonia medicinalis	6	24	0,3
4	Gedi Leaf	Abelmoschus manihot (L.) Medik	6	24	0,3

The UV value is categorized as high if the plant is used by respondents in large quantities or the UV value is more than 0.1 (Chaachouay et al. 2019), and the UV value is categorized as low if it is used by few respondents (Sarquis et al. 2019) or UV is close to 0 (Zahoor et al. 2017). so that the results of the use value (UV) on Tree Benalu are 0.3, Bidara Leaf is 0.3, Benalu Stone is 0.3, and Gedi Leaf is 0.3. All UV values < 1.

Table 2. Informant Consensus Factor Value (ICF)

Disease Category	Disease Type	Plant Type	Nat	Na	TCR $= \frac{nar - n}{nar - 1}$
Cancer	Breast cancer	Tree Benalu	3	2	0,5
		Bidara Leaf			
		Benalu stone			
		Gedi Leaf			

Informant Consensus Factor (ICF) is a value that expresses the consistency of information between informants who sampled the study based on disease categories. ICF has a low value (close to 0) when plants are randomly selected or no information about the use of plants is exchanged by each informant, and a high value (close to 1) when plants are used by many informants and information is exchanged (Sarquis et al., 2019).so that the informant consensus factor value (ICF) in cancer disease obtained the results 0.5 < 1.

Research Results (Flores Tribe)

Based on community interviews in Satarteu Village, Satarpunda Sub-district, East Manggarai Regency, NTT. There are 9 types of plants known to be used in traditional medicine, 4 of which are used as anticancer drugs.

Table 3. The Use Value (UV) of Flores Tribe

Lokal Name	Scientific Name	U	n	Uv
Ndusuk	Melastoma malabathrium L.	6	9	0,3
Nggeduk	Equisetum hyemale L.	6	9	0,3
Soursop	Annona muricata	6	9	0,3
Black Grape Stem	Imperata cylindrica	6	9	0,3

Plants used by the community as anticancer treatments obtained UV values on Ndusuk plants are 0.3, Nggeduk is 0.3, Soursop is 0.3 and Black Grape Stem is 0.3. All UV Values <1

Table 4. Informant Consensus Factor Value (ICF)

Disease Category	Disease Type	Plant Type	Nar	Na	TCR $= \frac{nar - n}{nar - 1}$
Cancer	Breast cancer	Ndusuk	6	9	0,3
Cancer	Breast cancer	Nggeduk	6	9	0,3
Cancer	Breast cancer	Soursop	6	9	0,3
Cancer	Breast cancer	Black Grape Stem	6	9	0,3

CONCLUSIONS

1. The plants used by the Makassar tribe (Bollangi Hamlet, Pattallassang District, Gowa Regency) as breast cancer are Benalu Tree (*Lorantus* sp), Bidara Leaf (*Ziziphus mauritiana* Lam.), Benalu Batu (*Begonia medicinalis*), Gedi Leaf (*Abelmoschus manihot* (L.) Medik. While the Flores Tribe (Satarpunda Village, North Lambaleda District, Manggarai Regency, NTT) is Ndusuk (*Melastoma malabathricum* L). Nggeduk (*Equisetum hyemale* L), Soursop (*Annona muricata*), Black Vine Stem (*Imperata cylindrica*).
2. The people of the Makassar tribe and the Flores tribe process medicinal plants by boiling (most widely used), pounding and consuming directly.
3. The use of plants as traditional medicine in these two tribes is by drinking, rubbing/pasting, eating/chewing.
4. Based on the results of the analysis using the calculation method of Use Value (UV) and Informant Concentrus Factor (ICF), the plants that have the potential to be tested for bioactivity in the Makassar Tribe are the Use Value (UV) of Benalu Pohon (*Lorantus* sp), Bidara Leaf (*Ziziphus mauritiana* Lam.), Benalu Batu (*Begonia medicinalis*), Gedi Leaf (*Abelmoschus manihot* (L.) Medik), which is 0.3 and the Informant Value (ICF) of Gedi Leaf (*Abelmoschus manihot* (L.) Medik), which is 0.3 and the Informant Consensus Factor (ICF) value of breast cancer (0.5). while the Flores tribe Ndusuk (*Melastoma malabathricum* L). Nggeduk (*Equisetum hyemale* L), Soursop (*Annona muricata*), Black Vine Stem (*Imperata cylindrica*) which is 0.3 and the Informant Consensus Factor (ICF) value of breast cancer is 0.3.

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Potential Drug-Drug Interactions in Prescriptions to Hypertensive Patients at Apotek Farmasi Airlangga Surabaya

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ABSTRACK

Hypertension defined as a systolic blood pressure greater that 130 mmHg or a diastolic blood pressure greater than 80 mmHg. Antihypertensive agents are frequently used in combination in order to reach the suggested blood pressure or the goal therapy. Concomitant usage of antihypertensive agents or with other medications leads to the increase of potential drug-drug interactions (PDDIs) which could interfere the medication effectivity or harm the patients. This study was purposed to analyze the PDDIs in prescriptions written for hypertensive patients at Apotek Farmasi Airlangga Surabaya in December 2022. The PDDIs of 91 hypertensive patients' prescriptions was analyzed using online Medscape drug interaction checker. The results showed that 91 of 196 (46.4%) prescriptions analyzed had a total of 176 PDDIs. The vast majority of PDDIs recorded was between antihypertensive agents at 47.2% while 38.1% of the PDDIs occurred at the concomitant use of antihypertensive agents with other medication and the remaining 14.7% of PDDIs were the consequence of inter usage of other medications. Additionally, most of PDDIs occurred were pharmacodynamic reactions at 78.4% while based on its severity, most of PDDIs needed to be monitored closely at 82.6%. Amlodipine-bisoprolol was the most frequent potential drug interaction pair that found at 13.6% followed by candesartan-bisoprolol at 11.4%. Closely monitoring on blood pressure and potassium serum levels are essentials in order to preven the incident of PDDIs.

Keywords: Hypertension, Drug-drug Interaction, Prescription, Antihypertensive agents.

INTRODUCTION

Hypertension defined as a systolic blood pressure greater that 130 mmHg or a diastolic blood pressure greater than 80 mmHg [1]. The high blood pressure condition lead to the cardiovascular disease and death globaly. In 2010, 1.39 billion of adult worldwide had hypertension with systolic blood pressure greater that 140 mmHg and/or diastolic blood pressure greater than 90 mmHg [2]. The prevalence of hypertension is increase globally due to the exposure of lifestyle risk factors such as sedentary lifestyle and the ageing of the population [3].

The goal therapy of hypertension is to achieve blood pressure <140/90 mmHg, or <130/80 mmHg for patients with diabetes or chronic kidney disease [4]. Multiple antihypertensive drugs including thiazides, calcium channel blockers, β-blockers, angiotensine receptor blockers (ARBs), etc are prescribed to achieve the goal therapy. Additionally, medications for other co-morbid conditions which are much frequent with hypertension such as myocardial infarction, diabetes mellitus, chronic kidney disease, and congestive cardiac failure, make hypertensive patients are exposed to potential drug-drug interactions (PDDIs) which could interfere the medication effectivity or harm the patients.

A cross sectional study conducted in Czech Republic revealed that manifest drug-drug interactions (DDIs) contributed to 3,3% drug-related hospital admissions in elderly [5]. Moreover, a study in France reported that elderly patients admitted to emergency departments are particularly exposed to high-risk potential DDIs [6]. Beside its impact to the hospital and emergency admissions DDIs also had significant contribution to the length and cost of hospitalization. An analysis in PDDIs would prevent any adverse drug reactions caused by DDIs. This study was purposed to observe PDDIs on medications prescribed to hypertensive patients.

METHODS

This was a prospective cross-sectional study conducted among prescriptions written for hypertensive patients during December 2022 at Apotek Farmasi Airlangga Surabaya. The study was commenced after getting permission from the official of the pharmacy. Adult hypertensive patient's prescriptions of both genders with and without comorbidities were included in the study. Prescriptions which only contained of one medication were excluded. The screening of PDDIs of each prescriptions was conducted through Medscape drug interaction checker. Based on Medscape drug interaction checker, the PDDIs severity were classified as minor (current medications can be continued), monitor closely (close monitoring is required), and serious (suggested for alternative medication). PDDIs were further categorized based on the mechanism as pharmacodynamic interactions, pharmacokinetic interactions, and unspecified using Medscape drug interaction checker. Additionally, the clinical manifestation of the PDDIs were also presented descriptively.

RESULTS AND DISCUSSION

A total of 196 hypertensive prescriptions contained 2 or more medications were analyzed. Among the samples, 91 (46.4%) had PDDIs (Figure 1).

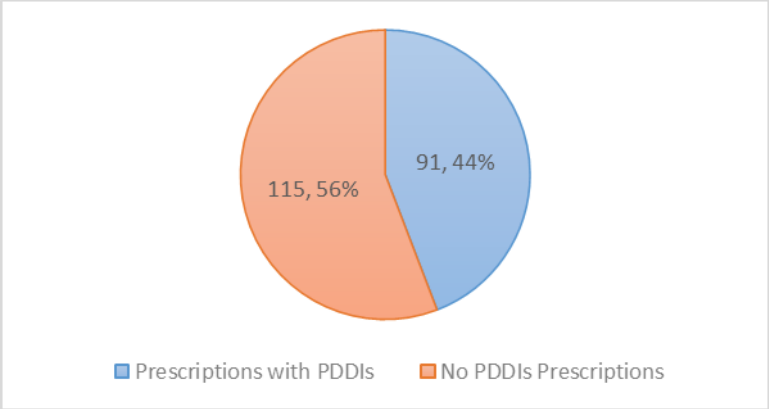

Figure 1. The proportion of Prescriptions with PDDIs

The characteristics of prescriptions with PDDIs is illustrated in Table 1. Of 91 prescriptions, males were slightly predominant than females at 51.7%. Related to age, unfortunately, 47.3% of prescriptions had no information about the patient's age while 33.0% of them belonged to the age group of >60 years. The majority of prescriptions contained of 3 items of drug at 38.5% with the average number of drugs per prescription were 3.65 ± 1.11. Beside that, 78.0% of the hypertensives prescriptions were prescribed with other class of drug with the most frequent other class drugs prescribed was oral hypoglycemic agents (31.9%).

Table 1. Characteristics of the prescriptions with PDDIs

	Chategories	Number of prescriptions	Percentage (%)
Gender	Male	47	51.7
	Female	44	48.3
Age (years)	<40	4	4.4
	40-60	14	15.4
	>60	30	33.0
	No information	43	47.3
Number of drugs prescribed	2	11	12.1
	3	35	38.5
	4	29	31.9
	>4	16	17.6
Prescribed with other class of drug	Yes	71	78.0
	No	20	22.0
Other class drug prescribed	Oral hypoglycemic drugs	29	31.9
	Insulins	4	4.4
	Antihyperuricemic	6	6.6

	agents		
	Expectorants	2	2.2
	Antibiotics	4	4.4
	Gastrointestinal medications	11	12.1
	Vitamins	20	22.0
	NSAIDs	12	13.2
	Antiplatelets	12	13.2
	Corticosteroids	6	6.6
	Direct vasodilators	9	9.9
	Anticholesterol	12	13.2

Among 91 antihypertensives prescription analyzed, there were 176 PDDIs observed from Medscape online drug interaction checker. Of that figure, 47.2% was PDDIs that occurred between antihypertensive agents while 38.1% and 14.7% remaining were PDDIs observed between antihypertensive drugs and other class drugs and between non-antihypertensives drugs, respectively (Figure 2). This finding was higher than those reported by Subramanian *et al.*that PDDIs among hypertensive agents was 38 out of the total PDDIs observed (30.9%) [7]. This present results was also higher than the results of two studies conducted in Indonesia by Herliany *et al.* and Swandari *et al.* that revealed 32.9% and 3.9% of PDDIs among antihypertensive medications, respectively [8,9]. This difference probably caused by the different in drugs prescription profiles and drug interaction tool used since study by by Herliany *et al.* and Swandari *et al* used drugs.com or Lexicomp. In Subramanian *et al*. study, Medcape drug interaction checker also used to screen the PDDIs. However, different drugs prescription profiles and time of study might cause the discrepancy in results.

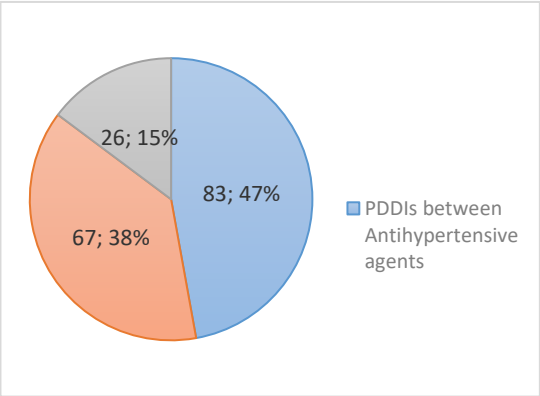

Figure 2. The Proportion of PDDIs

The proportion of PDDIs based on its mechanism is shown in Figure 3. The vast majority PDDIs observed were pharmacodynamic reactions which calculated at 78.4% followed by unknown mechanism of interactions that counted at 14.7%. Meanwhile, pharmacokinetic interactions only observed at 6.9% of the total PDDIs. The figures of pharmacodynamic reactions in the present study was higher than previous studies which counted at 37.3% and 40% [7,10]. Oppositely, the proportion of pharmacokinetic PDDIs in our study was much lower compared to the previous study that counted 22.7% and 60% [7,10]. Unsimilar results in types of PDDIs is also closely related to the prescription profiles and drug interaction tools used because the information related to drug interactions varies significantly from one resource to another [11].

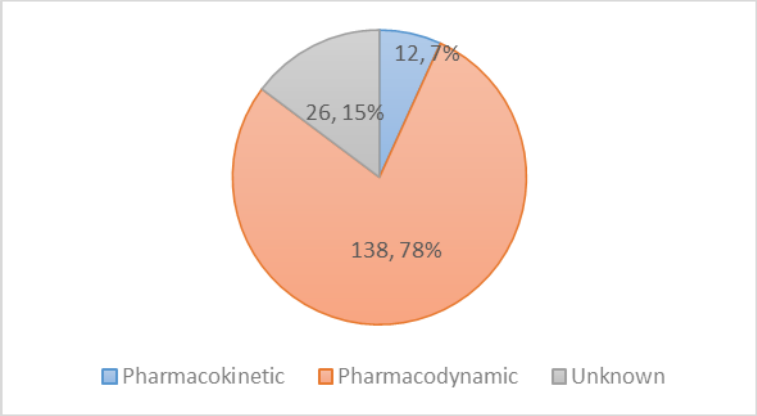

Figure 3. The Prevalence of PDDIs based on its Mechanism

The distribution of the PDDIs based on its severity revealed that the majority of PDDIs had moderate severity which need to be monitored closely at 82.6%. Additionally, 9.7% of the total PDDIs were serious reactions while the remaining 6.9% were minor interactions (Figure 4). Our findings in the severity of PDDIs were in line with other studies which indicated predominantly moderate severity PDDIs among hypertensive prescriptions although the percentage vary from 63.11% to 89.4% [7–9,12]. However, serious PDDIs observed in our study was similar with the study by Saraswati *et al* at 9.8%. but lower than those documented by Herliany *et al.* at 15.1% [8,12]. The serious PDDIs was dominated on the use of amlodipine with simvastatin which increase the potential of myopathy. The risk of muscular toxicity is caused by the increase of simvastatin's bioavailability due to the presence amlodipine as CYP3A4 inhibitor [13]. However, a study by Fuhrmann reveal no evidence for a clinically relevant interaction between amlodipine and simvastatin although muscular discomfort was detected in 6.20% of the patients receiving amlodipine and simvastatin [14].

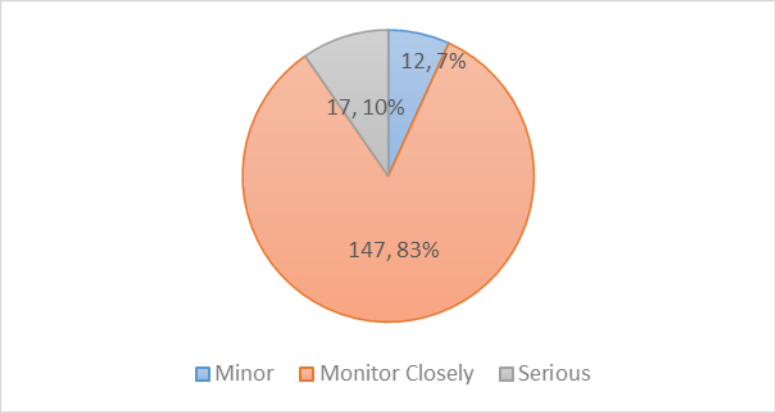

Figure 4. The Prevalence of PDDIs based on its Severity

The distribution of potential drug interacting pairs among anti hypertensive drugs group and with other class of drugs are presented in Tabel 2 and Table 3. Among hypertensive drugs, amlodipine-bisoprolol and candesartan-bisoprolol were the most frequent drug interaction pairs that constituted 13.6% and 11.4% of the total PDDIs, respectively. This interaction pairs had potential manifestation hypotension and hyperkalemia which need closely monitor on blood pressure and potassium serum level. Regarding the evidence, there is still lack of data about the incidence of hyperkalemia on the concomitant use of candesartan and bisoprolol. However, a recent study assessing the onset of hyperkalemia following the administration of ARBs showed that the overall incidence of hyperkalemia was very low, and there was no statistically significant difference according to the type of ARBs [15]. Moreover, a systematic review and metaanalysis indicated that bisoprolol treatment for 8 weeks resulted in a slight but statistically significant change in potassium levels [MD: −0.10; 95% CI: −0.16, −0.04; P<0.01] among HT patients [16]. Hence, closely follow up on the potassium levels of patients receiving candesartan and bisoprolol is needed.

The increasing risk of hypotention due to the combination of amlodipine and bisoprolol could be harmful for particular hypertension patients. However, multicenter, randomized, comparative phase III study reported that the concomitant use of amlodipine and bisoprolol was beneficial to reach blood pressure improvements in patients with essential hypertension following monotherapy failure [17].

In regards to potential drug interacting pairs among anti hypertensive with other class of drugs, the most common PDDIs occurred in the concomitant use of amlodipine-metformin at 10.2% with potential manifestation hyperglycemia. Nevertheless, a study by Yongyi *et al.* evaluating the effect of amlodipine combined with metformin in treatment of patients with obesity-related hypertension showed opposite results. It was reported that fasting blood glucose (FBG), glycated hemoglobin (HbAlc), as well as fasting insulin (FINS) in group of patients receiving amlodipine and

metformin were significantly lower than those who only received amlodipine (P<0.05) [18]. The study confirmed that the combination of amlodipine and metformin in the treatment of patients with obese-related hypertension has a better clinical effect, which can improve blood lipid level, alleviate obesity and insulin resistance, and has higher safety in medication compared to metformin monotherapy [18].

Table 2. Distribution of potential drug interacting pairs among anti hypertensive drugs group

Drug Interaction Pairs		Mechanism of PDDIs	Severity	Potential Manifestation	n	(%)
Amlodipine	Bisoprolol	Pharmacodynamic Synergism	Monitor Closely	Hypotension	24	13.6
Candesartan	Bisoprolol	Pharmacodynamic Synergism	Monitor Closely	Hyperkalemia	20	11.4
Nifedipine	Bisoprolol	Pharmacodynamic Synergism	Monitor Closely	Hypotension	8	4.5
Furosemide	Bisoprolol	Pharmacodynamic Antagonism	Monitor Closely	Preventing Hypokalemia	6	3.4
Candesartan	Spironolacton	Pharmacodynamic Synergism	Monitor Closely	Hyperkalemia	6	3.4
Bisoprolol	Spironolacton	Pharmacodynamic Synergism	Monitor Closely	Hyperkalemia	5	2.8
Furosemide	Candesartan	Pharmacodynamic Antagonism	Monitor Closely	Preventing Hypokalemia	3	1.7
Carvedilol	Amlodipine	Pharmacodynamic Synergism	Monitor Closely	Hypotension	2	1.1
Spironolacton	Furosemide	Pharmacodynamic Antagonism	Monitor Closely	Preventing Hypokalemia	2	1.1
Telmisartan	Bisoprolol	Pharmacodynamic Synergism	Monitor Closely	Hyperkalemia	2	1.1
Candesartan	Hydroklorothiazide	Pharmacodynamic Antagonism	Monitor Closely	Preventing Hypokalemia	2	1.1
Carvedilol	Candesartan	Pharmacodyn	Monitor	Hypotension	1	0.6

		amic Synergism	Closely			
Carvedilol	Nifedipine	Pharmacodynamic Synergism	Monitor Closely	Hypotension	1	0.6
Diltiazem	Bisoprolol	Unknown	Serious	Bradicardia	1	0.6

Table 3. Distribution of potential drug interacting pairs among anti hypertensive with other class of drugs

Drug Interaction Pairs		Mechanism of PDDIs	Severity	Potential Manifestation	n	(%)
Amlodipine	Metformin	Pharmacodynamic antagonism	Monitor Closely	Hyperglycemia	18	10.2
Amlodipine	Simvastatin	Uknown	Serious	Myopathy	10	5.7
Bisoprolol	Aspirin	Pharmacodynamic antagonism	Monitor Closely	Hypertension	6	3.4
Candesartan	Aspirin	Pharmacodynamic antagonism	Monitor Closely	Hypertension	5	2.8
Amlodipine	Dexamethasone	Pharmacokinetic metabolism	Minor	Hypertension	4	2.3
Candesartan	Insulins	Uknown	Monitor Closely	Hypoglycemia	4	2.3
Nifedipine	Metformin	Pharmacodynamic antagonism	Monitor Closely	Hyperglycemia	3	1.7
Nifedipine	Simvastatin	Pharmacokinetic metabolism	Serious	Myopathy	2	1.1
Spironolactone	Aspirin	Uknown	Monitor Closely	Hypertension	2	1.1
Furosemine	Metformin	Uknown	Minor	Hypoglycemia	2	1.1
Bisoprolol	Antacid	Pharmacokinetic absorbtion	Monitor Closely	Hypertension	1	0.6
Bisoprolol	Mefenamic Acid	Pharmacodynamic antagonism	Monitor Closely	Hypertension	1	0.6
Captopril	Antacid	Pharmacokinetic absorbtion	Monitor Closely	Hypertension	1	0.6
Captopril	Mefenamic Acid	Pharmacodynamic antagonism	Serious	Hypertension	1	0.6
Captopril	Metformin	Uknown	Monitor Closely	Hypoglycemia and lactate	1	0.6

				acidosis		
Diltiazem	Aspirin	Uknown	Minor	Bleeding	1	0.6
Diltiazem	Metformin	Pharmacodynamic antagonism	Monitor Closely	Hyperglycemia	1	0.6
Lisinopril	Glimepiride	Pharmacodynamic sinergism	Monitor Closely	Hypoglycemia	1	0.6
Lisinopril	Metformin	Uknown	Monitor Closely	Hypoglycemia and lactate acidosis	1	0.6
Furosemide	Aspirin	Pharmacodynamic antagonism	Monitor Closely	Hypertension	1	0.6
Telmisartan	Simvastatin	Uknown	Minor	Myopathy	1	0.6

This study had limitation since the analysis of PDDIs was only conducted descriptively and no evidence evaluation. A further study analyzing the predictors of the PDDIs followed by the investigation of the occurrence of PDDIs in clinical setting are needed.

CONCLUSION

The results of this study highlighted that hypertensive patients are exposed to PDDIs mainly pharmacodynamic reactions that need to be monitored closely. Since the most frequent potential drug interaction pair were amlodipine-bisoprolol and candesartan-bisopolol, closely monitoring on blood pressure and potassium serum levels are essentials in order to prevent the incident of PDDIs.

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The Effectivity of The Gelatin Extract Baronang's Bone (*Siganus canaliculatus*) as Drug Of The Healing Burns In Rabbits (*Oryctolagus cuniculus)*

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ABSTRACT

Utilization of waste had Baronang fish bones (*Siganus canaliculatus*) into gelatin is an alternative to increase the economic value of waste, also because of the lack of attention to testing the activity of gelatin extract from fish bones which has medicinal properties. Gelatin basically is a pure protein, obtained from thermal denaturation of collagen contained in the skin and bones fish's. Baronang fish bones contain collagen which functions to regenerate skin in burns. The aims of this research to determine the effectiveness of Baronang fish bone gelatin extract (*Siganus canaliculatus*) as drug of the healing burn in rabbits (*Oryctolagus cuniculus*). The gelatin extraction from baronang fish bones with concentration of 3% acetic acid for about 18 hours, followed by a characteristics of organoleptic test. The effectiveness test of baronang fish bone gelatin extract (*Siganus canaliculatus*) with variations in the concentration of 0.5%, 1% and 2%. The result obtained was organoleptic test showed the gelatin extract of baronang fish bones (*Siganus canaliculatus*) are chewy, brownish in color, had a distinctive odor and homogeneous. In the effectiveness test of Baronang fish bone gelatin extract (*Siganus canaliculatus*), which has effectiveness against burns and the best concentration for burns was obtained 2%, it can be concluded that baronang fish bone gelatin extract (*Siganus canaliculatus*) has the effectiveness of the healing burns.

Keywords :*Siganus canaliculatus*, Gelatin, Healing Burns

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INTRODUCTION

Burns are a serious injury that can affect anyone. It is estimated that one in approximately 3.5 million people will experience burns (Sheridan, 2011). *World Health Organization* (WHO) estimates that there are 265,000 deaths occurring every year worldwide due

to burns (WHO, 2014). In Indonesia, it is reported that more than 250 people die per year due to burns and the symptoms tend to increase.

Treating burns with natural ingredients is a safe way to treat burns and at the same time reduces the cost of treating burns. Currently, the use of burn medicine comes from plants, so innovation is needed in the use of marine resources (Lasabuda, 2013).

Indonesia as a tropical country is rich in high biodiversity. The potential of Indonesia's marine fisheries resources is approximately 6.4 million tons per year (Lasabuda, 2013). One type of fish that is found or widespread in Indonesia is the rabbitfish (*Siganus canaliculatus*) and is found in Tondonggeu Waters in the Abeli District, Kendari City, Southeast Sulawesi Province, which is located on the coast. This fish is an economically important fish and is usually processed into it *fillet*. Utilization as *fillet* can produce waste in the form of bones (Songchotikunpan *et al*., 2008).

About 30% of fish consists of skin, bones and scales which contain collagen protein (Walters & Stegemann, 2014). Based on research (Mohtar *et al*., 2011) states that rabbitfish bones contain collagen. Research that has been carried out (Are *et al.*, 2016) tuna fish bone collagen gel preparation formulation (*Albacore tuna*) with a concentration of 2% can be used for healing burns and research (Tungadi, 2020) administration of 2% snakehead fish extract showed significant results in accelerating the healing process of wounds on rabbit skin.

The use of conventional gelatin is a limiting factor in the widespread application of gelatin, such as gelatin originating from pigs which is haram in Islam and gelatin originating from cow skin and bone is also haram for Hindu society. Therefore, research is starting to look for various sources of alternative gelatin raw materials from fish that can replace conventional gelatin, including extracting gelatin from fish skin (Karim & Bhat, 2009); (Abd Elgadir *et al*., 2013), Fishbone (That *et al*., 2013; Sanaeus*et al*., 2013), even including gelatin from new sources viz*edible insect* (Mariod, 2013).

Gelatin is obtained through an extraction process obtained from thermal denaturation of collagen from animals (Haryati *et al*., 2019). Acetic acid is an organic acid that can be used in collagen extraction and has high extractability (Astiana & Nurjanah, 2016). Study (Trilaksani *et al*., 2012) shows that the best gelatin extraction results are an acetic acid concentration of 3% and a soaking time of 18 hours.

Based on the background that has been explained, researchers are interested in utilizing waste from rabbitfish bones (*Siganus canaliculatus*) has economic value and can be used as a raw material for healing medicines for burns.

METHOD

Research Tools and Materials

The tools used include acid-resistant containers, digital scales, pipettes, beakers, ovens, pH meters, measuring cups, thermometers, metal plates with a diameter of 2 cm, and calipers.

The materials used include Baronang fish bones (*Siganus canaliculatus*) obtained from waste (processing residue *fillet*) at Labakkang restaurant, Aroma Sulawesi, Sarappo, Fisherman's Village, Kendari City Seafood restaurant, distilled water, acetic acid (CH_3COOH) 3%, gel Bioplacenton®.

The test animals used in this research were rabbits (*Oryctolagus cuniculus*) 2 healthy males with a body weight of 2.3 kg aged 6 months.

Works procedure

1. Baronang Fish Bone Preparation (*Siganus canaliculatus*)

Baronang fish bone waste (*Siganus canaliculatus)* 5 kg fresh washed clean, separated from the remains of meat and fat that are still attached by soaking in water at a temperature of 80^0C for 60 minutes while stirring. Then dried in the oven at 50^0C for 60 minutes and the bones are cut into small pieces (1.5-2 cm).

2. Making Baronang Fish Bone Gelatin Extract (*Siganus canaliculatus*)

Rabbitfish bones (*Siganus canaliculatus)* which has been dried and cut into small pieces of 5 kg is soaked with 500 ml of 3% acetic acid in a glass jar for 18 hours. Experienced fish boness *welling* (bulging) forms soft bones (*ossein*) washed repeatedly until it reaches pH 5-6. Heating is carried out at an extraction temperature of 80-83^0C for 3 hours with a ratio of fish bones and distilled water of 1:3. The filtrate obtained from the extraction is filtered using a filter cloth and dried in an oven at a temperature of 40-50^0C for 48 hours. The resulting gelatin is then ground until dry gelatin is obtained in the form of fine granules.

3. Gelatin Characteristics Testing

Gelatin characteristics include organoleptic tests, acidity (pH) tests, water content tests, and gelatin yield measurements.

a. Organoleptic Test

Organoleptic tests are carried out to see the physical appearance of the preparation by observing the shape/consistency, color, smell and taste (Allen & Ansel, 2013).

b. Use pH

Gelatin was weighed as much as 1 g dissolved in 10 mL of distilled water then the pH was measured using a pH-meter, left for a few moments and the results were adjusted to the universal pH standard. The pH of the preparation that meets the skin pH criteria is in the interval 4.5 – 6.5 (Tranggono & Latifah, 2007).

c. Test the Water Rate

Testing the water content of gelatin is intended to determine the water content contained in gelatin. The water content of gelatin will affect its shelf life, because it is closely related to the metabolic activities that occur while the gelatin is stored (Trilaksani *et al*., 2012).

d. Gelatin Rend

Determination of gelatin yield aims to see how effective and efficient the extraction process is to make gelatin. A high yield value will indicate a high level of efficiency during the manufacturing process (Huda *et al*., 2013; Ridhay *et al*., 2016).

4. Percentage of Burn Wound Healing in Baronang fish bone gelatin extract (*Siganus canaliculatus*)

The data to be analyzed is the percentage of burn wound healing obtained by measuring the average diameter of the burn wound. Measurements are carried out once every day after treatment with:

$d_{x\,(1,2,3\ldots10)}$ = average burn wound diameter for each treatment repetition

d = number of treatments

Calculated using the formula:

$$d_x = \frac{d1+d2+d3+d4+d5+d6+d7+d8+d9+d10}{10}\ x\ 100\%$$

Burn wound healing percentage:

$$P\% = \frac{do-dx}{dx}\ x\ 100\%$$

Information :

P% : percentage of wound healing

do : initial wound diameter

dx : wound diameter on the day of observation

5. Testing the Effectiveness of Extracts from Baronang Fish Bones (*Siganus canaliculatus*) as a medicine for burns in rabbits (*Oryctolagus cuniculus*)

Test the effectiveness of Baronang fish bone extract (*Siganus canaliculatus*) in healing wounds begins by making a burn on the skin of the rabbit's back (*Oryctolagus cuniculus*). First, the back was anesthetized with lidocaine hydrochloride gel. After that, the skin is attached to a heat inducer with a temperature of 80^0C for 7 seconds. The heat induction tool is a metal plate with a diameter of 1.9 cm which is heated with a blue flame.

The next stage is to evaluate the ability to heal burn wounds using 6 treatment groups on Rabbits (*Oryctolagus cuniculus*) namely Group I which was not treated, Group II was given liquid extract of rabbitfish bone gelatin (*Siganus canaliculatus*), Group III was given Bioplacenton® gel as a positive control, group IV was given a thick extract of 0.5% baronang fish bone gelatin, group V was given a thick extract of 1% baronang fish bone gelatin, and group VI was given a thick extract of baronang fish bone gelatin (*Siganus canaliculatus)* 2%. Wound treatment is carried out by administering the extract twice a day in the morning and evening for 10 days by applying it to the wound and the wound is covered with gauze and the diameter of the wound is measured using a caliper.

Data analysis

Data on physical characteristics and healing time of burn wounds up to 100% were analyzed statistically using the Kolmogorov-Smirnov test to determine the data distribution and continued with one-way analysis of variance (ANOVA) with SPSS 17.0*for window* to determine differences between treatment groups due to the influence of the test preparation. Then an LSD analysis test was carried out (*Least Significant Different*) to determine significant differences between the two treatments.

RESULTS AND DISCUSSION

Burns are caused by the transfer of energy from a heat source to the body. This heat is transferred through conduction or electromagnetic radiation. Skin with burns will experience damage to the epidermis, dermis and subcutaneous tissue depending on the causal factors and the length of time the skin is in contact with the heat source or cause. The depth of the burn will cause damage or disruption of skin integrity and cell death (Moenadiat, 2005).

In this research, the initial stage was carried out to test burn wounds on test animals in the form of rabbits (*Oryctolagus cuniculus)* First, Baronang fish bone samples were taken (*Siganus canaliculatus*) at several processed fish restaurants in Kendari City. Baronang fish bone samples (*Siganus canaliculatus*) collected as much as 5 kg, the samples were processed by washing the fish bone samples clean, separating them from the remains of meat and fat that were still attached to facilitate the process of removing the gelatin from the fish bones, by soaking them in water at a temperature of 80^0C for 60 minutes while stirring. The purpose of using this temperature is in accordance with the solubility point of fat and the coagulation temperature of albumin, which ranges from 32-80°C. Using temperatures of more than 80°C can reduce the amount of collagen produced (Atma & Ramdhani, 2017). Dried in the oven at 50^0C for 60 minutes and the bones are cut into small pieces (1.5-2 cm) with the aim of expanding the surface area so that during the immersion process with acetic acid and the main extraction, the reaction takes place more quickly (Atma & Ramdhani, 2017).

The dried samples were then soaked with 500 ml of 3% acetic acid in a glass jar for 18 hours. This is based on research conducted (Trilaksani *et al.*, 2012) shows that the extraction of collagen into gelatin from fish bones will turn them soft (*ossein*) with a combination of treatments that will produce good gelatin, namely an acetic acid concentration of 3% and a soaking time of 18 hours. This soft bone will be easier to extract the collagen tissue into gelatin because the acid is able to change the collagen fibers *triple helix* into a single chain. Soaking the bones in this acid solution aims to demineralize or remove calcium salts and other minerals contained in the bones (Main, 1997).

Fish bones that experience swelling (*swelling)* forms soft bones *ossein*) washed repeatedly until it reaches pH 5-6. Heating is carried out at an extraction temperature of 80-83^0C for 3 hours with a ratio of fish bones and distilled water of 1:3 according to research (Trilaksani *et al.*, 2012). The purpose of extraction is to convert collagen into gelatin. Extraction of collagen into gelatin using distilled water is carried out at pH 4-5 because generally this pH is

the isoelectric point of non-collagen protein components. The isoelectric point is the point at which the amino acids that make up noncollagenous proteins become dipolar and have a net charge of zero (Hart *et al*., 2003). The amino acids will not move to any electrode, so that when*ossein* extracted, non-collagen protein components are not extracted. The filtrate obtained from the extraction is filtered using a filter cloth and dried in an oven at a temperature of 40-50^{0}C for 48 hours. The gelatin produced was then ground to obtain 100 grams of dry gelatin in the form of fine granules, then continued with organoleptic testing. The organoleptic test results are in the following table.

Table 1. Organoleptic Test Results of Baronang Fish Bone Gelatin Extract (*Siganus canaliculatus*)

No.	Organoleptic Examination	Baronang Fish Bone Gelatin Extract
1.	Form/Consistency	Springy
2.	Color	Brownish yellow in color
3.	Smell	A bit fishy
4.	Feel	Not Feeling

(Primary data source: 2019)

The organoleptic test results in Table 1 are in accordance with the gelatin quality standards according to the Indonesian National Standard (SNI-06-3735-1995), namely colorless, sometimes yellowish, smell/taste normal/acceptable to consumers. Results of the yield of Baronang fish bone gelatin extract (*Siganus canaliculatus*) obtained as much as 16.6% w/w. Determination of gelatin yield aims to see how effective and efficient the extraction process is to make gelatin. The higher the yield value the longer the soaking time (Ridhay *et al*., 2016). This is because the longer the contact time, the more acid molecules on the bone increase, so that more collagen is dissolved in the acid, the water content obtained is 12.5% and is acceptable because the maximum water content of gelatin according to (SNI-06-3735-1995) is 16%, and obtained a pH value of 5, which means the pH is acceptable according to the standard (SNI-06-3735-1995), namely a maximum pH value of 6 (National, 1995).

The gelatin extract was obtained and then tested with 2 test animals in the form of rabbits (*Oryctolagus cuniculus)* with an age of approximately 6 months and a weight of 2.3 kg, by shaving the fur on the rabbit's back at 6 points, then preparing a tool to make burns on the rabbit's back by means of an iron plate with a diameter of 1.9 cm heated over alcohol for 3 minutes with blue flame and attached to the rabbit's back for 7 seconds, making 6 the burn wound points were on the left back 3 and the right back 3. After the burn treatment, the rabbit test animals were given extract on the left back as treatment and the right back as control, where on the left back they were given extracts of concentrations of 0.5%, 1% and 2%, This concentration was chosen to determine the effectiveness of gelatin from baronang fish bones (*Siganus canaliculatus*) on test animals and there is still a lack of research on the use of

Baronang fish bones (*Siganus canaliculatus*) was used from the lowest concentration to the highest concentration, while on the right back Bioplacenton® gel was given as a positive control, liquid extract from rabbitfish bone gelatin, this extract was obtained before further extraction using heating with distilled water and without treatment, this was intended to determine effects caused after burns by not giving Baronang fish bone gelatin extract (*Siganus canaliculatus*) on the right back, the burn wound was covered with gauze during observation over a period of 10 days, measuring the burn wound using a caliper.

During 10 days of observation in the 0.5%, 1%, and 2% concentration treatment groups, there was a change in the diameter of burn wounds in rabbits (*Oryctolagus cuniculus)*, on the 3rd day of observation, the 2% concentration gelatin extract experienced a change in wound diameter of 0.5 cm so that the wound diameter at the 2% concentration became 1.85 cm, on the 4th day the 1% extract treatment group experienced a reduction in the diameter of the burn wound by 0.5 cm to 1.85 cm, then On the 5th day of observation, the concentration of 2% gelatin extract reduced the diameter of the burn wound to 1.8 cm. On the 6th day of observation, the 0.5% gelatin extract concentration experienced a reduction in the diameter of the burn wound by 0.5 cm so that the burn wound diameter was reduced to 1.85 cm. 10 observations of a 2% extract concentration resulted in a reduction in the size of the wound diameter to 1.7 cm, while extract concentrations of 0.5% and 1% changed the size of the wound diameter on the 10th day of observation to 1.85 cm. This shows that from the concentration tested on rabbits (*Oryctolagus cuniculus)*, the 2% concentration showed better changes in burn wound diameter than the 0.5% and 1% concentrations. These results are in accordance with research that has been carried out (Tungadi*et al.*, 2011) using extracts from Snakehead Fish (*Ophiocephalus streaked*) on Rabbit Skin Wounds Histopathologically. The results of measuring the diameter of burn wounds in rabbits can be seen in the following table.

Table 2. Average Measurement Results and Percentage of Wound Healing from Baronang Fish Bone Gelatin Extract (*Siganus canaliculatus*)

No	Treatment Group	Average Healing of Burn Wounds Day 1 – 10 (cm)	Burn Wound Healing Percentage (%)
1.	Gelatin Extract 0.5%	1.87	1,60
	Gelatin extract 1%	1.86	2,15
	Gelatin extract 2%	1.79	6,14
2.	Positive control (Gel Bioplacenton®)	1.71	11,11
3.	Liquid gelatin extract	1.9	0
4.	No treatment	1.9	0

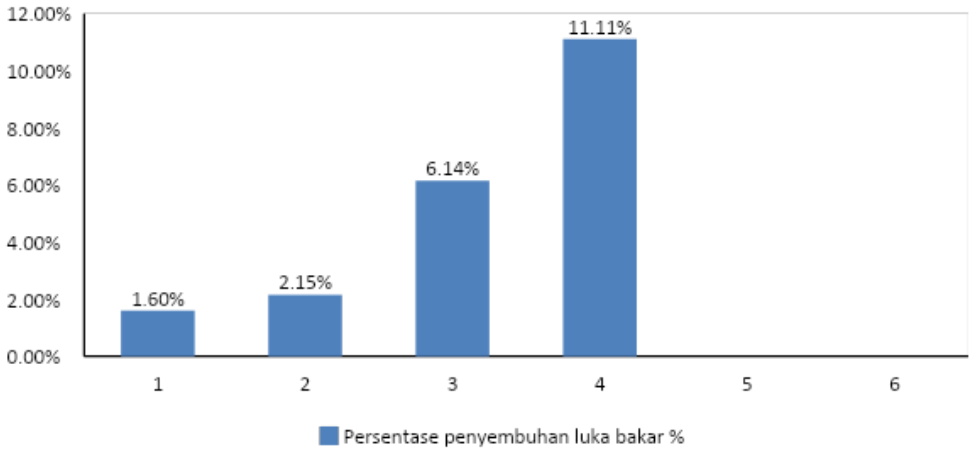

Figure 1. Percentage of healing of burn wounds in each treatment

The results of statistical tests show that the period of time the extract is administered has an effect on the area of the wound that has healed, this can be seen from the significance value which is close to 0.00. Furthermore, it can also be seen that the interaction between the period of administration and the concentration of the extract used also influences the area of the wound that has healed at the significance level. ($P<0.05$). This shows that the longer the gelatin extract is given, the faster the healing, which is indicated by the smaller the wound area, besides that there is an interaction between the time period and the administration of Baronang fish bone gelatin extract (*Siganus canaliculatus*). 2% concentration of Baronang fish bone gelatin extract (*Siganus canaliculatus*) has a good healing effect according to the length of time it is administered, this is in accordance with research that has been carried out (Nasution *et al*., 2018) that the biochemical components of Baronang fish (*Siganus canaliculatus*) can improve the wound healing process, such as the amino acids contained in the form of glycine, proline, arginine and alanine, where arginine is a polyamine precursor for collagen synthesis in wound healing. The role of arginine in the body's immune system is mainly mediated by formation*nitric oxide* which is one of the mediators of secondary inflammation. Arginine supplementation of total calories in experimental burn animals resulted in a significant increase in survival (Moenadiat, 2009).

Results from testing the effectiveness of Baronang fish bone gelatin extract (*Siganus canaliculatus*) as a medicine for burns on rabbits (*Oryctolagus cuniculus*) was carried out using statistical test methods *One-Way* ANOVA and analyzed with the LSD test (*Least Significant Difference*) to find out whether there is a difference between the treatment averages based on the results of statistical tests*One-Way* ANOVA on test animals obtained significant results with a value of 0.003 which

means it is smaller than 0.05 (P<0.05) so Ho is rejected and Ha is accepted so it can be concluded that Baronang fish bone gelatin extract (*Siganus canaliculatus*) has burn activity. LSD test results (*Least Significant Difference*) shows that there is a difference between treatment averages, the 0.5% concentration is significantly different from the 2% concentration which is marked with an asterisk (*) in the LSD test data, and the concentration of 1% extract and 2% extract has a significant difference which is marked with an asterisk (*) on LSD test data.

CONCLUSION

Based on the results of the research that has been carried out, it can be concluded that:

1. Results of organoleptic examination of Baronang fish bone gelatin extract (*Siganus canaliculatus*) produces a chewy shape/consistency, brownish yellow color, slightly fishy odor and no taste and the yield obtained is 16.6% with a water content obtained of 12.5% and the pH value obtained is 5 indicating an acceptable value according to the standard (SNI-06-3735 -1995)
2. Gelatin extract from Baronang fish bones (*Siganus canaliculatus*) at concentrations of 0.5%, 1% and 2% have an effect as a wound healer. Concentration of extract from Baronang fish bones (*Siganus canaliculatus*) 2% has a good burn healing effect.

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Formulation Of Loose Powder Preparation And Evaluation Of The Anti-Fungal Activity Of The Ethanol Extract Of Water Pacar Leaves (*Impatiens balsamina* L) Against Candidiasis

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ABSTRACT

Henna leaves are a plant that can be used to cure various diseases. Henna leaves contain chemical compounds that are important for skin health, including alkaloids, flavonoids, saponins, tannins and steroids which have antifungal potential. This study aims to determine the physical characteristics and antifungal activity of a loose powder preparation of ethanol extract of henna leaves (*Impatiens balsamina* L) against the growth of the fungus Candida albicans. Laboratory experimental is a type of this research. Extraction of chemical compounds used the maceration method with 96% ethanol solvent which produced a thick extract of 45 grams. The thick extract is formulated into loose powder preparations with extract concentrations of 6.25%, 12.5% and 25%. The evaluation of the preparation includes organoleptic tests, pH, homogeneity, degree of smoothness, adhesion and Cycling test. The antifungal activity test used measurement of the diameter of the inhibition zone using the well method. Data analysis for testing physical characteristics and antifungal activity was carried out using Post Hoc LSD (sig. $p<0.05$). The results of this study indicate that the ethanol extract of henna leaves (*Impatiens balsamina* L) loose powder preparation meets the requirements for the physical characteristics of the preparation. The ethanol extract of henna leaves has antifungal activity against Candida albicans with an extract concentration of 6.25% producing an inhibitory zone diameter of 7.3 mm, a concentration of 12.5% producing an inhibitory zone diameter of 11.6 mm and a concentration of 25% producing the diameter of the inhibition zone was 15.4 mm, where FII and FIII were significantly different from the positive control ($p<0.05$). Loose powder formulations containing ethanol extract of henna leaves can be used as a treatment for skin diseases caused by the fungus Candida albicans.

Keywords: Henna Leaves, Loose Powder Preparation, Candida albicans, Antifungal

INTRODUCTION

Skin disease is a disease that is often found in tropical countries like Indonesia. Generally, skin diseases are often ignored because skin diseases are not deadly diseases. However, if ignored continuously and without the use of appropriate therapy, it can cause discomfort and reduce life quality (Hay et al., 2017). A skin disease caused by a fungal infection is candidiasis. Candidiasis is a fungal infection caused by the fungus Candida albicans.

Commonly used drug therapies for fungal infections include ketocenazole, nystatin, miconazole, amphotericin B, griseovulvin and flucanazole. However, continuous use of chemical drugs causes the fungus to become resistant. Topical use of chemical drugs can cause side effects such as burning, itching and even irritation. One alternative that is effective against fungi is the use of traditional medicines derived from plants.

Medication therapy is commonly used for fungal infections such as ketocenazole, nystatin, miconazole, amphoteresin B, griseovulvin and flucanazole. However, continuous use of chemical drugs causes the fungus to become resistant. Topical use of chemical drugs can cause side effects such as burning, itching and even irritation. One alternative that is effective against fungi is the use of traditional medicines derived from plants..

Henna leaves (*Impatiens balsamina* L) have antifungal activity. Based on research (Sutanto, 2021) states that ethanol extract of henna leaves has inhibition against Candida albicans fungi at a concentration of 6.25% with an average inhibition of 11 mm, a concentration of 12.5% of 14.83 mm and a concentration of 25% of 21.33 mm. This is because henna leaf extract contains secondary metabolite compounds in the form of alkaloids, flavonoids, saponins, tannins and steroids which have the potential as antifungals.

The lack of utilization of henna leaves makes researchers interested in innovating by developing bioactive compounds contained in water henna leaves into antifungal drugs in the form of loose powder preparations. Loose powder is a very light powder, usually used to apply makeup or as a skin medication. The advantages of loose powder preparations are that they have a fragrant aroma, relatively cheap prices and are suitable for all skin types and can be used for children or adults (Arsalan, 2017). The use of loose powder with active substances remains safe to use even in the long term, this is because natural ingredients are able to influence pathogenic processes that can weaken bacteria, fungi and viruses in developing resistance to plants (Gupta, 2014).

This study aims to determine the physical characteristics of ethanol extract of water henna leaves (*Impatiens balsamina* L) and antifungal activity against Candida albicans fungus with extract concentrations of 6.25%, 12.5% and 25%.

METHODS

Tools and Materials

40, 60, 100 mesh sieve (Test Sieve Analysys), stirring rod, maceration vessel, porcelain cup, petri dish, erlenmeyer, beaker, hairdryer, hot plate, label paper, parchment paper, pH paper (Universal Test Paper), filter paper, LAF (Laminar Air Flow), mortar and pestle, ose, oven (memmert), bunsen burner, tweezers, drop pipette, tube rack, rotary evaporator (Biobase), horn spoon, sudip, spoit, test tubes, digital scales (Ohaus), Ethanol Extract of Henna leaves (*Impatiens balsamina* L), distilled water (PT. Cognis, Indonesia), methyl paraben (PT. Bratachem), menthol, talc, zinc oxide, magnesium stearate (PT. Bratachem), calcium

carbonate (PT. Bratachem),, Mycorine powder, Potato Dextrose Agar (PDA), Candida albicans fungus, NaCl (PT. Cognis, Indonesia).

Formulation of Loose Powder Preparations of Ethanol Extract of Henna leaves

The powder preparation formula of ethanol extract of water henna leaves was made into four powder formulas with a weight of 30 grams each. The formula components are presented in table 1.

Table 1. Design of loose powder preparation formula

Ingridients	Function	Formula (b/v)			
		F0	F1	F2	F3
Ethanol Extract of Henna leav	Active agen	-	6,25%	12,5%	25%
methyl paraben	Preservative	0,2%	0,2%	0,2%	0,2%
magnesium stearate	Adhesives	5%	5%	5%	5%
zinc oxide	Aderen substance	3%	3%	3%	3%
calcium carbonate	Absorbent	4%	4%	4%	4%
menthol	Flavor	1%	1%	1%	1%
talc	Base	ad 100%	ad 100%	ad 100%	ad 100%

The preparation of loose powder begins with sieving zinc oxide and talc. The sifted ingredients were then weighed at their respective concentrations. Calcium carbonate, magnesium stearate, zinc oxide were crushed together and some talc was added (mass I) then menthol was crushed in another mortar, methyl paraben and ethanol extract of water henna leaves were added and the remaining talc was added (mass II). Mass I and Mass II were combined and homogenized. The preparation was then sieved using a 100 sieve to produce a very fine preparation. Next, the loose powder was packaged and tested.

Evaluation of the Characteristics of Loose Powder Preparations of Ethanol Extract of Water Girlfriend Leaf

Organoleptic Test

A loose powder preparation was taken, using visual observation to observe the color, smell and shape of the preparation (Nisa et al., 2020).Uji pH

Homogeneity Test

A total of 0.5 grams of preparation was placed on the preparation glass, then covered using another preparation glass. Homogeneity testing is done by looking at the coarse grains contained in the preparation. Powder is said to be homogeneous if there are no coarse grains (Warnida, 2016).

Evaluation of Fine Powder Degree

The test was carried out by sieving the loose powder preparation using sieves No. 40, 60 and 100 accompanied by horizontal shaking of the sieve. Shaking of the loose powder is accompanied by vertical tapping until all the powder is perfectly sifted (Lau, 2020). The more powder that is able to pass through a 100 mesh sieve, the finest the powder (Isnawati, 2017).

Adhesion Test

The adhesion test was conducted by applying 0.1 gram of powder on the surface of the hand. Allow to stand for 5 minutes, the powder was blown using a rubber blower for 1 minute. Weighed the powder that adhered and the powder that fell. Calculate the percentage of powder that falls off. Preparations that have good adhesion are those that fall less than the powder that sticks (Aprilia et al., 2020).

Cycling Test

A total of 20 grams of loose powder was put into a beaker. The stability test was conducted by storing the loose powder for 24 hours at 4℃ and 40℃. The test was conducted for 6 cycles or 12 days. The observed characteristics include organoleptic parameters, homogeneity, and pH (Pradiningsih, 2019).

Antifungal Testing

Testing the inhibitory power of the powder of ethanol extract of water henna leaves (*Impatiens balsamina* L) against the growth of Candida albicans fungi was carried out by the agar diffusion method using the pits method. Petri dishes were prepared as required. Potato Dextrose Agar (PDA) was poured into a sterile Petri dish as much as 15 mL then added 1 mL of fungal suspension culture mixed well so that the fungus was evenly distributed. Then a well was made and the positive control (mycorine powder), negative control and each powder preparation formula as much as 2 grams, incubated at 37 ℃ for 48 hours. After 48 hours, it was observed whether there was an inhibition zone in the pitting area. The presence of a clear zone area around the pits indicates the presence of antifungal activity. The inhibition area formed was measured with a bar (Hartini, 2017).

RESULTS AND DISCUSSION

The formulation research and antifungal activity test of the powder preparation of ethanol extract of water henna leaves (*Impatiens balsamina* L) against Candida albicans fungi are intended to determine the physical characteristics of the powder preparation of ethanol

extract of water henna leaves (*Impatiens balsamina* L) and determine the inhibition of the powder preparation against the growth of Candida albicans fungi..

Research on the formulation and antifungal activity test of a loose powder preparation of ethanol extract of water henna leaves (*Impatiens balsamina* L) against Candida albicans fungi is intended toSamples used in this study were water henna leaves obtained from Mandonga village, Mandonga sub-district, Kendari city, Southeast Sulawesi. Henna leaves are plants that are commonly used by the community as ornamental plants, over time henna leaves are now used as plants that have medicinal properties. Water henna leaves (*Impatiens balsamina* L) contain active compounds that are antibacterial, anti-inflammatory, antifungal, antiviral and anticancer (Hardiana & Safrida, 2020).uk know the physical characteristics of the preparation of loose powder ethanol extract of water henna leaves (*Impatiens balsamina* L) and determine the inhibition of the preparation of loose powder against the growth of Candida albicans fungi.Daun pacar air (*Impatiens balsamina* L*)* diekstraksi dengan menggunakan metode maserasi dengan pelarut etanol 96%. Maserasi salah satu metode penarikan senyawa aktif dalam suatu tanaman dengan cara merendam sampel menggunakan pelarut yang sesuai (Chairunnisa *et al*., 2019). Etanol 96% adalah jenis pelarut yang digunakan, pemilihan pelarut tersebut karena etanol merupakan pelarut yang mudah didapat, selektif, tidak toksik dan memiliki kemampuan penyarian yang relatif tinggi sehingga akan mampu menyaring senyawa aktif yang bersifat polar, semi polar dan non polar. Selain itu pelarut etanol 96% lebih mudah untuk masuk atau berpenetrasi ke dalam dinding sel sampel dibandingkan dengan etanol yang memiliki konsentrasi lebih rendah, sehingga akan menghasilkan ekstrak yang pekat (Trifani, 2012).

In the formulation of loose powder preparations, methyl paraben is also used as a preservative that serves to reduce the occurrence of microbial contamination, magnesium stearate as an adhesive material that has good adhesive properties so that the preparation is able to stick well to the skin, zinc oxide as a local antiseptic that helps cover the skin from defects, calcium carbonate as an astringent that can provide a soft effect and coat the skin. To cover up the bad odour of the extract, a flavouring ingredient, menthol, is used, which also gives a cooling sensation. Talc is the basic ingredient used as a formulation of loose powder preparations (Tari, 2022). The preparation of water henna leaf ethanol extract loose powder can be seen in Figure 1.

Figure 1. Preparation of ethanol extract of water henna leaf powder

The physical characteristics test of the preparation consists of several tests to see the physical characteristics of the preparation. Organoleptic testing was carried out for 4 weeks at room temperature, aiming to determine the effects caused during storage. The results of observations on organoleptic testing can be seen in Table 2.

Table 2. Organoleptic observation results of loose powder preparations

Sample	Organoleptic Evaluation			
	F0	FI	FII	FIII
Color	White	Greenish white	light green	deep green
Flavor	Menthol	Menthol	Menthol	Menthol
Form	powder	powder	powder	powder

The results of organoleptic observations showed that the positive control and negative control (F0) were white, while FI was greenish white, FII light green, and FIII deep green. The colour of the preparation produced is in accordance with the colour of the extract, which is green. The formation of colour differences in each preparation is caused by different extract concentrations. The higher the concentration of an extract used, the more intense the colour of the preparation will be. The powder preparation formulation uses menthol as a flavouring so that it will produce a distinctive smell/aroma of menthol.

pH test is carried out to ensure that the formulated preparation has a pH that is suitable for the skin. Based on the results of pH testing, it shows that the preparation of water henna leaf ethanol extract loose powder in FI has a pH of 5, FII has a pH of 6 and FIII has a pH of 6, while the positive control has a pH of 6 and the negative control (F0) has a pH of 5. The difference in pH produced from each formula is caused by different extract concentrations. It is known that ethanol extract of water henna leaves contains alkaline secondary metabolite compounds, namely alkaloids and saponins (Sujarno et al., 2007). Preparations that have a pH

<4.5 can cause skin irritation while preparations that have a pH >6.5 cause the skin to become scaly (Kharisma & Safitri, 2017).

The test for homogeneity was carried out to determine whether the powder preparation had the same composition containing the same amount of medicine. Based on the observation of the homogeneity test, it shows that the preparation of sprinkling powder of ethanol extract of water henna leaves (*Impatiens balsamina* L) in formula I, formula II and formula III during storage for 4 weeks at room temperature there are no coarse particles or lumps in the preparation so that it can be said to be homogeneous. The preparation is said to be homogeneous if the active substance and additives can mix and blend well, besides that no coarse grains are found in the preparation (Warnida, 2016). Homogeneous preparations will produce good quality because they show that the medicinal ingredients are evenly dispersed in the basic ingredients, so that each part of the preparation contains the same amount of medicine.

The preparation of loose powder ethanol extract of water henna leaves (*Impatiens balsamina* L) in FI, FII and FIII each produced a fine loose powder preparation which was marked that the preparation of loose powder ethanol extract of water henna leaves (*Impatiens balsamina* L) could pass through sieves 40, 60 and 100. The more powder that can pass through the sieve, the finest the powder (Isnawati, 2017). The fine degree of powder is very important for comfort on the skin. Generally, a fine powder should pass through a 100 mesh sieve. Less fine powder will reduce comfort and cause irritation to the applied skin (Nisa et al., 2020).

The ethanol extract of water henna leaves (*Impatiens balsamina* L) loose powder preparation has good adhesion, this is characterised by the amount of powder that falls off less than the amount of powder that adheres to the skin. The more powder that falls off during the test, the worse the adhesion of the powder. Preparations that have good adhesion are preparations in powder tests that fall less than the amount of powder that sticks (Aprilia et al., 2020). Good adhesion to loose powder will make drug penetration into the skin better, provide ease of use, and produce powder that is not easily lost due to friction from outside. The results of the adhesion test of the loose powder preparation can be seen in Table 3.

The cycling test aims to determine the physical stability produced by the preparation of water henna leaf ethanol extract loose powder at different environmental temperatures, namely 4 ° C and 40 ° C (hot and cold) which are extreme temperatures that occur in a country every year and even every day which allows preparations to be stored at these temperatures (Slamet et al., 2020). Based on the results of the Cycling test, it shows that all loose powder formulations have the same colour, composition, texture and pH when the preparation is made. This shows that the preparation of water henna leaf ethanol extract loose powder is a stable preparation at 4°C and 40°C storage.

Table 3. Results of the stickiness test of the loose powder preparation

Formul	Powder Weight (g)	Weight of Fallen Powder (g)	Adherent Powder (g)	Percentage of Adherent Powder (%)
F0	0,1	0,0203	0,0797	79,7%
FI	0,1	0,0207	0,0792	79,3%
FII	0,1	0,0228	0,0771	77,2%
FIII	0,1	0,0247	0,0753	75,3%
PC	0,1	0,0187	0,0813	81%

Table 4. Measurement of the diameter of the inhibition zone of the powder preparation of ethanol extract of water henna leaves (*Impatiens balsamina* L) against the growth of Candida albicans fungi.

Formula	Inhibition zone diameter (mm)	Category
F0	0±0.00	Nothing
FI	7.3±1.48	Medium
FII	11.6±2.99	Strong
FIII	15.4±1.65	Strong
Positif Control	6±0.68	Medium

According to the results of antifungal testing, there are differences in the diameter of the inhibition zone in each preparation. Loose powder preparation in FI produces an average diameter of inhibition zone of 7.3 mm with moderate category, FII produces an average diameter of inhibition zone of 11.6 mm with strong category, and FIII produces an average diameter of inhibition zone of 15.4 mm with strong category. The positive control (Mycorine powder) produced an average inhibition zone diameter of 6 mm which is a moderate category. While the negative control (F0) did not produce an inhibition zone diameter against Candida albicans fungal growth. The negative control used is a loose powder preparation without the content of water henna leaf extract (*Impatiens balsamina* L), in this case there is no antifungal content contained in the preparation so that the preparation is unable to produce an inhibition zone diameter. So it can be said that the preparation of loose powder containing ethanol extract of water henna leaves can be used as a treatment for candidiasis because it has biological activity against the growth of Candida albicans fungi.

Differences in the diameter of the inhibition zone produced are caused by differences in the concentration of each extract. In this case, the preparation of loose powder in FIII with

25% extract concentration produces a larger diameter of inhibition zone against Candida albicans fungi when compared to other concentrations. The difference in the concentration of the extract used will affect the amount of antifungal compounds contained in it so that it will produce different abilities of antifungal activity (Sulistyawati et al., 2019)..

CONCLUSION

The results showed that the ethanol extract of water henna leaves with concentrations of 6.25%, 12.5% and 25% (FI, FII and FII) met the requirements of the physical characteristics of the powder preparation and significantly ($p<0.05$) had antifungal activity against Candida albicans fungi.

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Determination Of Total Flavonoid Content And Sun Protection Factor (SPF) Value Of Maja Leaves (*Aegle marmelos L.*) Ethanol Extract Using UV-VIS Spectrophotometry Method

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ABSTRACT

Maja leaves (Aegle marmelos L.) are one of the plants that have potential as SPF. The size of the SPF value is influenced by the antioxidant content, maja leaf extract has potential as an SPF due to the antioxidant content originating from secondary metabolite compounds of the flavonoid group, which have chromophore groups that are able to absorb UV light thereby reducing its intensity on the skin. Maja leaves contain various secondary metabolites such as flavonoids, alkaloids, steroids, tannins and saponins. The aim of this research was to determine the total flavonoid levels and SPF values contained in maja leaf extract. This research uses experimental analytical methods. Simplisia leaf powder 500 grams of maja was extracted using the maceration method using ethanol solvent 96%, obtained a thick extract of 51 grams and a yield value of 10.2% which is includedin the good yield category. The quantitative test was carried out to determine total flavonoid levels and SPF values using a UV-Vis Spectrophotometer. The results of this study show that maja leaf extract (Aegle marmelos L.) has a total flavonoid content of 154.857 mgQE/g or 15.4857% and has an SPF value at concentrations of 100 ppm, 200 ppm, 300 ppm, 400 ppm and 500 ppm respectively, namely 3.33 (minimal protection), 4.27 (medium protection), 5.86 (medium protection), 7.07 (extra protection) and 8.5 (maximum protection). Based on research results from maja leaf extract samples, it is comparable to 154,857 mg of quercetin in 1 gram of extract and the highest SPF value at a concentration of 500 ppm cannot optimally protect the skin from UV-B rays.

Keyword : SPF, Maja leaves (Aegle marmelos L.), Total Flavonoid, UV-VIS Spectrophotometry Method

INTRODUCTION

In Indonesia, from previous generations until now, plants have been used as medicine to treat health problems. One of the plants used is maja (Aegle marmelos L.). Maja plants contain various groups of compounds such as alkaloids, terpenoids, vitamins, coumarins, tannins, carbohydrates, flavonoids, fatty acids and essential oils (Bhar et al, 2019). The antioxidant activity of this plant is due to the presence of flavonoids, flavones, isoflavones, anthocyanins, coumarin lignans, catechins and isocatechins. Aegle marmelos is widely reported to have antioxidant activity against various free radicals. Aegle marmelos leaves have been reported to contain alkaloids,

cardiac glycosides, terpenoids, saponins, tannins, flavonoids and steroids (Dinesh et al, 2011).

The size of the SPF value is influenced by the antioxidant content of the active ingredients used to make sunscreen preparations. Antioxidants are substances that can neutralize free radicals thereby protecting the body from various diseases by binding free radicals (Suryadi, et, al: 2021). Sun Protection Factor (SPF) is a universal indicator to describe the efficiency of sunscreen products which shows the sunscreen product's ability to reduce erythema caused by Ultraviolet rays. The amount of a compound's ability to protect skin from sunlight can be seen based on its SPF value, namely the UV protection value that can protect skin from sunburn(Atika, 2021).

The high potential metabolite compounds contained in maja plants, especially phenol-derived antioxidant compounds, namely flavonoids, which have the potential to ward off free radicals from the effects of exposure to UV rays from the sun, it is necessary to carry out tests to determine total flavonoid levels and SPF (Sun Protecting Factor) values in leaf extracts. maja (Aegle marmelos L.) which was extracted with 96% ethanol by using the UV-Vis spectrophotometer method.

METHODS AND MATERIALS

A. Tools and materials

The materials and tools used in this research were 96% ethanol, aluminum foil, distilled water, 10% aluminum chloride, hot water, concentrated hydrochloric acid, 2N hydrochloric acid, 5% iron (III) chloride, chloroform, quercetin standard solution, methanol p.a, sodium acetate 1 M, Liebermann-Bouchard reagent, n-hexane, magnesium powder, Wagner's reagent and Dragendorff's reagent and the tools are the UV-Vis spectrophotometry equipment (Shimadzu), beaker glass (pyrex), porcelain cup, glass funnel, measuring cup 50 ml (pyrex), scissors, hotplate (IKA C-MAG HS7), watch glass, filter paper, measuring flask 25 mL (pyrex), volumetric flask IO0 mL (pyrex), analytical balance, dropper pipette (pyrex), wooden clamp, rotary evaporator, spatula, maceration container, test tube (pyrex),and test tube rack.

B. Methods

500 gram are macerated using 3000 ml of 96 % ethanol solvent and stored at room temperature for 24 hours. The maserate is then concentrated using a rotary evaporator, then the Phytochemical screening is carried out to identify compounds including phenols, flavonoids, saponins, alkaloids, steroids and terpenoids from the macerates. A total of 20 mg of sample was weighed and dissolved in 10 ml of methanol to obtain a concentration of 2000 ppm. Then put the filtrate into a cuvette and measure the absorbance value using a UV-Vis spectrophotometer at a wavelength of 436. The five dilutions concentration , namely 100 ppm,200 ppm, 300 ppm, 400 ppm and 500 ppm, read the absorbance of the maja leaf extract solution at a wavelength between 290-320 nm using a 1 cm cuvette and ethanol as blank. Futhermore,the Determination of Sun Protection Factor (SPF) activity was carried out in vitro using the UV-Vis

spectrophotometric method. A total of 0.1 g of ethanol extract of maja leaves (Aegle marmelos L.) was dissolved with ethanol to 100 mL to obtain an extract solution with a concentration of 1000 ppm (stock solution). The stock solution was diluted by taking 2.5; 5; 7.5; 10; and 12.5 mL of solution and each solution was put into a 25 ml measuring flask, ethanol was added up to the mark. Then 5 dilution concentrations were obtained, namely 100 ppm, 200 ppm, 300 ppm, 400 ppm, and 500 ppm. The absorbance of the maja leaf extract solution was read at a wavelength between 290-320 nm using a 1 cm cuvette and ethanol as a blank. Each interval is 5 nm with repetition 3 times

RESULT AND DISCUSSIONS

500 grams of simplicia powder was macerated with 6 liters of 96% ethanol solvent then concentrated using a rotary evaporator and a thick extract of 51 grams was obtained. The results of the extraction of maja leaves were then calculated and the yield was 10.2% with the phytochemical sreening result is showed in table 1 the metabolite sekunder contains in the extracts.

Table 1. the phytochemical screening test of maja leaves extract

Extracts	Test	Reagen	Result	Note
Maja leaves (*Aegle marmelos* L)	Saponin	Heating water	+	Forming Foam
	Tannin	Fecl	+	Strong blue colour
	Flavonoid	Magnesium powder and HCL p.a	+	Green forming ring
	Steroids	Lieberman boucard	+	Brown
	Alkaloid	Dragendorf Wagner	+	- -

On screening phytochemistry carried out by Asha et al (2019) the content of chemical compounds in the ethanol extract maja fruit namely alkaloids, saponin glycosides, flavonoids and polyphenols, tannins. Bring it on according to Varegeshe and Tripathi (2013) extract Maja fruit ethanol contains tannins, phenols,carbohydrates, proteins, flavonoids, alkaloids, steroids, terpenoids and triterpenoids.

Absorbance measurements were carried out using a UV-Vis spectrophotometer with a wavelength of 436 nm. From the five concentrations, the average absorbance was obtained at concentrations of 20 ppm (0.1195), 40 ppm (0.1645), 60 ppm (0.1735), 80 ppm (0.1875) and 100 ppm (0.1915). The results obtained from measuring the standard curve, namely the higher the concentration, the higher the absorbance value. And the respective absorbance values obtained for the 96% ethanol extract were 0.483, 0.457, and 0.156. According to the Director General of POM (2014) the range of total flavonoid levels based on their absorbance value is between 0.2-0.8 The results obtained from the 96% ethanol extract (table 2) can be concluded that the sample contains flavonoid levels.

Tabel 2. The result of determination compounds levels

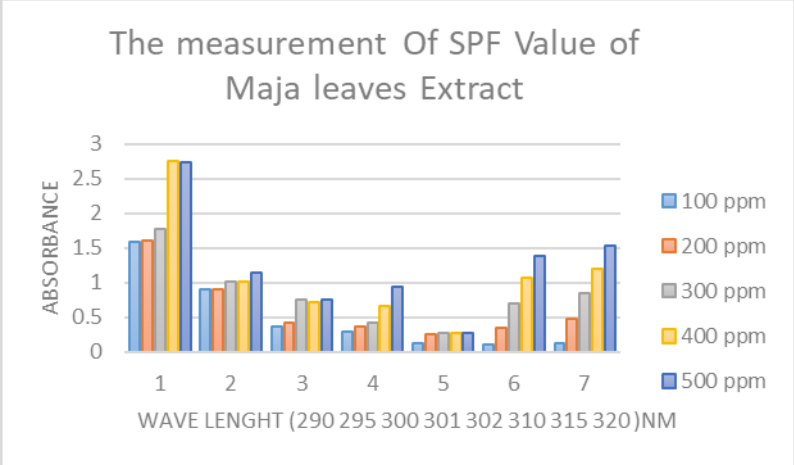
Sample	absorbance	Contents Flavonoid (%)
Maja Leaves Extract	0,365	15,4875

The measurements of the total flavonoid content (table 2) first the absorbance of the samples that had been made in triplicate was calculated as an average. The average absorbance results of quercetin are included linear regression equation and obtained y = 0.0008x + 0.1172 with correlation coefficient of R = 0.8388. The average absorbance result of the maja leaf extract sample with a concentration of 2000 ppm was 0.365. Then the quercetin concentration was calculated and the result was 309.75 mg/L. The total flavonoid content for the ethanol extract of maja leaves (Aegle marmelos L.) was 154.875 mgQE/g or 15.4857%.

The average SPF value with varying concentrations of 100, 200, 300, 400, and 500 ppm respectively is 3.33, 4.27, 5.86, 7.07 and 8.5 (table 3) so it can be seen from determining the SPF value of the sample that the SPF value increases as the concentration of the sample solution increases.

Table 3. The measurement of SPF Value Of Maja Leaves Extract

Sample	concentration	Spf value	Category
Maja leaves extract	100	3,33	Minimal protection
	200	4,27	Minimal protection
	300	5,82	Medium
	400	7,07	Extra
	500	8,5	Maximal



The SPF value is obtained from the results Absorbance measurement at lengthwave 290-320 nm based on Mansur's equation. in this research, value The Correction Factor (CF) used is 10 , This CF value is obtained by measuring the absorbance of sunscreen preparations whose SPF value is known. Determining the SPF value of a sample where the SPF value increases as the concentration of the sample solution increases. From the data obtained, it can be categorized into variations in concentrations of 100, 200, 300, 400 and 500 ppm respectively, namely minimum protection, medium protection, extra protection and maximum protection. From the results obtained at the highest concentration of the sample, namely 500 ppm with an

SPF value of 8.5 in the maximum category, it was not able to protect the skin well from excessive exposure to UV-B rays, because the SPF value that is considered good is above 15 in the ultra category (Suryadi, 2021).

CONCLUSION

The maja leaf extract sample (Aegle marmelos L.) had a total flavonoid content of 154.875 mgQE/g or 15.4875%. Every 1 gram of maja leaf extract is equivalent to 0.154875 g of quercetin. The SPF value of maja leaf extract (Aegle marmelos L.) at each concentration is 100 ppm, 200 ppm, 300 ppm, 400 ppm and 500 ppm respectively, namely 3.33 (minimal protection category), 4.27 (medium protection category), 5.86 (medium protection), 7.07 (extra protection) and 8.5 (maximum protection category). From the average concentration range from 100-500 ppm, the highest to lowest SPF values are obtained, namely maximum protection at a concentration of 500 ppm and minimum protection at a concentration of 100 ppm. The highest concentration of the sample was not able to maximally protect the skin from excessive exposure to UV-B rays.

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The Study Compares Different Solvents' Effects On The Flavonoid Content In The Total Extract Of Nampu Leaf Plants (*Homalomena latifrons* Engi.).

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ABSTRACT

The plant utilized in this study is the Nampu leaf (*Homalomena latifrons* Engi.), which belongs to the Araceae family. The research aims to determine the flavonoid content in the extract of Nampu leaves (*H. latifrons* Engi.) based on solvent variations. The extraction of Nampu leaf plants (*H. latifrons* Engi.) was carried out using a multi-step maceration method with solvents such as 70% ethanol, methanol, and distilled water. Qualitative analysis was performed using thin-layer chromatography (TLC) characterized by the appearance of fluorescent spots on the TLC plate under a UV lamp at 366 nm, with positive spraying of AlCl3 forming yellow-greenish spots indicating the presence of flavonoid compounds.

Subsequently, the determination of the total flavonoid content was conducted using a UV-Vis spectrophotometer method employing quercetin standards measured at a maximum wavelength of 431 nm. The research findings indicate that the total flavonoid content in the Nampu leaf extract (*H. latifrons* Engi.) is 27.69 mgQE/g extract or 2.76% (w/w) for 70% ethanol extract, 58.29 mgQE/g extract or 5.82 % (w/w) for methanol extract, and 12.12 mgQE/g extract or 1.21% (w/w) for water extract. The most optimal solvent for seeking flavonoid compound content in Nampu leaf plants (*H. latifrons* Engi.) is methanol.

Keywords: Flavonoid, 70% ethanol extract, methanol, Nampu leaf plant (*Homalomena latifrons* Engi.)

INTRODUCTION

The diverse chemical compounds present in various plants range from roots and rhizomes. Some of the chemicals found in Nampu leaves include saponins and flavonoids(1), while the roots contain saponins, flavonoids, tannins, and polyphenols(2). The pharmacological effects of Nampu include aphrodisiac and tonic properties(3).

Flavonoids belong to the group of phenolic compounds widely found in plant pigments(4). Currently, there are over 6,000 different compounds classified as

flavonoids(5). Flavonoids have numerous benefits, such as acting as antioxidants(6), protecting cell structures(7), anti-inflammatory properties(8), preventing bone loss(9), functioning as antibiotics(10), and many other advantages.

Flavonoid extraction, vital for unlocking the therapeutic potential of these plant compounds(11), involves various methods(12). Traditional solvent-based extraction using ethanol or methanol(4) is common but may co-extract undesired substances(13). Supercritical fluid extraction (SFE) with carbon dioxide offers a cleaner alternative, while water-based techniques are environmentally friendly(14). Researchers strive to develop sustainable and efficient extraction methods for diverse applications in pharmaceuticals and nutraceuticals(15). In this discussion, we will explain how the difference in solvent usage can affect flavonoid extraction from Nampu leaves (*H. latifrons* Engi.). variations.

MATERIAL AND METHODS

The experimental equipment used includes glassware (Pyrex), stirring rods, vials, a Camag chamber, a freeze dryer (Christ), measuring flasks (IwakiCTE33) of 5 and 10 mL, UV lamps at 254 and 366 nm (Philips), micropipettes (Dragon Lab), capillary tubes (Nesco Lab), graduated pipettes (IwakiCTE33), a rotary vacuum evaporator (Rotavapor R-220), ultraviolet-visible spectrophotometer (Thermo Scientific GENESYS 10S), reaction tubes (Pyrex), analytical balance (Carat Series), and a rough balance. $AlCl_3$ 10%, distilled water (aquadest), n-hexane:ethyl acetate eluent (2:8), ethanol, potassium acetate 10%, quercetin, thin-layer chromatography (TLC) plates F254 (E. Merck), methanol, p.a-grade methanol, and the plant material (simplisia) of Nampu leaves (*Homalomena latifrons* Engi.) were utilized.

Sample extraction was conducted using a multi-step maceration method with 70% ethanol, methanol, and water as solvents(16). 80 grams of Nampu leaf powder were dissolved in 1 L of ethanol, stirred, and left for 3 x 24 hours. The extract was filtered through filter paper, resulting in filtrate and residue. The residue underwent re-maceration twice. The produced residue was then macerated with 1 L of methanol for 3 x 24 hours. The methanol extract was filtered, underwent re-maceration twice, yielding filtrate and residue. Subsequently, the residue from the second extraction process underwent maceration with 1 L of distilled water, stirred, and left for less than 24 hours. The extract was filtered, producing filtrate and residue. The extracts from 70% ethanol and methanol solvents were evaporated using a rotary vacuum evaporator, and the water solvent was evaporated until all solvents separated, resulting in a concentrated extract.

The ethanol, methanol, and water extracts of Nampu leaves were each dissolved in ethanol, spotted on TLC plates with silica gel F254 as the stationary phase. The TLC plates were then placed in a chamber containing *n*-hexane:ethyl acetate eluent (2:8)

until complete elution. Subsequently, the spots were observed under UV light at 254 and 366 nm, and $AlCl_3$ spraying was performed, revealing a yellow-green color(17).

The quantitative flavonoid test involves the preparation of reagent solutions, a quercetin standard solution, and the determination of total flavonoid content in Nampu plant leaf extracts. The test utilizes UV-Vis spectrophotometry at a wavelength of 431 nm to measure absorbance and quantify flavonoids in terms of quercetin equivalents(18). The process includes the creation of standard solutions and three replications for accurate measurement(19).

RESULTS AND DISCUSSION

After performing extraction with various types of solvents, diverse results were obtained, as indicated in Table 1.

Table 1. Yield results of leaf extract from Nampu plant (*H. latifrons* Engi.)

No.	Sample	Sample Weight (g)	Solvent Amount (mL)	Extract Results (g)	Extract Yield (%) w/w
1	Ethanol (70%) extract	80	1,000	11.98	14.98
2	Methanol extract	80	1,000	4.49	5.61
3	Water extract	80	1,000	1.33	1.66

The yield results obtained from each Nampu leaf extract are as follows: for the 70% ethanol extract, it amounted to 11.981 g with a yield of 14.98 % (w/w), for the methanol extract, it was 4.49 g with a yield of 5.61 % (w/w), while for the water extract, the obtained extract was 1.33 g with a yield of 1.66 % (w/w). These results are presented in Table 1. The determination of these yields aims to assess the concentration of secondary metabolites successfully carried by the solvents. However, the specific type of secondary metabolite attracted has not been identified.

The qualitative test was conducted to ascertain the presence of flavonoid content in Nampu plant leaves (*H. latifrons* Engi.) using the thin-layer chromatography (TLC) method. Separation with TLC involves employing various eluents with different polarities to obtain a solvent that provides effective separation and produces clear colour spots(20). The common application of TLC is to determine the number of compounds in a mixture and identify compounds(21). The results of this qualitative test indicate that the Nampu plant leaf extract positively contains flavonoid compounds.

Quantitative testing for the quercetin standard solution utilizes p.a-grade methanol as a solvent, serving to dissolve the quercetin standard(22). $AlCl_3$ plays a crucial role in inducing a bathochromic effect by causing a shift towards higher wavelengths, allowing the quercetin standard's wavelength to fall within the visible wavelength range(23). Additionally, the addition of potassium acetate acts as a stabilizing agent to maintain the bathochromic effect that has occurred(24). The mixture of quercetin standard solutions is homogenized and left for 30 minutes to facilitate the complete formation of reactions between the quercetin standard solution and other reagents(25). The results of the quantitative test are indicated in Table 2.

Table 2. Results of determining the total flavonoid content in the leaf extract of Nampu plant (*H. latifrons* Engi.).

Sample	Replications	Absorbace (y)	Initial flavonoid contents (x)	Total flavonoid content (mgQE/g extract)	Average total flavonoid content (mgQE/g extract)	Percentage flavonoid content (w/w)
Ethanol (70%) extract	1	0.19	2.78	27.78	27.69	2.77 %
	2	0.19	2.79	2.90		
	3	0.19	2.74	27.39		
Metanol extract	1	0.42	5.69	56.94	58.29	5.83 %
	2	0.44	6.00	59.97		
	3	0.43	5.80	57.95		
Water extract	1	0.07	1.25	12.50	12.12	1.21 %
	2	0.06	1.16	11.62		
	3	0.07	1.23	12.25		

The flavonoid content results obtained from each extract of the Nampu plant (*H. latifrons* Engi.) are as follows: the 70% ethanol extract recorded 27.69 mgQE/g extract, constituting a percentage content of 2.77% (w/w). Similarly, the methanol extract exhibited a flavonoid content of 58.29 mgQE/g extract, accounting for 5.83% (w/w), while the water extract yielded a content of 12.12 mgQE/g extract, representing a percentage of 1.21% (w/w).

CONCLUSION

Based on the findings from the conducted study, it can be summarized that the total flavonoid content in the respective extracts of Nampu plant leaves (*H. latifrons* Engi.) is as follows: the 70% ethanol extract contains 27.69 mgQE/g extract, constituting

a percentage of 2.77 % (w/w); the methanol extract has a content of 58.29 mgQE/g extract, representing 5.83 % (w/w); and the water extract exhibits a content of 12.12 mgQE/g extract, equivalent to 1.21 % (w/w). Consequently, it can be concluded that methanol is the most effective solvent for extracting flavonoids from Nampu plant leaves (*H. latifrons* Engi.).

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Total Flavonoid Content and Antioxidant Activity Test of Ethanol Fraction of Papaya Seed (*Carica papaya* L.) With DPPH (2,2-diphenyl-1-picrylhydrazyl) Method

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ABSTRACT

The aging process involves the gradual weakening of cells and organs, starting from adulthood and accelerating after the age of 50, marked by the body experiencing various ailments and wrinkling of the skin. Free radicals are the primary cause of premature agingOne of the efforts to slow down premature aging due to free radicals is the use of antioxidants. This study aimed to determine the total flavonoid content in the ethanol fraction of papaya seeds (Carica papaya L.) and to assess the antioxidant activity of the ethanol fraction of papaya seeds (Carica papaya L.) measured using the DPPH method. This study was experimental research. The population in this study consists of papaya plants (Carica papaya L.) obtained from Konda Satu Village, Konda District, South Konawe Regency, Southeast SulawesiThe sample in this study is the seeds of California papaya (Carica papaya L.), which were extracted to obtain a concentrated extract and then fractionated to obtain a concentrated ethanol fraction. The total flavonoid content was measured using UV-Vis spectrophotometry at 436 nm, and antioxidant activity was assessed using the DPPH method and measured using UV-Vis spectrophotometry at 517 nm. The results of the study showed that the ethanol fraction of papaya seeds has a total flavonoid content of 1.75 mgQE/g or 0.175% and possesses strong antioxidant activity with an IC_{50} value of 91.98 µg/ml. It is expected for future researchers to continue research on total flavonoid content testing using different fractionation methods and to conduct antioxidant activity tests using alternative methods.

Keyword : Total Flavonoid, Antioxidant Activity, Papaya Seed (Carica papaya L.), DPPH

INTRODUCTION

The aging process is the weakening of cells and organs as a whole starting from adulthood slowly and rapidly accelerating after the age of 50, marked by the body becoming prone to illness and wrinkled skin. Naturally, the aging process occurs in every human being, but the process varies; some age quickly (premature aging process) and some age slowly (stay youthful). Although the aging process occurs due to several factors, free radicals also contribute to accelerating someone's aging process. Free

radicals are the main cause of premature aging. One effort to slow down premature aging due to free radicals is antioxidants. As an active ingredient, antioxidants are used to protect the skin from damage caused by oxidation and prevent premature aging. Antioxidants used, especially vitamin C and E, function to repair skin damage caused by free radicals from ultraviolet radiation and cigarettes (Aizahh, 2016).

Free radicals are atoms or molecules that have unpaired electrons, are unstable, and highly reactive; as a result, free radicals will attract electrons from molecules inside cells and cause damage to cells and tissues. Free radicals can cause various diseases, such as diabetes mellitus, Parkinson's disease, emphysema, acute kidney failure, premature aging, and cancer. Therefore, the body needs antioxidants that can stabilize free radicals (Qazi & Khursid, 2018).

Antioxidants are compounds capable of inhibiting the oxidation rate of other molecules or neutralizing free radicals (Fajriah, et al., 2007). Exogenous antioxidant intake is needed because it helps restore body balance and slow down the oxidation process of free radical compounds. Antioxidants provide at least one or more hydrogen/electron atoms to free radicals so that free radical compounds can be more stable (Kuncahyo I and Sunardi, 2007). Antioxidants stabilize free radicals and complement the deficiency of electrons that have free radicals, inhibiting the chain reactions of free radical formation that can cause oxidative stress. According to Prabantini (2010), based on their source, antioxidants are distinguished into two types: natural antioxidants (antioxidants derived from natural material extractions) and synthetic antioxidants (antioxidants obtained from chemical synthesis reactions). Examples of natural antioxidants include flavonoid compounds, tannins, vitamin C, vitamin E, and others. Meanwhile, examples of synthetic antioxidants include Butylated Hydroxyanisole (BHA) and Butylated Hydroxytoluene (BHT). Research conducted by Basma et al., (2011) found that synthetic antioxidants (BHA and BHT) can cause liver damage and carcinogenesis.

It is necessary to note that among the community, there are many antioxidant-labeled products available for sale, but these products are often sold at high prices, making them difficult to afford for all segments of society. Therefore, exogenous antioxidants are highly needed as alternative antioxidants originating from plants, one of which is the papaya plant, specifically its seed part (Senet, 2018).

The Papaya plant (Carica papaya L.) has properties as an antioxidant in several parts. One of them is in the papaya seed. Papaya seeds have antioxidant benefits from phytochemical substances contained in them, namely phenolics, beta-carotene, and vitamin C (Amaliah, et al., 2018; Najmudin, 2021). Based on the results of phytochemical screening that has been conducted, ripe papaya seed extracts positively contain flavonoids, where flavonoids have the ability to reduce free radicals (Tambunan, et al., 2018). Antioxidant testing is conducted because according to Sugiani (2012),

antioxidants have been proven to delay premature aging processes, such as Vitamin A, C, and E, as well as trace elements such as selenium, molybdenum, and zinc.

The extraction method used is the maceration method because the process is relatively easy and the equipment is readily available. The solvent used in the extraction process is ethanol. In the extraction process, flavonoid compounds are polar with ethanol solvents, so flavonoids will dissolve in a solvent solution that is appropriate to their polarity (Bangun, et al., 2021).

Antioxidant activity testing can be performed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method. The DPPH method is used to test the ability of an antioxidant component to scavenge free radicals in a substance or extract and also has advantages such as being quick, simple, proven accurate, and efficient. Observations can be made using a spectrophotometer to determine the free radical scavenging activity by the sample (Pratiwi & Wahdaningsih, 2018).

Based on research conducted by Najmudin, et al., (2021), the total flavonoid content of papaya seeds is 0.23 mg QE/g or 0.23%, and its antioxidant activity is considered strong because the IC50 value obtained is 41.85 ppm. Based on research conducted by Bangun, et al., (2021), the 96% ethanol extract of papaya seeds (Carica papaya L.) with an average absorbance of 0.5603 falls into the good category, where the good absorbance value range is between 0.2-0.8 in the ultraviolet or visible light region (Rohman, 2009), with a total flavonoid content of 15.8181 mg QE/g or 1.5818%. Meanwhile, based on research conducted by Sari, et al., (2018), the 70% ethanol fraction of papaya fruit flesh has very weak antioxidant activity because the IC50 value obtained is 309.06 ppm.

Based on the above research, there has been no research using papaya seed ethanol fractions, so researchers are interested in conducting more specific antioxidant activity testing on fractions using ethanol solvents. In papaya seed extracts, complex chemical compounds are still present, so further separation needs to be done with the fractionation process. Fractionation with ethanol is intended to search for polar compounds that have the potential as antioxidants, such as flavonoids, phenols, tannins, and glycosides that have the potential as antioxidant activities (Sutomo, et al., 2021). Based on this background, it is necessary to conduct total flavonoid content tests and antioxidant activity tests on papaya seed ethanol fractions (Carica papaya L.) in premature aging using the DPPH method (2,2-diphenyl-1-picrylhydrazyl).

METHOD

Sampling and Sample Preparation

Samples in the form of papaya seeds (Carica papaya L.) were directly taken from Desa Konda Satu, Konda District, South Konawe Regency, Southeast Sulawesi. Sampling was conducted in the morning before sunrise. Papaya seeds used as samples were obtained

from ripe California papaya fruits. Papaya seeds were separated from the skin and fruit flesh, then washed thoroughly under running water and drained. Subsequently, they were dried to reduce the moisture content in the seeds. The seeds were ground and sieved to obtain crude powder (Tambunan, et al., 2018).

Sample Extraction

Dried papaya seed crude powder (Carica papaya L.) was then extracted. Extraction was performed using the cold maceration method, soaking the papaya seeds with 96% ethanol solvent in a maceration container. This process was carried out for 3x24 hours in a maceration container with solvent filtration every 24 hours, stored in a place not exposed to direct sunlight, and occasionally stirred. The maceration results were filtered, and then the maceration results were evaporated using a Rotary Evaporator until a thick extract was obtained (Doughari, 2012).

Fractionation of Papaya Seed Ethanol Extract

Papaya seed extract weighing 35 g was dissolved in 100 mL of ethanol until evenly mixed. The extract was then placed into a separating funnel, followed by the addition of n-hexane solvent as much as 100 mL. The mixture was partitioned and left to stand until two layers were formed. The n-hexane layer was separated, then the water layer was partitioned again with n-Hexane solvent as much as 100 mL until a clear solution was obtained. The ethanol fraction obtained was evaporated using a Rotary Evaporator until a thick fraction was obtained. The thick fraction was evaporated again using a hairdryer until a constant weight was obtained.

Determination of Total Flavonoid Content

Preparation of Quercetin Solution

A total of 10 mg of quercetin (Standard) was weighed and dissolved in 10 mL of methanol as a stock solution. Then, quercetin dilution was made with concentrations of 20, 40, 60, 80, and 100 ppm as standard quercetin solutions (Ipandi, et al., 2016).

Blank Absorbance Measurement

The measurement was carried out by mixing 1.0 mL of quercetin solution with p.a methanol until the volume reached 5.0 mL in a measuring flask, then the mixture was shaken and allowed to stand for 30 minutes at room temperature. The absorbance was then measured at the maximum wavelength, repeated three times.

Measurement of Total Flavonoid Content in Papaya Seed Ethanol Fraction (Carica papaya L.)

A total of 10 mg of papaya seed ethanol fraction was weighed and dissolved in 10 mL of methanol to obtain a concentration of 100 µg/mL. A volume of 0.5 mL of the test

sample was measured and added with 1.5 mL of ethanol, then 5 drops of 10% aluminum chloride, 5 drops of 1 M sodium acetate, and 2.8 mL of distilled water were added. Incubated for 30 minutes. The absorbance was then measured using a UV-Vis spectrophotometer at a wavelength of 436 nm.

Antioxidant Activity Test

Preparation of DPPH Stock Solution (2,2-Diphenyl-1-picrylhydrazyl)

DPPH powder of 10 mg was dissolved with 50 mL of p.a ethanol solvent in a measuring flask to obtain a stock solution with a concentration of 200 ppm.

Blank Antioxidant Power Measurement

The test was carried out by taking 2 mL of p.a ethanol, then adding 3 mL of 200 ppm DPPH solution, and then the absorbance was measured at the maximum wavelength.

Measurement of Sample Antioxidant Activity

Papaya seed ethanol fraction (Carica papaya L.) of 10 mg was dissolved with 50 mL of p.a ethanol (200 ppm) in a measuring flask while stirring until homogeneous. Then dilution was performed with a concentration series of 20, 40, 60, 80, and 100 ppm. Each concentration series made was pipetted 1 mL, then added with 1 mL of 200 ppm DPPH solution, and then added 3 mL of ethanol. The mixed solution was incubated for 30 minutes. Then, the absorption was measured at a wavelength of 517 nm.

Measurement of Vitamin C Standard Sample Antioxidant Activity

Vitamin C powder was weighed at 10 mg each and dissolved with 100 mL of p.a ethanol (100 ppm) in a measuring flask while stirring until homogeneous. Then, dilution was performed with a concentration series of 20, 40, 60, 80, and 100 ppm. Each concentration series made was pipetted 1 mL, then added with 3 mL of 200 ppm DPPH solution. The mixed solution was incubated for 30 minutes, and then the absorption was measured at a wavelength of 517 nm.

Data Processing and Analysis

The antioxidant activity of the sample was determined by the magnitude of the DPPH radical absorption inhibition through the calculation of DPPH absorption inhibition using the formula:

% Inhibition = (absorbance blank - absorbance sample) / (absorbance blank) x 100%

The calculation results were entered into the regression equation with the fraction concentration (ppm) as the X-axis and the % inhibition value (antioxidant) as the Y-axis. From the equation Y = bx + a, the IC50 value could be calculated using the formula: IC50 = (50-a) / b.

RESULT

The yield of papaya seed ethanol extract (Carica papaya L.).

Extraction Yield of Papaya Seed Ethanol Extract (Carica papaya L.) obtained using the maceration method can be seen in table 1.

Table 1. Percentage yield of papaya seed extract.

Sample	Crude Powder Weight	Extract Weight	Extract Yield (%) (%)
Papaya Seed	750 gram	54 gram	7,2 %

Fraction Yield (Carica papaya L.)

The yield results of papaya seed ethanol fraction (*Carica papaya* L.) can be seen in table 2.

Table 2. Yield of papaya seed ethanol fraction (*Carica papaya* L.)

Sampel	Extract Weight	Ethanol Fraction Weight	Yield
Papaya Seed	30 gram	8 gram	26,6 %

Flavonoid Evaluation

The results of quercetin standard absorbance measurements as a comparison at a wavelength of 436 nm.

Table 3. Absorbance of quercetin calibration curve

Sample Type	Concentration (ppm)	Absorbance I	Absorbance II	Absorbance III
quercetin	20	0,108	0,111	0,1095
	40	0,118	0,12	0,119
	60	0,16	0,16	0,16
	80	0,165	0,163	0,164
	100	0,185	0,181	0,183

Based on the table 3, the average absorbance of each quercetin concentration is obtained, where 20 ppm concentration has an average absorbance of 0.1095, 40 ppm has an average absorbance of 0.119, 60 ppm has an average absorbance of 0.16, 80 ppm has an average absorbance of 0.164, and 100 ppm has an average absorbance of 0.183.

Total flavonoid content of papaya seed extract (Carica papaya L.)

Table 4. Total Flavonoid Content

Sample Weight	Absorbance	Average Absorbance	Equivalent Content (ppm)	Flavonoid Content (%)
0,02	0,092	0,093	3,5	0,175%
	0,107			
	0,082			

Based on the table 4, the flavonoid content was found to be 1.75 mgQE/g or 0.175%.

Antioxidant Evaluation

Antioxidant data obtained were determined by looking at the IC_{50} value, by creating a curve relationship between the concentration of papaya seed ethanol fraction solution with the percentage inhibition that resulted in a linear regression equation. After obtaining the inhibition percentage, a graph was made between the concentration solution x and the inhibition percentage y. Furthermore, it was analyzed with a linear regression equation to obtain the IC_{50} value to know how many antioxidant parameters were seen in the table 5

Table 5. Antioxidant evaluation of papaya seed ethanol fraction and vitamin C

Sample	Concentration (ppm)	Blank Absorbance	Sample Absorbance	Antioxidant Activity (% Inhibition)	IC_{50} Value (μg/mL)
Papaya seed ethanol fraction	20	0,683	0,544	20,351	91,98
	40	0,683	0,504	26,207	
	60	0,683	0,423	38,067	
	80	0,683	0,388	43,191	
	100	0,683	0,312	54,319	
Vitamin C	20	0,406	0,188	53,694	11,67
	30	0,406	0,162	60,098	
	40	0,406	0,151	62,807	
	50	0,406	0,125	69,211	

Based on the table 5 it is found that the IC50 value of papaya seed ethanol fraction is 91.98, which is classified as strong because the IC50 value is below <100..

The relationship between quercetin and papaya seed ethanol fraction, can be seen in Figure 1.

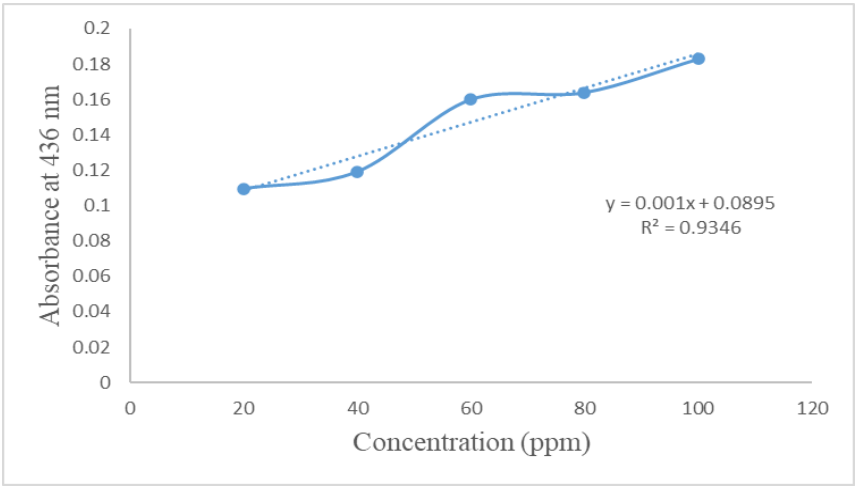

Figure 1. Graph of the results between quercetin and papaya seed ethanol fraction

Relationship between % inhibition and the concentration of papaya seed ethanol fraction solution, can be seen in Figure 2.

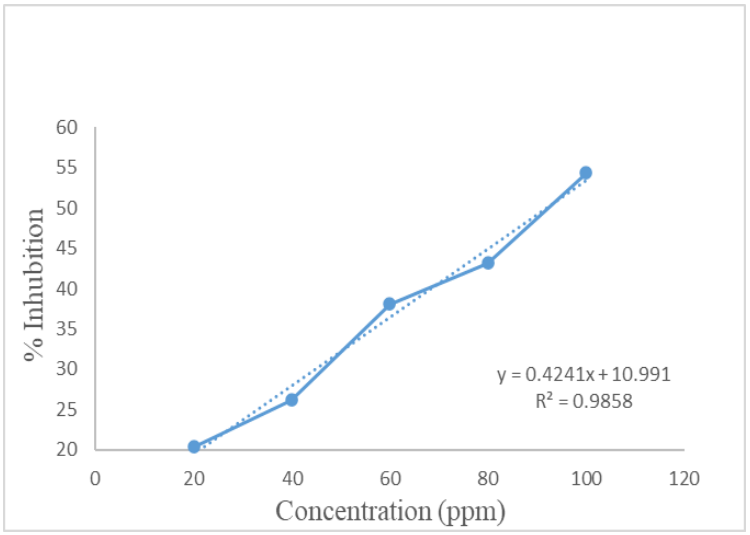

Figure 2. Graph of the Relationship between % Inhibition and the Concentration of Papaya Seed Ethanol Fraction Solution.

The relationship between % inhibition and the concentration of vitamin C solution, can be seen in figure 3.

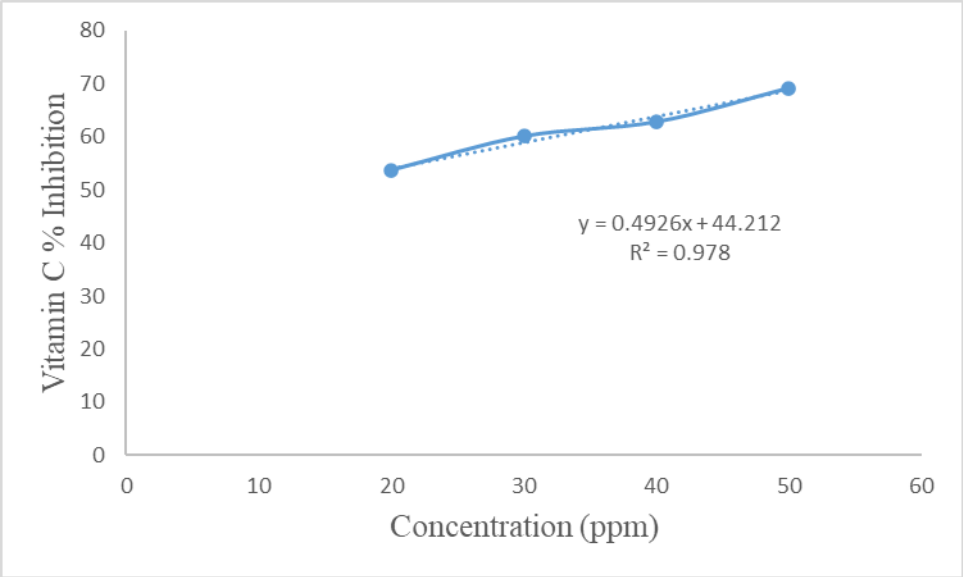

Figure 3. Graph of the results between % Inhibition and the Concentration of Vitamin C.

DISSCUSSION

The Aims of this research were to determine the total flavonoid content in the ethanol fraction of papaya seeds and to assess the antioxidant activity of this fraction, which has potential antioxidant properties.

The samples used in this study were papaya seeds (Carica papaya L.) obtained from Konda Satu Village, Konda District, South Konawe Regency, Southeast Sulawesi. The papaya seeds used were obtained from ripe California papaya fruits. The papaya seeds were separated from the fruit pulp and skin, washed thoroughly under running water, and then drained. They were then air-dried to stop enzymatic reactions that could lead to chemical changes in the compounds present in the papaya seeds. This drying process also aimed to reduce the moisture content in the crude drug to prevent microbial growth and spoilage. Subsequently, the dried seeds were ground and sieved to obtain papaya seed powder (Tambunan et al., 2018).

The dried crude drug was then weighed to obtain 750 grams. It was then extracted using the cold extraction method, specifically the maceration method. This method was chosen for its simplicity and the ease of obtaining equipment. Ethanol 96% was used as the solvent during the maceration process. Ethanol was chosen because flavonoid compounds, generally in the form of glycosides, are polar in nature and need to be dissolved in a polar solvent. Additionally, ethanol has the ability to dissolve a wide range of compounds, from polar to non-polar, effectively extracting the compounds present in the crude drug. The maceration process involved soaking the papaya seed powder in a maceration vessel filled with ethanol until the powder was completely submerged. It was soaked for 3x24 hours, with agitation and solvent replacement every 24 hours. The mixture was then filtered using filter paper, and the resulting liquid

extract was concentrated using a rotary vacuum evaporator to obtain a thick extract. The concentration aimed to separate the ethanol solvent from the extract until a concentrated extract was obtained. The concentrated extract was then dried to remove any residual moisture. The yield of the concentrated extract was found to be 7.2%.

From the maceration process of papaya seeds (Carica papaya L.) with a dry crude drug weight of 750 grams, a concentrated extract of 54 grams was obtained with a yield of 7.2%. The calculation of the yield aimed to determine the percentage of compounds extracted from the papaya seeds using the solvent, but it could not determine the specific types of compounds extracted (Ukieyanna, 2012).

Fractionation was then carried out to obtain pure natural components free from other unwanted chemical components (Nugroho et al., 2013). The purpose of fractionation was also to separate liquid substances based on their polarity levels. Fractionation was conducted step by step based on the polarity level of the solvent. Fractionation was performed using the liquid-liquid extraction method with n-hexane and ethanol solvents. 10 grams of papaya seed ethanol extract (Carica papaya L.) were weighed and suspended in n-hexane: ethanol (1:1) solution, each 100 ml, then shaken for 10-15 minutes, followed by settling until two layers were formed: n-hexane and ethanol layers. The n-hexane layer was separated, and fresh n-hexane was added again, 100 ml each time. This process was repeated three times using the same method. The fractionation product was dried using a hairdryer until thick. A hairdryer was used to accelerate the drying process (Diba et al., 2021). The fractionation process resulted in 30 grams of concentrated extract with a yield of 26.6%. The higher the yield obtained, the more secondary metabolites were attracted. Determination of this yield functioned to determine the secondary metabolite content but did not identify the specific types of compounds contained within (Sari, Yasti. et al., 2021). The yield obtained met the requirements of the Indonesian Herbal Pharmacopoeia, which stipulates a yield of not less than 7.2%.

Quantitative analysis of total flavonoid compounds using UV-Vis spectrophotometry was performed to determine the amount of flavonoid content in the papaya seed ethanol fraction (Carica papaya L.). Flavonoid analysis was conducted using UV-Vis spectrophotometry because flavonoids contain a conjugated aromatic system, resulting in strong absorption bands in the ultraviolet and visible light spectrum regions (Harbone, J.B. 1987).

In this study, the ethanol fraction of papaya seed and quercetin standard solutions were reacted with sodium acetate and AlCl3. The determination of total flavonoid content using the AlCl3 reagent is known as the colorimetric method, where the AlCl3 reagent forms complexes with the keto group on the C-4 atom of flavonoids. The formation of complexes, usually indicated by a yellowish coloration of the solution, occurs after the addition of the AlCl3 reagent (Azizah, 2014).

The test for the total flavonoid content of the papaya seed ethanol fraction (Carica papaya L.) was conducted using the UV-Vis spectrophotometric method to determine the total flavonoid content present in the papaya seed ethanol fraction (Carica papaya L.). In this study, quercetin solution was first prepared as a standard at concentrations of 20 ppm, 40 ppm, 60 ppm, 80 ppm, and 100 ppm. Quercetin was selected as the standard solution because it is a flavonol-type flavonoid with a keto group at C-4 and hydroxyl groups at C-3 or C-5 neighboring the flavone and flavonol (Aminah et al., 2017). Moreover, quercetin is one of the flavonoid compounds that can react with aluminum chloride to form complexes (Bangun et al., 2021). Various concentrations were used because the method used to determine the content was based on a standard curve equation. To create a standard curve, several concentration series were first prepared to obtain a linear regression equation to calculate the percentage content.

The maximum wavelength absorption measurement at a wavelength of 400-800 nm showed that the maximum wavelength of the quercetin standard curve was at 436 nm. This wavelength was used to measure the absorption of the papaya seed ethanol fraction sample. From the measurement results, the quercetin standard between the absorbance of quercetin and concentrations of 20, 40, 60, 80, and 100 ppm, a linear regression equation was obtained, namely y = 0.001x + 0.0895, with an obtained R2 value of 0.9346. According to Sugiono (2009), the interpretation of the coefficient of determination R2 intervals is said to be very strong if it ranges from 0.8 -1, strong if it ranges from 0.60 - 0.799, moderately strong if it ranges from 0.40 - 0.599, low if it ranges from 0.20 - 0.399, and very low

CONCLUSION

Based on the results of the conducted research, it can be concluded that the total flavonoid content in the ethanol fraction of papaya seeds (Carica papaya L.) is 1.75 mg QE/g or 0.175%. The ethanol fraction of papaya seeds (Carica papaya L.) exhibits strong antioxidant activity with an IC50 value of 91.98 µg/mL.

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