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RESEARCH ARTICLE
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Biochemical and Hematological Assessment in Patients With Thyroid Dysfunction

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ABTRACT

This investigation conducted for biochemical and hematological assessment in patients with thyroid dysfunction. The research was carried out at a laboratory in Baghdad, Iraq, and it is a case-control study. The sample was gathered from February 2023 through the years 2021 and 2022. About 250 individuals were a part of this study; 100 were hypothyroid, 100 were hyperthyroid, and 50 had normal thyroid function and complete blood counts. A total of three millilitres of whole blood and two millilitres of EDTA were obtained from each subject in an aseptic manner for the CBC. Also, 2 ml of blood was drawn for serum separation, these sera were used for evaluation T3, T4, TSH, AST, ALT, Urea and Creatinine by using kits purchased from Linear company, the procedures for all were done according to manufacturer instructions. The control group had considerably greater mean RBC, Hb, HCT, and MCH concentrations, while the hypothyroid group had significantly lower values. The hyperthyroid group showed no significant difference between MCV and MCH. There were no statistically significant MCHC results in either the hypothyroid or hyperthyroid groups compared to the control group. There was little difference in total lymphocyte and platelet counts between the control, hyperthyroid, and hypothyroid groups. Differential leukocyte count showed statistically significant differences between the hypothyroid and hyperthyroid groups. As seen in Table 2, the hypothyroid group had higher serum TSH levels ($p<0.05$), in contrast to the hyperthyroid group which exhibited lower levels ($p<0.05$). Alternatively, when contrasted with the control group, the hypothyroid group had lower T3 and T4 levels ($p<0.05$), whereas the hyperthyroid group had higher levels ($p<0.05$). Improvements in hepatic and kidney functions were seen in patients with hypo or hyperthyroidism as compared to the control group in this investigation. In conclusion, both hypothyroidism as well as hyperthyroidism affects on hematological and biomarkers for thyroid, liver and kidney

Keywords: Biochemical, Hematology, Kidney, Liver, Thyroid

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INTRODUCTION

When it comes to hematopoiesis and other aspects of metabolic processes, the thyroid gland is crucial. Patients with thyroid problems often have blood abnormalities as well, due to the critical role that thyroid hormones play in the RBC production and metabolism as well as all other components of blood Chandel et al., (2015); Jafarzadeh (2010).

In addition to subclinical thyroid function abnormalities, many patients present with other abnormalities, such as irregular lipid profiles or blood tests, heart problems, atherosclerosis, or other symptoms that can indicate a thyroid hormone shortage or abnormalities Cinemre (2009); Erikci (2009); Gullu et al., (2005); B. Qasim (2018); Qasim et al., (2019).

A prevalent clinical disease, anaemia affects a disproportionate number of women of childbearing age and the elderly, and its frequency in the general population may approach 10% in certain regions of the globe. A decrease in the quantity of Hb or RBCs causes the blood to be less able to transport oxygen to bodily tissues, a condition known as anaemia. According to the WHO standards, a haemoglobin level below 12.0 g/dL for men and 14.0 g/dL for women is considered anaemia. Normocytic anaemia is defined as an MCV between 80 and 100 fl, microcytic anaemia as an MCV between 80 and 100 fl, and macrocytic anaemia as an MCV of 100 fl or more Organization (2011); Szczepanek-Parulska et al., (2017); Tefferi, (2003).

Both directly and indirectly, thyroid hormones affect blood parameters by increasing erythropoietin synthesis and boosting erythrocyte precursors Szczepanek-Parulska et al., (2017).

Low iron levels impact haemoglobin levels in patients with thyroid abnormalities. Low folate and B12 levels impact haemoglobin and red blood cell counts in as many as 25% of these patients. Comorbid illnesses associated to bone marrow suppression may also induce anaemia Cinemre (2009).

Patients with thyroid problems may have many forms of anaemia; the most frequent of these is iron deficiency anaemia, while microcytic and macrocytic anaemia are less prevalent Szczepanek-Parulska et al., (2017)

According to several writers, thyroid dysfunction is often accompanied with anaemia; in fact, it is believed that over 50% of patients have blood abnormalities. Anaemia and other abnormalities of blood parameters are common symptoms of subclinical hypothyroidism Ashraf (2017)

Nutritional deficiencies and decreased thyroid hormones both contribute to anaemia in thyroid dysfunction patients. The latter causes a decrease in erythropoietin levels, which in turn reduces oxygen supply to tissues, and inhibits the stimulation of bone marrow erythrocyte precursors, all of which lead to anaemia Ashraf (2017); Schindhelm (2013)

This investigation conducted for biochemical and hematological assessment in patients with thyroid dysfunction.

METHOD

The research was carried out at a laboratory in Baghdad, Iraq, and it is a case-control study. The sample was gathered from February 2023 through the years 2021 and 2022. a total of around 250 individuals were involved: 50 of the patients had normal thyroid function and full blood counts, whereas the 100 had hypothyroidism and 100 with hyperthyroidism. Under strict aseptic circumstances, two millilitres of EDTA-anticoagulated blood and three millilitres of whole blood were drawn from these subjects for the CBC, respectively. Also, 2 ml of blood was drawn for serum separation, these sera were used for evaluation T3, T4, TSH, AST, ALT, Urea and Creatinine by using kits purchased from Linear company, the procedures for all were done according to manufacturer instructions. The SPSS version 24 statistical package was used for the study.

RESULT AND DISCUSSION

The control group had a considerably greater mean RBCs, Hb, platelet count, and total cellular volume, whereas the hypothyroid group had a significantly lower mean RBC count, MCV, and MCH. When comparing MCV and MCH, the hyperthyroid group could not identify any significant differences. There were no statistically significant MCHC results in either the hypothyroid or hyperthyroid groups compared to the control group. In terms of total leukocyte count and platelet count, neither the control group nor the hypothyroid group, nor the hyperthyroid group differed significantly from the other two (Table 1). Statistical analysis revealed that the hypothyroid and hyperthyroid groups had significantly different differential leukocyte counts (Table 1).

Table 1. Hematological Analysis In Studied Groups

Hematological Parameters	Control	Hyperthyroid	Hypothyroid
RBC (N x $10^6/\mu l$)	4.61 ± 0.52A	3.89 ± 0.68B	3.42 ± 0.38C
Hemoglobin (g)	14.01 ± 0.61A	11.4 ± 1.94B	9.17 ± 2.54C
Hematocrit (%)	40.96 ± 2.4A	33.83 ± 6.7B	28.27 ± 3.04C
MCV (fl)	84.68 ± 3.29A	81.63 ± 1.2B	80.39 ± 5.41C
MCH (pg)	28.96 ± 0.51A	28.32 ± 0.74A	27.15 ± 2.41B
MCHC (g/dl)	30.23 ± 1.05A	29.61 ± 5.91B	29.36 ± 1.35B
RDW (%)	12.59 ± 0.62B	13.78 ± 0.20A	13.29 ± 0.71A
TLC ($Nx10^3/\mu l$)	8.23 ± 1.40A	7.47 ± 0.98B	7.84 ± 1.63B
Platelet ($Nx10^3/\mu l$)	259.3 ± 108.2A	296.0 ± 24.5A	188.48 ± 36.07B

Table 2 shows that serum TSH levels were greater in the hypothyroid group ($p<0.05$) and lower in the hyperthyroid group ($p<0.05$). In contrast, Table 2 shows that compared to the control group, the hypothyroid group had lower levels of T3 and T4 ($p<0.05$) while the hyperthyroid group had higher levels ($p<0.05$).

Compared to the control group, individuals with hypo and hyperthyroidism exhibited improved liver and kidney functioning, according to the present research (Table 3).

Table 2. Thyroid Hormones Level In Studied Groups

Thyroid indices	Control	Hyperthyroid	Hypothyroid
T3 (ng/ml)	1.39 ± 0.01B	3.58 ± 0.13A	0.94 ± 0.01C
T4 (µg/dl)	8.93 ± 0.63B	15.24 ± 0.21A	3.01 ± 0.74C
TSH (µIU/ml)	2.14 ± 0.12B	0.32 ± 0.01C	8.95 ± 1.6A

Table 3. Some Biomarkers In Studied Groups

Biomarkers	Control	Hyperthyroid	Hypothyroid
AST (U/L)	8.27 ± 0.76C	28.3 ± 5.19A	22.6 ± 3.37 A
ALT(U/L)	14.20 ± 0.38 C	29.1 ± 8.85 A	21.54±4.41B
Urea (mmol/L)	13.1 ± 2.6 C	21.52±1.6 A	18.34±2.1 B
Creatinine (mg/dl)	0.85 ± 0.01 C	1.93 ± 0.24 A	1.65±0.02 B

Anaemia may be caused by thyroid dysfunctions, which impact red blood cells. Importantly, hypothyroidism and hyperthyroidism are these dysfunctions. Also, pancytopenia might be a result of them. Thyroid dysfunction is also associated with changes in haematological parameters such as RBC count, Hb, HCT, MCV, MCH, WBC, as well as platelet count Das et al., (1975). Therefore, in order to ascertain the association between thyroid disorders and alterations in blood counts, this descriptive cross-sectional research was conducted at a tertiary care facility. In comparison to the control group, the hypothyroid group exhibited a notable drop in Mean RBC count, haemoglobin, hematocrit, MCV, and MCH, as well as an increase in RDW (P-value 0.05). In contrast, the hyperthyroid group exhibited a decrease in Mean RBC count, haemoglobin, and hematocrit, as well as an increase in RDW. The hyperthyroid group showed no significant difference between MCV and MCH. Results from the MCHC did not vary substantially (P 0.05) between the control group and the hypothyroid and hyperthyroid groups. The total leukocyte count and platelet count did not vary significantly (P-value 0.05) among the hypothyroid, hyperthyroid, and control groups, according to the statistical analysis.

Das et al., (1975) found anaemia in individuals with hypothyroidism and hyperthyroidism when they examined peripheral blood smears. Golde et al., (1977). In their investigation to associate haematological parameters with thyroid hormones, Dorgalaleh et al., (2013) found no significant differences in RBC, TLC, or platelet count between the hypothyroid and hyperthyroid groups as compared to the control group. On the other hand, Hb, HCT, MCV, MCH, MCHC, and RDW were all significantly different. ("Clinical Relevance of Thyroid Dysfunction in Human Haematopoiesis: Biochemical and Molecular Studies," 2010) While the euthyroid group did not vary substantially in Hb and HCT, Geetha J P et al. found that RDW and MCV were significantly different from euthyroid individuals in hypothyroid and hyperthyroid patients Geetha & Srikrishna (2012).

Research by Kawa et al., (2010) found that, comparing the control group with individuals suffered from hyperthyroidism and hypothyroidism, the results had significantly different levels of RBCs, HCT, MCV, MCH, as well as MCHC Dorgalaleh et al., (2013)

As seen in Table **2**, the hypothyroid group had higher serum TSH levels ($p<0.05$), in contrast to the hyperthyroid group which exhibited lower levels ($p<0.05$). On the other hand, T3 and T4 levels decreased ($p<0.05$) in the hypothyroid group compared to the control group, and increased ($p<0.05$) in the hyperthyroid group. Consistent with other research, our investigation found that experimental groups had well-established hypothyroidism and hyperthyroidism based on changed TSH, T3, and T4 levels on the third and sixth weeks Klecha et al., (2006); Serakides et al., (2005). This study's findings corroborated those of Altaher et al., (2013), which found that hyperthyroid patients had significantly higher T3 and T4 levels as comparing with control. In contrast, hyperthyroid patients had significantly lower TSH levels. A lower TSH level in individuals with hyperthyroidism is consistent with previous studies showing that higher thyroid hormones and a lower TSH level are key indicators of thyrotoxicosis. Graves' disease as well as toxic nodular goitre, which is linked to hyperthyroidism, account for the majority of thyrotoxicosis cases Gilbert (2017). According to prior research by Fadel, et al., (2000) on the onset of hyperthyroidism, a significant drop in TSH levels is often seen with an additional rise in T3, T4 levels. This agreed with the most recent findings.

Consistent with our findings, Ahmed et al., (2013) observed elevated SGOT and SGPT levels in 55 individuals with a diagnosis of hypothyroidism, 24 of whom were male and 31 of whom were female. In a study conducted at Manipal Teaching Hospital in Pokhara, Pandey Pandey et al., (2013) discovered that SGOT, SGPT, and ALP enzyme levels were higher in 30 individuals diagnosed with obvious hyperthyroidism and 30 individuals diagnosed with hypothyroidism. The increase was more noticeable in the group with hyperthyroidism than in the group with hypothyroidism. One hundred twenty-four women were identified as hypothyroid (77 as subclinical hypothyroid and 47 as overt hypothyroid), whereas one hundred twenty-two were listed as euthyroid (control). Consistent with their increase, our results for SGOT, SGPT, and ALP in the liver were reported by Yadav et al., (2013)

Results showed that urea levels were greater in 198 euthyroid people than in overtly hypothyroid subjects Tayal et al., (2009). Their results showed that elevated TSH levels and decreased T3 and/or T4 levels affected serum creatinine levels. Additionally, 98 subclinical and 89 overthypothyroid patients had elevated creatinine levels. The overt hypothyroid individuals in our research had elevated urea levels. This might be because of a reduction in renal clearance of uric acid or an increase in production caused by myopathy associated with hypothyroidism Yokogoshi & Saito (1996).

Two studies found that uric acid and blood creatinine levels were higher in 80 hypothyroid people Arora et al., (2009). There was a statistically significant increase in

creatinine and uric acid levels among 47 patients with overt hypothyroidism and 77 patients with subclinical hypothyroidism when compared to 120 healthy controls, which is in line with the findings of Saini et al., (2012). Our findings were in agreement with those of Wedaatalla and Abdella Wedaatalla & Abdella (2012), who found that urea levels were significantly higher in Sudanese women with hypothyroidism and hyperthyroidism (P< 0.05) when compared to those of the control group. Thirty individuals with newly diagnosed primary hypothyroidism had elevated blood creatinine and uric acid levels, as compared to euthyroid patients, according to Kumar Kumar (2013). Low glomerular filtration rate (GFR) and changes in renin activity may explain why hypothyroidism is linked to increased levels of uric acid and creatinine, according to the research. The hypothyroid person's rennin levels drop, which causes an increase in vascular resistance and a reduction in glomerular filtration rate (GFR) due to the effects of thyroid hormones on SVR and VSMC Ojamaa et al., (1996).

CONCLUSION

Both hypothyroidism as well as hyperthyroidism affects on hematological and biomarkers for thyroid, liver and kidney.

AUTHOR CONTRIBUTIONS

All authors played a role in the preparation of this article.

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The Protective Role of Nigela Sativa Volatile Oil on Antioxidant and Oxidative Stress Enzymes

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Hepatotoxicity is the outcome of a paracetamol overdose.In this study, 40 adult male rats, weighing 180–260 gm and aged between 9 and 13 weeks, were given doses of Nigela sativa oil and their liver enzymes were examined to see how paracetamol affected them. The rats were kept in an environment at a temperature of 25 Co in an animal house. ,There were four groups of rats, and food was provided for them. (G). (G1) The only injections given to the control group are food and regular saline (0.9% of the time). (G2) 200 mg/kg of N. sativa and (G3) 400 mg/kg of paracetamol Addition of 400 mg/kg body weight of paracetamol. supplying 300 mg/kg B.W. of N. sativa to (G4)400 milligrams/kg B.W. of aspirin. Rat G2, GPT 87.80 IUL, and GOT hepatic enzyme concentration (129.32 IUL) all exhibit significant increases in comparison to the control. Furthermore, the levels of the liver GSH enzyme in rats (G2)1.24 IUL shown a substantial increase, whereas G3 (GOT) 91.9 IUL, (GPT)76.70 IUL, and group (G4) GOT 109.88 IUL and enzyme (GPT) 55.66 IUL exhibited significant declines as compared with control.While MDA enzyme in rats G2 (0.259 IUL) shows a major increase in comparison to the control, rats G3 (0.139 IUL) and G4 (0.112) show a noticeable decrease in comparison to G2. While body weight indicates that G2's outcomes are significantly lower than those of the other three groups. Groups G3 (2.55 gm) and G4 (2.7 gm) demonstrated a significant rise in liver weight compared to controls and G2, whereas groups G2 (24.23 gm) demonstrated a significant increase in liver weight compared to controls and group G2. Male rats in groups G2 (1.7 gm) demonstrated a significant drop in liver weight compared to controls. This the study the antioxidant and protective volatile oil extract from N. sativa. again overdose of paracetamol.

Keywords: Nigela sativa, Hepatotoxicity , Paracetamol, Volatile Oil

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INTRODUCTION

Herbalists use a variety of plants referred to as alternative or therapeutic herbs.These plants are regarded as an abundance of secondary metabolism in motion. substances that can be employed in the research and manufacturing of drugs (Kapoor dkk., 2013). Examples of active secondary metabolites substances include polysaccharide, flavone, terpenoid, and phenol (Kumar & Yadav, 2009).

N.Sativa holds a special place among Southeast Asian herbal items as a non-prescription treatment for a variety of ailments.There have been reports of its antibacterial, hypolipidemic, antidiabetic, and antihypertensive effects (Saha dkk., 2004). The phytochemical, pharmacological, and toxicological properties of N.Sativa. Recently, have been reviewed (Ali & Blunden, 2003). The plant is widely cultivated throughout the world.Nigella sativa, an oriental spice, has been used for a very long time as a natural remedy for the treatment of numerous acute and chronic illnesses (Usmanghani dkk., 1997). sativa have recently been the focus of a variety of pharmacological studies.These research revealed a broad range of actions, including antibacterial, anticancer, anti-inflammatory, mutabagani, hypoglycemia, smooth muscle relaxant, cytotoxic, and immunostimulant properties (Sezik dkk., 2001).

It was shown that N. sativa is highly bioavailable and offers noticeably better defense against free radical-induced DNA damage and lipid peroxidation (Mansour dkk., 2002), To protect cells from the destructive effects of reactive oxygen species (ROS), antioxidant enzymes defense the mechanisms that protect cells from the cellular a free radicals as well as restore and stop the growth of molecules damaged by oxidative stress. are crucial (Plaa & Hewitt, 1982). Typically, ROS results in the oxidation of proteins, lipids, and DNA (Burits & Bucar, 2000) .A product is the malondialdehyde enzyme (MDA). that is regarded to be a symptom of cell damage from peroxidation under the majority of oxidative stress circumstances. The free radicals and ROS combine to produce lipid peroxidation in membrane lipids (Mates dkk., 1999).

The human liver can efficiently and swiftly break down naphthalene into stable protein-reactive and cytotoxic metabolites, but if microsomal proteins do not act promptly to detoxify these metabolites, they can harm DNA, proteins, and lipids found in cell membranes and other tissues.Additionally, it has been shown that intracellular reduced glutathione may effectively detoxify naphtha (Tingle dkk., 1993).

The most popular OTC drug, paracetamol (acetaminophen) was only developed in the 1960s after being discovered 100 years earlier (Cranswick & Coghlan, 2000). Due to its accessibility and wide availability, self-poisoning with paracetamol is a common occurrence throughout the world (Guidet & Shah, 1989). In cases of symptomatic fever temperature (T> 38.5°C), a dose of 15 mg/kg every 6 hours (60 mg/kg/day) of oral or rectal paracetamol is advised (*New South Wales (NSW) Therapeutic Advisory Group Inc*, 2008). Although prolonged administration of supratherapeutic doses of paracetamol (>90 mg/kg/day) to a sick child under the age of two has been identified as a significant risk for hepatotoxicity, acute ingestion of a higher dose of 150 mg/kg/day of paracetamol has been found to be safe (Rumack & Matthew, 1975)the recommended dosage for analgesia is 15 mg/kg every 4-6 hours, up to a maximum of 60-90 mg/kg/day. 60% of cases of acute liver failure in the United States and the United Kingdom are caused by paracetamol overdoses, either on their own or in conjunction with other medicines, necessitating orthotropic liver transplants.

METHOD

A. Plant C

Plant material from the plant N. sativa that was purchased from Karbala, Iraq, on May 18, 2022, was a mechanically processed in to a powder and used straight away (Al-Ibrahemi dkk., 2020).

B. Extraction of Volatile Oil

Using 60 grams of N.sativaof 70% methanol and were combined in a thimble before being placed in a flask.A rotary evaporator operating at 45°C was then used to evaporate the extract(Al-Ibrahemi dkk., 2022).

C. Study of Secondary Metabolism Regulators:

1. Saponins

Foam formed after agitating the extract aqueous solution for an extended period of time(Al-Ibrahemi dkk., 2020).

2. Phenolics

Five milligrams of dill extract and 0.5 milliliter of a 1% lead acetate solution were used to create a precipitate, which was used to identify lead acetate.

3. Glycosides

After the extractor (0.5 mg) was dissolved in 1.0 ml of water, an aqueous solution of sodium hydroxide (NaOH) was added.

4. Tannins

After adding distilled water, A water bath equipment was used to boil 5 cc of extract for 10 minutes at a temperature of 80–100 °C. After the liquid was filtered, five drops of 1% ferric chloride were added to give it a dark green hue(Geissman, 1962)

5. The Alkaloids

A reddish-brown precipitate was produced by combining the recovered filtrate with Wagner reagent, which is a potassium iodide and iodine solution(Pandey dkk., 2011)

6. Flavonoids in

After combining, a few drops of concentrated HCl were added, and the liquid was heated with magnesium until a red hue emerged. 4 mL of extracts were mixed with 1.5 ml of

50% methanol. There are flavonoids when a color is red(Al-Bazaz dkk., 2020)

D. Experiment Design

The rats were kept in an environment at a temperature of 25 Co in an animal house. , There were four groups of rats, and food was provided for them. (G). (G1) The control group receives injections with only meal and saline solution (0.9%), 400 mg/kg of paracetamol (G2), and 200 mg/kg of N. sativa (G3) coupled with 400 mg/kg B.W. of paracetamol (G3) were used. giving (G4) 400 mg/kg B.W. of paracetamol and 300 mg/kg B.W. of N. sativa.

E. Biochemical Analyses

To separate the blood serum, blood was taken using the cardiac puncture procedure then centrifuged at 3000 rpm for 10 minutes. At 40 degrees, the blood serum was maintained until the enzyme assays were run, and the blood was drawn after 30 days.

1. The creation of glutamate pyruvate for GPT and glutamate oxaloacetate for GOT, which results in the transfer of an amino group from alanine or aspartate to oxoglutarate, is the technique for measuring GOT and GPT. The GPT and GOT were measured using a kit technique. (Reitman France colorimetric method, linear chemical, S.L., Spain) (Guidet & Shah, 1989)

2. Malondialdehyde (MDA) µmol/ L:

   Procedure :

   Following was a sample and a blank represented by two tubes.
   The solution was centrifuged at 450 x g for 15 minutes after standing at room temperature for 20 minutes. The absorbance of all sample was measured at 532 nm (Ellman, 1959)

3. Glutathione or GSH measure (µmol/ L):

   Solution for Tris Buffer

   The result of dissolving them was 6.57 grams.0.0292 grams and 900 milliliters of Tris hydroxyl methyl aminoethane water distillationAdd 0.1 ml of EDTA (Ethylenediaminetetraacetic Acid) to bring the volume up to 1 L. Refrigerate the mixture until you need to utilize the acidic distill water substrate (pH 7.6) (Ghosh & Sil, 2009)

RESULT AND DISCUSSION

A. Chemical Analysis of Phytochemistry:

The active phytochemical components in the N .sativaextract were found this results of the chemical compound screen analysis. Table shows sativa (1). were negative results for tannins but positive results for phenol, glycosides, alkaloids, saponins, and flavonoids (Ijaz dkk., 2017).

Table 1. Phytochemical Screening of N. Sativa Extract

Reagent	Sample	Blank
Sample	150µ	……
TCA (17.5%)	1ml	1ml
TBA (0.6%)	1ml	1ml
All tubes were combined using a vortex, then heated in an 80°C water to bath for 15 minutes before being allowed to cool to 25°C.		
TCA (70%)	1ml	1ml
D.W	……	150µl

B. Biochemical Test

Table 2. GOT and GPT enzyme percentages for the rat groups given the concentrations (U/L) of the research

Parameter/ Treatment Groups	GOT	GPT
G1 Control	50.21	87.12
G2	87.80	129.32^{a}
G3	67.70^{b}	91.9^{b}
G4	51.66^{b}	109.88^{b}
LSD	0.17	

(a) indicates a significant increas
(b) denotes a significant fall) p≤ 0.05

Table 3. GSH and MDA enzyme percentages for rat groups administered the study's concentrations levels (U/L)

Parameter/ Treatment Groups	GSH	MDA
G1 Control	0.176	2.29
G2	0.259^{a}	1.24^{b}
G3	0.139^{b}	2.12^{a}
G4	0.112^{b}	2.28^{a}
LSD		0.19

An important change is indicated with a small letter

(a) indicates a discernible increase
(b) denotes a significant fall) p≤ 0.05

Table 4. both the liver and overall body weight of Rat

Parameter/ Treatment Groups	Weight of Body (gm)	Liver Weight (gm)
G1 Control	2.88	29.76
G2	1.77^{b}	24.23^{b}
G3	2.55^{a}	26.95^{a}
G4	2.81^{a}	28.01^{a}
LSD		0.13

An important change is indicated with a small letter.

(A) indicates a significant rise
(B) denotes a significant drop. p≤ 0.05

C. Result

The results showed that there was a significant increase of levels of the liver enzymes in rats G2,GOT (129.32 IUL) and GPT (87.80 IUL) when compared with the control group . When compared to the control, G3 GOT (91.9 IUL), GPT (76.70 IUL), and G4 GOT (109.88 IUL) and GPT (55.66 IUL) all demonstrated a substantial decline. Paracetamol (400 mg/kg) showed hepatotoxicity after 24.

Table (3) indicates Rats in groups G2 (1.24 IUL) had a substantial drop in liver GSH enzyme levels compared to controls, while groups G3 (2.12 IUL) and G4 (2.28) have significant increases. MDA enzyme levels are significantly higher in rats in G2 (0.259 IUL) compared to controls, but significantly lower in G3 (0.139 IUL) and G4 (0.112 IUL) than G2 (Mates dkk., 1999).

According to table (4)'s results, G2 significantly decreased when compared to the other three groups. G2, G3, and G4 male rats' body weights (24.23 gm, 26.95 gm, and 28.01 gm, respectively) showed a substantial rise in comparison to control and G2. Male rats in groups G2 and G4 had a significant higher liver weights than control and G2, but male rats in groups G3 and G4 had significantly higher liver weights than control.

D. Discussion

The goal of the current study was to examine the antioxidant and protective effects of N. sativa extract on liver enzyme damage caused by paracetamol.

It is commonly recognized that some paracetamol is converted by the cytochrome P450 pathway into the dangerous metabolite N-acetyl-Pbenzoquinamine, which, if swallowed unintentionally at a height, can result in substantial liver cell death (NAPQI) (Schmidt & Dalhoff, 2002)

High dosages of paracetamol cause liver GSH depletion (because GSH combines with NAPQI to form mercapturic acid), which raises lipid peroxidation by absorbing hydrogen from polyunsaturated fatty acids and finally damages the liver (Itadt & Krauss, 2000). When the liver or heart are injured, The liver and cardiac cells ordinarily contain the enzyme serum glutamic pyruvic transaminase (sGPT) (EC 2.6.1.2), which is released into the bloodstream.Thus, blood sGPT levels rise in response to damage to the liver or heart. Some drugs, such as aspiri diclofen sodium, and paracetamol, can also increase sGPT levels(Kushwah dkk., 2013).

It is well known that hepatic parenchyma cells are adversely affected by toxins such as paracetamol to the extent that the total level of plasma protein lowers. The consumption of paracetamol significantly increased GPT and protean (Plaa & Hewitt, 1982). pointing out that paracetamol use lowers uric acid and total protein levels while raising the levels of the GPT, GOT enzymes and the glucose, all of which enhance the risk of paracetamol toxicity (Kadhim dkk., 2013)

The present study's objective was to determine whether Nigella sativa oil has a protective impact on liver functions against paracetamol-induced acute toxicity. An increase in serum levels of liver enzyme is a biochemical manifestation of paracetamol hepatotoxicity (Yarmohammadi dkk., 2012). The reduction of liver enzyme levels (ALT and AST) in mice treated with Nigella sativa may be linked to the slowing of body weight increase, indicating a potential therapeutic benefit of Nigella sativa administration (Pari & Sankaranarayanan, 2009).

The volatile oil of a Nigella sativa was examined for the antioxidant activity and found to contain high levels of thymoquinone carvacrol, t-anethole, and 4-terpineol.These findings demonstrated that TQ has positive impacts on hepatic enzyme activities, which may have an anti-hyperglycemic effect (Burtis dkk., 1999).

The first indication that the intestinal absorption of glucose is directly restricted by Nigella sativa (black seed) through electrogenic processes in culture.additional to the documented increase in body weight and glucose tolerance in rats after receiving continuous oral therapy in vivo.Many studies have shown that Nigella sativa and its active component, thymoquinone, have hepatoprotective qualities (Li dkk., 2009)

The neutralization of free radicals in lipids and the prevention of hydrogen peroxide's breakdown in to free radicals are the mechanisms by which phenolic compounds (flavonoids) exert their antioxidant activity(Durgo dkk., 2007). Due to their redox characteristics and nucleophilic thiol groups, flavonoids play a significant role in plants' overall antioxidant activities. They can detoxify compounds by donating protons or hydrogen atoms to reactive metabolites or free radicals, conjugating substances with the aid of glutathione-S-transferase (GST), or chemically reacting with reactive metabolites to create conjugates.Reactive intermediates may be able to prevent cell death by interacting with GSH either directly or through a process mediated by GST (Javanmardi dkk., 2003)

Status of Antioxidants Consuming black seeds has been shown to reduce oxidative stress and enhance the activity of antioxidant enzymes in all studied tissues.Black seeds' multifunctional properties as an anticancer, antioxidant, anti-inflammatory, and antibacterial "drug" come from their oral usage (Harborne, 1984).

CONCLUSION

According to the current research, paracetamol's hebatotoxic effects may be reduced by dill and flavonoid extract's antihebatotoxic properties.

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Role of House Fly, *Musca domestica* (Diptera: Muscidae) as a Mechanical Vector of pathogenic Bacteria in Thi Qar Province

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ABTRACT

The common house fly, known as *Musca domestica*, has been acknowledged to carry and spread numerous contagious illnesses. The purpose of this investigation is to uncover the extent of the house fly's involvement as a carrier of harmful bacteria that can lead to diseases in both humans and animals. To accomplish this goal, a collection process was conducted using manual traps from March 2023 until July 2023. I collected house flies from various locations in Thi Qar province. A total of 370 samples were gathered, out of which 270 flies were utilized to isolate bacteria from both the outer and inner surfaces. To identify the house flies, I sent 100 of them to the Natural History Museum at the University of Basra. The current study recorded among 270 flies 104 (38.52%) have not bacteria, while 166 (61.48%) have bacteria. The results were recorded the highest isolated bacteria from outer surface 94 (34.81%) and 72 (26.67%) from inner surface, in contrast the lowest negative bacteria were from outer surface 41 (15.19%), and 63 (23.33%) inner surfaces. The study recorded the highest number of isolated bacteria were from Arido 23 (8.52%), followed in both Al-Sharqiyah and Altathhia 22 (8.15%), followed by Shuhadda, Ur and Summer 21 (7.78%), while the lowest isolated bacteria were in Shmoukh 4 (1.48%), followed in both Aledara almahaleia 14 (5.19%). The current study recorded the most isolated bacteria was *P. vulgaris* 21 (20.19%), followed by P. mirabilis 18 (17.31%), followed by S. aureus 11 (10.58%), in contrast the lowest isolated bacteria were *P. stuartii* 1 (0.96%), followed by both *S. paucimobilis* and *S. maltophilia* 2 (1.92%).

Keywords: Bacteria, House fly, Mechanical Vector, *Musca domestica*

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INTRODUCTION

The common house fly, scientifically known as *M. domestica* Linnaeus, can be found buzzing around in human houses, places where food is made, hospitals, restaurants, food markets, poultry and livestock farms, and various other domestic spaces or structures Klakankhai (2022). These little buggers are quite a nuisance to people, as well as to poultry, livestock, and other farm animals. Due to their habits and way of life, flies are known to be carriers of disease-causing microorganisms both mechanically and biologically. What makes the housefly particularly worrisome is its fondness for our food Wilson (2021). Flies have a natural inclination towards decomposing plants and animal organisms, which leads them to interact with various ecosystems, litter, and animal feces Davies et al., (2016). The areas that witness a substantial presence of manure or compost, particularly those lacking in human sanitation and kennels, serve as the primary breeding grounds for house flies and the concurrent propagation of bacteria El-Ghwas et al., (2021).

House flies are notorious carriers of various dangerous diseases, including anthrax, ophthalmia, tuberculosis, cholera, typhoid fever, and infantile diarrhea. They have the ability to transmit these illnesses between humans, animals, and vice versa Akter et al., (2017). Researchers have discovered over 130 pathogens on the surface of house flies, primarily bacteria. This highlights the critical role played by these insects in spreading harmful diseases Manickkam and Moses (2023).

When it comes to the transfer of disease-causing organisms by insects, such as house flies, every nook and cranny on their exterior can potentially serve as a mode of transportation for these tiny germs Ranasinghe (2023). Thus, the extent to which a specific body part of a house fly contributes to the spread of these microbes depends on its ability to carry out various tasks: capturing the germs, retaining them during travel, ensuring their infectivity remains intact, and ultimately depositing them onto either a new host or a surface that may come into contact with the host Yap et al., (2008). Grown-up flies function as carriers for certain diseases, carrying them from one place to another by interacting with the host. The transmission of diseases happens when the pathogens are dislodged from the fly's outer covering, through regurgitation or by contaminating surfaces with their feces. It has been reported that houseflies play a substantial role in spreading viruses Fayyaz et al., (2021).

The conduct of house flies heightens their ability to transmit bacterial pathogens. They dwell in close proximity to individuals (synanthropic) or within their abodes (endophilic cosmopolitan), and they chiefly sustain themselves by consuming animal and human excrement (coprophagic) as well as decaying substances, like trash Iqbal et al, (2014). Consequently, all stages of their life cycle may be exposed to various pathogens in unsanitary environments, which can then be mechanically transferred to humans. In the span of their lives, adult flies can traverse up to 20 miles, signifying their ubiquitous presence in the surroundings and their adeptness at dispersing pathogens from unhygienic zones into people's residences and places of employment and recreation Yin et al., (2022)

METHOD

Collection of flies

I went around various places in Thi Qar province, like Aledara almahaleia, Al Sharqiyah, Arido, Batha, Shmoukh, Shuhadda, Sumer, Tathhiah and Ur to gather house flies. I managed to collect a total of 370 samples by using a manual insect trap. As soon as I caught the flies, I sorted them into equal groups and placed them in glass tubes. Without wasting any time, I swiftly transported the tubes to the laboratory. To render the flies inactive, I put them in a freezer set at a chilling temperature of 0°C for a duration of 3 minutes Hasaballah (2021). House flies were identified by sending 100 flies to the Natural History Museum at the University of Basra. The flies have been identified as belonging to the class: Insect, order: Diptera, suborder: Cyclorrhapha, family: Muscidae, Genus: Musca, Species: *M. domestica*.

Culture and isolation of bacteria from house flies

The bacteria were cultured from 270 adult house flies that were collected in two ways:

The first: By adding 5 ml of distilled water to a test tube containing the adult house fly's external surface, we then cultured the bacteria. The next step involved shaking the mixture and taking a swab with a loop to streak it on MacConkey and blood agar. After incubating for 24 hours at 37°C, we obtained our results Hassan et al., (2022).

The second: The process involved collecting the bacteria from inside the adult flies, which was done by putting it in a test tube and sterilizing the outer surface with ethanol. After letting it dry, the fly was crushed and its internal entrails were extracted. Then, 5 ml of d.w. were added and shaken before taking a swab with a loop and streaking it on MacConkey medium and blood agar. Finally, it was incubated for 24 hours at 37°C Nwangwu et al., (2013)

Laboratory Diagnosis

Characteristic of Cultural Isolations

We examined the traits of the colonies that were separated and studied after we cultivated and purified the bacterial isolates on culture media. Our investigation involved using various types of agar, such as MacConkey Agar, Blood

Agar, and Mannitol Salt Agar. We looked at characteristics such as shape, size, texture, color, edges, and heights to understand more about these isolated bacterial colonies Yaseen et al., (2019).

Microscopic Characteristics

We took a closer look at the microorganisms by using a microscope once we had stained them with Gram stain. To do this, we spread a few bacteria from a colony onto a clean slide with a drop of normal saline. Then, we applied heat to fix them in place and proceeded to stain them with crystal violet, Iodine, alcohol, and safranine. Finally, we examined them under oil immersion.

Identification of Bacterial Isolates using Confirmatory Tests API-20 E System

According to the Manufacturer's Instruction Kit (BioMrieux), the API 20E system was utilized for Enterobacteriaceae. The 20 tests included in the kit were the Beta-galactosidase test (ONPG), Ornithine decarboxylase test (ODC), H2S production test (H2S), Lysine decarboxylase test (LDC), Urease test (URE), Indole production test (IND), Arginine dehydrolase test (ADH), Citrate utilization test (CIT), Tryptophan deaminase test (TDA), This test was conducted in accordance with the manufacturer's guidelines. (BioMerieux, France) as following:

A single isolated colony (from a pure culture) was suspended in sterile D.W.

1. Please fill these compartments completely with the bacterial suspension.
2. Please ensure that the ADH, LDC, ODC, H2S, and URE compartments are filled with sterile oil.
3. The container containing the strip was sealed shut and placed in an incubator set at a temperature of 37ºC. It remained there for a period of 18-24 hours.
4. After the incubation period, the necessary substances were introduced into their respective tubes: a drop of Kova's reagent was added into the IND tube, and a drop of TDA reagent was added into the TDA tube. The result was then observed without delay. Additionally, a drop of VP1 reagent followed by a drop of VP2 were directly introduced into the VP tube. After waiting for 10 minutes, the result was read. Subsequently, all the obtained results were carefully documented and compared with the company index based on numerical profiles. Every strip was divided into seven segments, and within each segment, there were three tests labeled as 1, 2, and 4. While the positive tests were assigned distinct numbers, the negative test was assigned the number zero. Ultimately, in the end result, there are seven numbers that can be compared with the index to determine the genus and species of the bacterial isolates.

Identification of Bacterial Isolates using Vitek 2 System

The Vitek 2 system was employed to verify the recognition of bacterial isolates belonging to both gram negative and gram positive categories. To accomplish this task, a distinctive diagnostic kit tailored for this particular system was utilized. This kit includes a diagnostic card specifically designed for gram-negative and gram-positive bacteria, comprising a total of 64 slots. Each slot contains a dry color indicator that interacts with the provided sample. The system meticulously registers these alterations, which are caused by the growth of bacteria within the holes. By analyzing the resulting changes in color, the Vitek 2 system then proceeds to identify the bacterial sample in accordance with the instructions laid out by BioMerieux.

RESULT AND DISCUSSION

Isolation Bacteria from Flies

The current study involved 270 flies were classified in to two part 135 of flies used for isolation bacteria from outer surface and the other 135 flies used for isolation bacteria from inner surface, the current study recorded among 270 flies 104 (38.52%) have not bacteria, while 166 (61.48%) have bacteria, the results also noted a significant difference between bacterial isolation at p. value < 0.05 as in Figure 1.

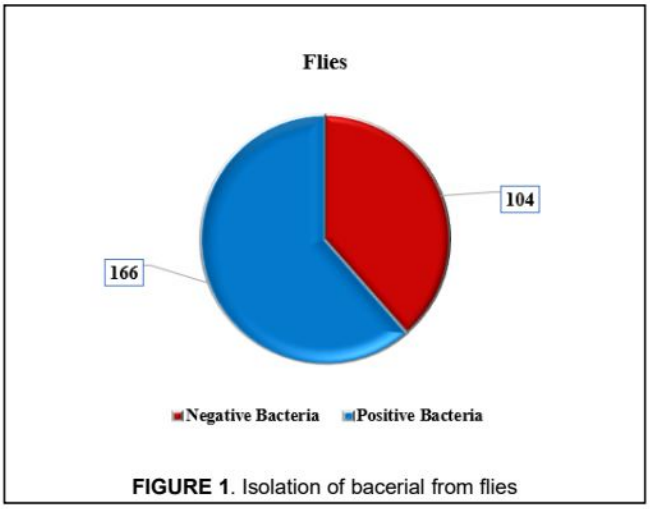

FIGURE 1. Isolation of bacerial from flies

Prevalence of Bacteria According to Their Site on Flies

The results of the current study recorded the highest isolated bacteria from outer surface 94 (34.81%) and 72 (26.67%) from inner surface, in contrast the lowest negative bacteria were from outer surface 41 (15.19%), and 63 (23.33%) inner surfaces, the study also a significant difference between bacterial isolation according to site of sample at p. value < 0.05, as in Table 1.

TABLE 1. Prevalence of bacteria according to site sample flies

Site / Sample	Outer surface		Inner surface		Total	
	No.	%	No.	%	No.	%
Negative bacteria	41	15.19	63	23.33	104	38.52
Positive bacteria	94	34.81	72	26.67	166	61.48
Total	135	50.0	135	50.0	270	100

CalX²= 7.52 TabX²= 3.84 DF=1 p. value 0.006

Prevalence of Bacteria According to the Site of the Flies Sample

TABLE 2. Prevalence of bacteria depends on location of fly sample

Sample Location	Negative bacteria		Positive bacteria		Total	
	No.	%	No.	%	No.	%
Ur	9	30.0	21	7.78	30	11.1
Sumer	9	30.0	21	7.78	30	11.1
Batha	12	40.0	18	6.67	30	11.1
Shmoukh	26	86.67	4	1.48	30	11.1
Al-Sharqiyah	8	26.67	22	8.15	30	11.1
Tathhia	8	26.67	22	8.15	30	11.1
Arido	7	23.33	23	8.52	30	11.1
Aledara almahaleia	16	53.33	14	5.19	30	11.1
Shuhadda	9	30.0	21	7.78	30	11.1
Total	104	38.52	166	61.48	30	100

CalX²= 41.4 TabX²= 15.51 DF=8 p. value < 0.001

The current results recorded the highest isolated bacteria were from Arido 23 (8.52%), followed in both Al-Sharqiyah and Altathhia 22 (8.15%), followed in Shuhadda, Ur and Summer 21 (7.78%), while the lowest isolated bacteria were in Shmoukh 4 (1.48%), followed in both Aledara almahaleia 14 (5.19%), the study also a significant difference between bacterial isolation according to location of sample at p. value < 0.05, as in Table 2.

Prevalence of Bacteria According to Location of Flies Sample

The current results recorded the most isolated bacteria were from outer surface of flies 94 (56.63%), while the lowest isolated bacteria from inner surface 72 (43.37%), also, recorded the most flies have bacteria were that collected from Shmoukh, Aledara almahaleia, Sumer, Ur, and Shuhadda, the study also a significant difference between bacterial isolation according to location of flies' sample at p. value < 0.05, as in Table 3.

TABLE 3. Prevalence of bacteria according to location of flies' sample

	Inner surface		Outer surface		Total	
	No.	%	No.	%	No.	%
Ur	9	42.86	12	57.14	21	12.65
Sumer	10	47.62	11	52.38	21	12.65
Batha	7	38.89	11	61.11	18	10.85
Arido	2	50.00	2	50.00	4	2.41
Al-Sharqiyah	15	68.18	7	31.82	22	13.25
Aledara almahaleia	5	22.73	17	77.27	22	13.25
Shmoukh	8	34.78	15	65.22	23	13.86
Tathhiah	8	57.14	6	42.86	14	8.43
Shuhadda	8	38.10	13	61.90	21	12.65
Total	72	43.37	94	56.63	166	100

CalX²= 55.7 TabX²= 15.51 DF=8 p. value < 0.001

Identification of Isolated Bacteria

The current study recorded the most isolated bacteria was P. Vulgaris 21 (20.19%), followed by P. mirabilis 18 (17.31%), followed by S. aureus 11 (10.58%), in contrast the lowest isolated bacteria were P. stuartii 1 (0.96%), followed by both S. paucimobilis and S. maltophilia 2 (1.92%), as in Figure 2.

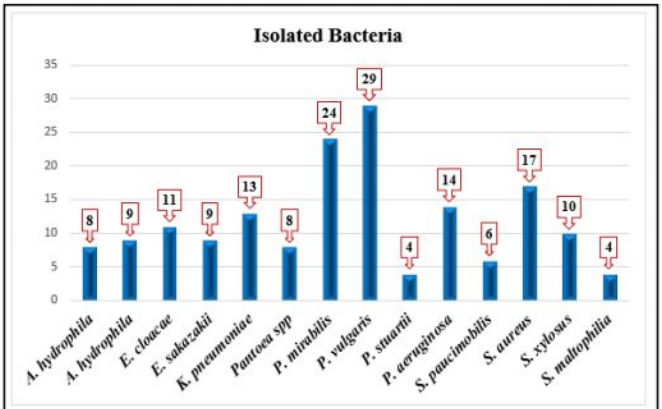

FIGURE 2. Identification of Isolated Bacteria.

Prevalence of Isolated Bacteria According to Site of Flies Sample

The results of present study showed that the most isolated bacteria were P. vulgaris and P. mirabilis from outer surface 17 (10.24%) and 14 (8.43%), respectively. Also, the most isolated bacteria from inner surface were P. vulgaris and P. mirabilis 12 (7.23%) and 10 (6.02%), while the lowest isolated bacteria were S. maltophilia 2 (1.20%), from both outer and inner surface, this study also a non-significant difference between bacterial isolation according to location of flies' sample site at p. value < 0.05, as in Table 4.

TABLE 4. Prevalence of isolated bacteria according to site of flies' sample

Bacterial Species	Inner surface		Outer surface		Total	
	No.	%	No.	%	No.	%
A. hydrophila	3	1.81	5	3.01	8	4.82
A.hydrophila	3	1.81	6	3.61	9	5.42
E. cloacae	5	3.01	6	3.61	11	6.63
E. sakazakii	4	2.41	5	3.01	9	5.42
K. pneumoniae	6	3.61	7	4.22	13	7.83
Pantoea spp	3	1.81	5	3.01	8	4.82
P. mirabilis	10	6.02	14	8.43	24	14.46
P. vulgaris	12	7.23	17	10.24	29	17.47
P. stuartii	3	1.81	1	0.60	4	2.42
P. aeruginosa	6	3.61	8	4.82	14	8.43
S. paucimobilis	3	1.81	3	1.81	6	3.61
S. aureus	8	4.82	9	5.42	17	10.24
S. xylosus	4	2.41	6	3.61	10	6.02
S. maltophilia	2	1.20	2	1.20	4	2.41
Total	72	43.37	94	56.63	166	100
CalX2= 2.68	TabX2= 22.23	DF=13	p. value 0.999			

M. domestica, commonly known as the common house fly, has a strong association with humans and can be found in various regions across the globe Dawaye (2024). The growth of its larvae occurs in decaying organic matter like fecal sources, while adults have a diverse diet that includes human and domestic animal food, waste, and excrement Salem and Attia (2021). Moreover, these adult flies serve as carriers for disease-causing agents that pose threats to both humans and domestic animals Hassan et al, (2021).

House flies possess the ability to mechanically transmit harmful pathogens that have an adverse effect on the well-being of both humans and animals. The daily flight behavior of house flies, which includes dispersal flights, undoubtedly plays a significant role in their capacity to not only cause annoyance but also propagate disease-causing agents Brewer et al., (2021).

House flies are most active during the day, but they prefer to find a place to rest overnight as the sun begins to set. During these resting hours, they take a break from their buzzing and flying, possibly because they sense the temperature changing with the arrival of night. Interestingly, their flight patterns differ depending on the season. In cooler months, their activity follows a single peak, while in hotter months, it shows a two-peaked.

CONCLUSION

1. The prevalence and occurrence of bacteria in all studied environments is evidence of house fly prevalence.
2. Healthcare and hygiene play an important role in reducing environmental pollution and limiting the spread of disease-carrying insects.
3. House flies can spread disease germs through various parts of the body, both internally and externally.

AUTHOR CONTRIBUTIONS

All authors played a role in the preparation of this article.

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RESEARCH ARTICLE
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Extraction and Identification of Certain Bioactive Compounds With Antibacterial Activity From The Green Algae *Cladophora glomerate*

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ABTRACT

Cladophora glomerata algae extract was used to inhibit the growth of five bacterial species: *Escherichia coli*, *Staphylococcus aureus*, *Proteus mirabilis*, Citrobacter, and *Klebsiella pneumoniae*. Algae samples were collected from the river Euphrates in Al-Fadhiliya/ Thi-Qar governorate and extracted into alcohol. The concentrations of the extracts were compared with five antibiotics. The 200% concentration showed the highest effectiveness against bacteria, with an inhibition zone of 28mm. The antibiotics Imipenem and Meropenem had an inhibition zone of 28mm, while Amikacin, Norfloxacin, and Piperacillin had an inhibition area of 12-21mm. The 20% concentration was least effective against *Escherichia coli*, with an inhibition diameter of 2mm. The alcoholic extract of *C. glomerata* alga had the highest inhibition region of 28 against *Klebsiella pneumoniae* and 13mm against *Proteus mirabilis*. The chemical algal contents were identified using mass GC technology, revealing compounds like 10-Undecyn-1-ol, n-hexadecanoic acid, 4,4-dimethyl-1-hex, and isoamyl nitrite, which were found to have the major function of inhibiting bacterial growth.

Keywords: Antibiotics, *Cladophora glomerata*, Pathogenic Bacteria

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INTRODUCTION

Algae are a group of thallophytes, or plants without stems, roots, leaves, fruits, or flowers. Because they contain auxiliary representative pigments like carotenoids, xanthophylls, and phycobiloproteins, as well as different types of chlorophyll pigments, algae are autotrophic organisms that can produce oxygen during the process of photosynthesis Winnacker (2015).

Algae can live in the ocean's dark, deep waters. The majority of them are separated into red, brown, yellow, green, and other types of algae based on the pigment components found in them. Not only are they useful in pharmacology, medicine, and the environment, but they are renewable living resources that are utilized as fertilizer and food in many parts of the world Nithya and Dhanalakshmi (2016).

Antibiotics have revolutionized therapeutics globally, but their uncontrolled use has led to antibacterial resistance, a major public health threat in the 21st century. Over 40 countries have shared reports on antimicrobial resistance surveillance with the World Health Organization, indicating its criticality. As a result, alternatives are needed to address this challenging situation and protect public health. Bhowmick et al., (2020).

Many alternatives, including algae, have been studied for their potent antibacterial qualities. Their capacity to endure harsh conditions and competitive settings enables them to produce a diverse array of bioactive substances through intricate metabolic processes that differ significantly from those of terrestrial animals. Algae metabolites are interesting sources of antibacterial chemicals because they have strong antibacterial action against a variety of bacteria, including ones that are resistant to drugs Surendhiran et al., (2021); Gao et al., (2023).

C. glomerata, a group of over 183 species of green algae. These algae are rich in antimicrobial, antioxidant, antidiabetic, and antitoxic cell activities, making them valuable for human health. They are classified as a healthy food by the World Health Organization. However, some antibiotics produced by microorganisms have become useless due to severe antimicrobial symptoms, high costs, and side effects like hypersensitivity. This has led to the development of new antibiotics in diverse environments, highlighting the importance of algae as active substances. Mohammed et al., (2021).

C. glomerata can be found in both fresh and saltwater habitats. It is well-known for its intense, recurring blooms. Saturated and unsaturated fatty acids with antibacterial properties are abundant in C. glomerata. Additionally, phenolic compounds-which are well-known for their antioxidant qualities-are present in it. In a rat study, its ethanolic extract also shown analgesic, anti-inflammatory, and anti-ulcer properties Petchsomrit et al., (2023).

The current study aims to extract the active substances from algae *C. glomerata* using the organic solvent of ethanol, and test its effect against the bacterial isolated from pathogenic samples.

METHOD

Collecting of algae

In January 2024, during the winter, C. glomerata was collected in plastic bags in the form of biomass algae from the Euphrates River in the city of Al-Fadhiliya/ Thi-Qar governorate. After that, the algae was brought into the lab and cleaned three times using distilled water after first being cleaned with regular water to get rid of any remaining dust, dirt, and living organisms. The algae mass was exposed to sunlight for five days, grinding into a powder, and then canning it. After that, it was kept at 0–4°C until used Peller et al., (2007); Weidman et al., (1984).

Preparation of alcoholic algal extracts

In a 500 cm3 volumetric flask, 10g of the dry weight of *C. glomerata* was combined with 250 ml of 70% ethyl alcohol. A Soxhelt device was used to extract the material for 12 hours at 78°C. After that, the mixture was allowed to cool and filtered through Whattman No. 1 filter paper. Finally, the mixture was concentrated using a rotary evaporator set at 50°C. The procedure was carried out multiple times to produce a sticky substance, which was then stored in the refrigerator until used Al-Saeed (2002). A stock solution of 200% was created by dissolving 2 g of the C. glomerata extract in 10 ml of distilled water. This solution was then diluted several times.

GC-Mass device analysis of the extract

This instrument is unique as a detector for volatile organic compounds and gaseous mixtures of different inorganic compounds due to the sophisticated technology used to link two gas chromatography and mass spectrometer devices. A sample of the substance's solution can be injected into the gas chromatography apparatus, which separates the mixture's constituent parts, in order to identify any pure unknown substance or mixture. After the materials have been separated, they are each fed into a mass spectrometer, which uses their molecular weights and the information library that is attached to it to identify the types of materials. This process does not require the use of a standard substance and produces results that can be compared to a database that has been stored, which numbers more than 400,000 compounds Kazem and Kraidy (2017).

Testing the biological effectiveness of the extracts

The effectiveness of the alcoholic extracts at concentrations (20, 40, 100, 200%) was tested against five pure pathogenic bacterial isolates (Escherichia coli, Staphylococcus aureus, Proteus mirabilis, Citrobacter, and Klebsiella pneumoniae). Bacteria cultured on Petri dishes containing Mueller-Hinton medium were inoculated using the spreading method. A holes were made using a cork porer with a diameter of 6mm, and the equivalent of 40 micrograms of extract was added to the holes and left to dry under sterile conditions.

The bacteria were then incubated at a temperature of 37°C for 24 hours, and the corona formed around the holes was measured with a ruler, which is an indication of growth inhibition Jeremiah et al., (2007).

RESULT AND DISCUSSION

The control group had a considerably greater mean RBCs, Hb, platelet count, and total cellular volume, whereas the hypothyroid group had a significantly lower mean RBC count, MCV, and MCH. When comparing MCV and MCH, the hyperthyroid group could not identify any significant differences. There were no statistically significant MCHC results in either the hypothyroid or hyperthyroid groups compared to the control group. In terms of total leukocyte count and platelet count, neither the control group nor the hypothyroid group, nor the hyperthyroid group differed significantly from the other two (Table 1).

C. glomerata extracts

TABLE 1. Displays the percentages of alcoholic and aqueous extracts produced from C. glomerata

Substance	Alcoholic extract
Wight of Powder	60g
Wight of extract	3g
Percentage	0.05

Identification the C. glomerata extracts by GC-mass spectrum

The absorption spectra of the ethanolic extract looked to be composed of seven components. There are four compounds that have a high percentage among the other compounds and are thought to be the key reason for the extract's potency against germs. The compounds that occupy these high proportion is:

The mass spectrum of the chemical separated by gas chromatography - the mass spectrum with a detention duration of 21,580 minutes - and by matching this component with the computer information base in The gadget demonstrated that it is the molecule 10-Undecyn-1-ol, with the chemical formula C11H20O and a molecular weight of 168 Daltons, occupying 51.99% of the total area of the separated compounds as in (Figure 1).

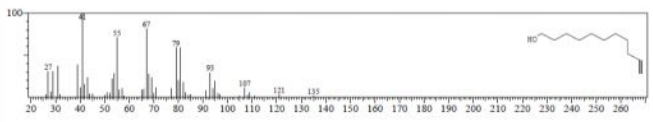

Figure 1. The mass spectrum of (10-Undecyn-1-ol) with chemical formula

The mass spectrum of the compound that was separated using the gas chromatography technique is depicted in Figure 2. The compound was found to be compound C16H3202, with a molecular weight of 256 Dalton and an area that accounted for 16.31% of the total area of isolated compounds, after being matched with the information base computerised in its chemical formula (Palmitic acid) n-Hexadecanoic acid.

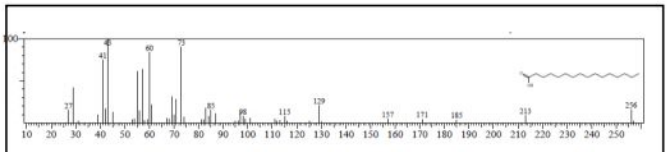

Figure 2. The mass spectrum of (n-Hexadecanoic acid) with chemical formula

The mass spectrum of the substance that was separated with a holding time of 21.103 minutes using the gas chromatography-mass spectrum technique is displayed in Figure 3. By comparing this component's weight of C8H16 and chemical formula, 4,4-Dimethyl-1-hexene, with the computerised information base, the gadget demonstrated that it is the molecular compound 112 Dalton and that it occupies 11.38% of the total area of separated compounds.

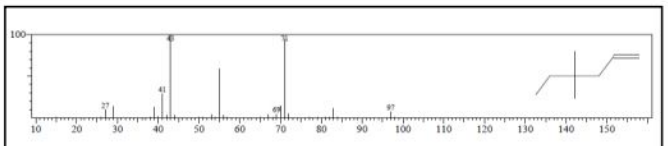

Figure 3.The mass spectrum of (4,4-Dimethyl-1-hexene) with chemical formula

The molecules that was separated using the gas chromatography technique is depicted in Figure 4 as its mass spectrum, which has a detention time of 17.571 minutes. It was established that this component is isoamyl nitrite by comparing it to the computer information base within the apparatus. Its molecular weight is 117 Daltons, its chemical formula is C5H11NO2, and its area of occupancy is 9.43%. of the entire space occupied by segregated automobiles.

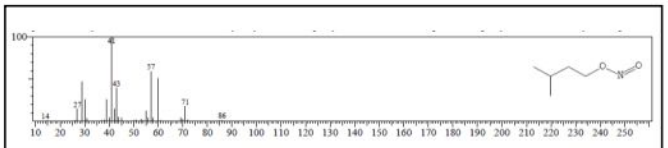

Figure 4. The mass spectrum of (isoamyl nitrite) with chemical formula

The effectiveness of the alcoholic extract

Table 2 shows the effectiveness of the size of the inhibition zone, estimated in (mm), as a result of the effectiveness of the alcoholic extract of C. glomerata algae, as it was shown from the results that the effectiveness of the concentration is 20% against the bacteria E. coli, Staph. aureus, Proteus mirbaili, Citrobacter, and K. pneumoniae, it was (4, 3, 4, 3, 4) mm, respectively. As for the 40% concentration, it was (7, 5, 7, 6, 8). As for the 100% concentration, its effectiveness was (10, 7, 10, 8, 14) mm. The concentration of 200% was equal to (12, 11, 13, 12, 28) mm, respectively.

TABLE 2. shows the effectiveness of the alcoholic extract of C. glomerata in increasing the size of the inhibition zone in millimeters.

Conc. Bacterial isolates	20%	40%	100%	200%
E. coli	4	7	10	12
Staph. aureus	3	5	7	11
P. mirbaili	4	7	10	13
Citrobacter	3	6	8	12
K. pneumoniae	4	8	14	28

Drug sensitivity to bacterial isolates

TABLE 3. The size of the inhabition zone, estimated in (mm), as a result of the effectiveness of antibiotics

Antibiotic Bacteria	Amikacin	Imipenem	Norfloxacin	Meropenem	Piperacillin
E. coli	18	25	15	30	20
Staph. aureus	13	25	0	15	3
P. mirbailis	18	30	20	32	0
Citrobacter	12	25	28	16	21
K. pneumonia	18	28	18	28	0

Table 3 shows the size of the corona formed, estimated in (mm), as a result of the effectiveness of antibiotics. From the table, the antibiotic Amikacin was effective against the pathogenic bacteria E coli K. pneumoni, Citrobacter, P. mirbailis, and Staph.aureus, and (18, 12, 13, 18, 18) mm, respectively. As for the antibiotic Imipenem, the results were (28, 25, 25, 30, 25) mm, respectively, and for the antibiotic Norfloxacin, the results were (28, 25, 25, 30, 25) mm, respectively. Its effectiveness against bacteria was (18,28,020,15) mm, respectively. As for the Meropenem, the results were (28,16,15,32,30) mm, respectively, and the Piperacillin was effective against bacteria (0,21,3,20) mm. Respectively.

The present research's results demonstrated that the green alga C. glomerata has a percentage of (0.05) for the alcoholic extract and a percentage of (0.2) for the aqueous extract, as indicated in Table 1. The variation in the polar nature of the materials that are used in the extraction is what causes a differences in the amount and quality of the extracted materials. This can also be linked to variations in the culture media and extraction techniques that was used, as well as growth variables like pH, temperature, and the volume of culture used in batch culture to yield biologically active compounds Shareef and AL-Salami (2011).

With the alcoholic extract of C. glomerata algae, E. coli bacteria colonies were inhibited within 24 hours; concentrations of 20%, 40%, 100%, and 200% mg/ml were used; the degree of inhibition reached 4, 7, 10, and 12 mm, respectively, which is close to what was obtained Al-Nasser (2011). These results were minimal in comparison to what Kamel et al. (2013) achieved with the alcoholic extract of Mougeati sp. Using the alcoholic extract of the moss Mougeotia sp., colonies of *E. coli* bacteria were inhibited within a period of 24 hours. The degree of inhibition reached 15 and 18 mm, respectively, at concentrations of 1 and 2 g/L.

The number of *S. aureus* bacteria colonies was inhibited within 24 hours to (3, 5, 7, 11) mm, respectively. These results are comparable to study of Danyal et al., (2013), which used Pithophora oedogonium's ethanolic extract to inhibit the pathogenic *S. aureus* bacteria. When contrasting the *C. glomerata* extract with the antibiotics listed above, as shown in Table 3, which contain inhibition zones 13, 25, 0, 15, and 3mm. The antibiotics' inhibition results make it evident that the extract concentrations that is used were more effective to those of norfloxacin and piperacillin, with the exception of the concentration 20%, which was equivalent to the latter.

When *Proteus mirbaili* bacteria were exposed to antibiotics, all concentrations of algae extract performed better than Piperacillin at inhibiting colony counts to (4, 7, 10, 13) mm, respectively. The colony counts of Citrobacter bacteria were found to be reduced to 3, 6, 8, and 12 mm. This is consistent with research conducted by Leeds et al., (2014) which used an ethanolic extract of *Myagropsis myagroides* algae to combat Listeria monocytogenes, and by Cakmak et al., (2014) which used an ethanolic extract of Dunaliella salina algae to combat the same bacteria.

As for the bacterium Klebsiella pneumonia, its colonies were inhibited to 4, 8, 14, and 28 mm, where the concentration was 200% better than the alcoholic extract of the Mougeotia sp. used by Kamel et al. (2013) that inhibited *K. pneumoniae* bacteria colonies to 18 mm when using concentrations of 2 g/L, and the inhibition zone decreased with a concentration of 1 g/L, reaching 16 mm. All of the concentrations used outperformed the antibiotic Piperacillin in comparison to the other antibiotics, and the 200% concentration performed better than all other antibiotics except Meropenem, which was equal.

These substances are responsible for the inhibition's efficacy: Among the total chemical compounds identified from *C. glomerata* by the GC-Mass analysis were Undecyn-1-ol-10 with a ratio of 51.99 and a retention time of 21.580, n-Hexadecanoic acid with a ratio of 16.31 and a retention time of 19.733, 4,4-Dimethyl-1-hexene with a ratio of 11.38 and a retention time of 21.103, and isoamyl nitrite with a ratio of 9.43 and a retention time of 17.571.

The fact that algae extracts contain steroidal fatty and protein compounds that inhibit bacteria is one of the causes of their inhibition. Furthermore, pigments and their derivatives, like chlorophyll and carotene, have antibacterial properties. Additionally, fatty acids possess anti-gram-positive and anti-gram-negative bacterial activity. Whether used singly or in combination, fatty acids can have either a bacteriostatic or bacteriocidal effect Aubert (1979).

CONCLUSION

Cladophora glomerata algae extract was used to inhibit the growth of five bacterial species: *Escherichia coli*, *Staphylococcus aureus*, *Proteus mirabilis*, Citrobacter, and *Klebsiella pneumoniae*. The concentrations of the extracts

were compared with five antibiotics. The 200% concentration showed the highest effectiveness against bacteria, with an inhibition zone of 28mm. The antibiotics Imipenem and Meropenem had an inhibition zone of 28mm, while Amikacin, Norfloxacin, and Piperacillin had an inhibition area of 12-21mm. The 20% concentration was least effective against *E. coli*, with an inhibition diameter of 2mm. The alcoholic extract of *C. glomerata* alga had the highest inhibition region of 28 against *K. pneumoniae* and 13mm against *P. mirabilis*. The chemical algal contents were identified using mass GC technology, revealing compounds like 10-Undecyn-1-ol, n-hexadecanoic acid, 4,4-dimethyl-1-hex, and isoamyl nitrite, which were found to have the major function of inhibiting bacterial growth.

AUTHOR CONTRIBUTIONS

All authors played a role in the preparation of this article.

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The Effect of Green Grape Extract on Serum Triglyceride Levels in Rats

Pengaruh Pemberian Ekstrak Anggur Hijau terhadap Kadar Trigliserida Serum pada Tikus Galur Wistar

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ABTRACT

Cardiovascular disease, one of which is coronary heart disease (CHD) is the main cause of death and morbidity caused by blockages in the coronary arteries (atherosclerosis). Elevated serum triglyceride levels can encourage the formation of atherosclerosis. Statins are the drugs most commonly used to lower triglyceride levels but have side effects, so new treatment are needed. Grapes are fruits that has many bioactive compounds such as proanthocyanidins and resveratrol which are believed to reduce triglyceride levels in serum. The purpose of this study was to determine the effect of green grape extract (*Vitis vinifera*) on triglyceride levels in male Wistar rats which were divided into 5 groups, namely P0 (fed with standard feed), P1 (fed with high cholesterol and then standard feed), P2 (fed with high cholesterol and simvastatin 0.2mg/200mgBW/day), P3 (fed with high cholesterol and grape extract at a dose of 500mg/200mgBW/day), and P4 (fed with high cholesterol and 250mg/200mgBW/day grape extract). Examination of triglyceride levels was carried out using the GPO-PAP method. Data analysis was performed with Annova test followed by Bonferroni test. The statistical test results showed that there were significant differences between each treatment group. The P3 group showed a lower average triglyceride level than P4 although it was still higher than P2. This showed that administration of green grape extract has an effect on decreasing serum triglyceride levels in all.

Keywords: Coronary Heart Disease, Green Grape Extract, Triglyceride

ABSTRAK

Penyakit kardiovaskular salah satunya penyakit jantung koroner (PJK) merupakan penyebab kematian dan kesakitan utama yang disebabkan adanya penyumbatan di arteri koroner (aterosklerosis). Peningkatan kadar trigliserida plasma dapat mendorong terbentuknya aterosklerosis. Statin merupakan obat yang paling umum digunakan untuk menurunkan kadar trigliserida tetapi memiliki efek samping sehingga diperlukan pengembatan pengobatan baru. Anggur (*Vitis vinifera*) merupakan buah yang memiliki banyak senyawa bioaktif seperti proantosianidin dan resveratrol yang diyakini dapat menurunkan kadar trigliserida dalam serum.

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Tujuan penelitian ini adalah untuk mengetahui pengaruh pemberian ekstrak anggur hijau terhadap kadar trigliserida pada tikus Wistar Jantan yang dibagi menjadi 5 kelompok yaitu P0 (diberi pakan standar), P1 (diberi pakan tinggi kolesterol lalu pakan standar), P2 (diberi pakan tinggi kolesterol dan simvastatin 0,2mg/200mgBB/hari), P3 (diberi pakan tinggi kolesterol dan ekstrak anggur dosis 500mg/200mgBB/hari), dan P4 (diberi pakan tinggi kolesterol dan ekstrak anggur 250mg/200mgBB/hari). Pemeriksaan kadar trigliserida dilakukan menggunakan metode GPO-PAP. Analisis data dilakukan dengan uji Annova yang dilanjutkan dengan uji Bonferroni. Hasil uji statistik menunjukkan bahwa terdapat perbedaan bermakna antar tiap kelompok perlakuan. Kelompok P3 menunjukkan rerata kadar trigliserida yang lebih rendah dari P4 meskipun masih lebih tinggi dari P2. Hal ini menunjukkan bahwa pemberian ekstrak anggur hijau memiliki pengaruh terhadap penurunan kadar trigliserida serum pada semua tikus.

Kata Kunci: Ekstrak Anggur Hijau, Penyakit Jantung Koroner, Triglierida

PENDAHULUAN

Penyakit kardiovaskular akhir-akhir ini meningkat karena perubahan gaya hidup, merokok, kurangnya aktivitas fisik dan faktor lainnya. Penyakit jantung koroner (PJK) masih merupakan penyebab kematian dan kesakitan utama yang berdampak secara sosioekonomi seperti juga stroke. PJK merupakan penyakit yang diakibatkan karena adanya penumpukan plak di dalam arteri koroner yang menyuplai oksigen ke otot jantung (aterosklerosis). PJK termasuk dari bagian penyakit kardiovaskuler yang paling umum terjadi Ghani et al., (2016).

Telah diketahui dengan baik bahwa gangguan lipid mendorong perkembangan aterosklerosis. Peningkatan konsentrasi trigliserida plasma berkontribusi terhadap peningkatan resiko penyakit kardiovaskular, serta penyakit lain seperti terjadi pada peningkatan resiko pankreatitis akut yaitu ketika ditemukan kadar trigliserida sangat tinggi (> 10 mmol/L). Dua sumber utama trigliserida plasma adalah eksogen yaitu dari lemak makanan dan dibawa dalam chylomicron dan endogen yaitu dari hati dan dibawa dalam partikel lipoprotein densitas sangat rendah (VLDL). Di kapiler dalam jaringan lemak dan otot, chylomicron dan lipoprotein ini dihidrolisis oleh lipoprotein lipase menjadi asam lemak bebas. Setelah makan, lebih dari 90% trigliserida yang bersirkulasi berasal dari usus dan disekresikan dalam chylomicron sedangkan selama periode puasa, trigliserida endogen disekresikan oleh hati sebagai VLDL. Peningkatan plasma lipoprotein kaya trigliserida dihasilkan dari peningkatan produksi dari hati dan usus (melalui jalur sintetik dan sekresi yang diregulasi) atau melalui penurunan katabolisme perifer (terutama dari penurunan aktivitas lipoprotein lipase) Yuan, Al-Shali and Hegele (2007).

Kadar trigliserida dapat diturunkan melalui beberapa cara seperti perubahan gaya hidup dan konsumsi obat-obatan. Perubahan gaya hidup dapat dilakukan dengan cara penurunan berat badan, kontrol gula darah, serta aktivitas fisik. Obat-obatan yang sering digunakan untuk menurunkan kadar trigliserida yaitu statin dan fibrat Parhofer and Laufs, (2019). Statin merupakan obat penurun lipid yang paling umum digunakan di Indonesia tetapi memiliki efek samping berupa rhabdomiolisis, hepatotoksik, dan myalgia. Oleh karena itu, perlu dikembangkan pengobatan baru Jo, Kim and Lim (2014).

Anggur (*Vitis vinifera*) merupakan sumber yang kaya senyawa bioaktif, tetapi akumulasi senyawa ini dalam anggur dipengaruhi oleh berbagai faktor termasuk varietas, kematangan, penyimpanan pascapanen, faktor lingkungan seperti lokasi, kondisi cahaya, suhu, nutrisi, air, mikroorganisme, dan praktik pemeliharaan anggur.positif, nilai ramal negatif, dan akurasi diagnostik Senyawa fenolik utama dalam buah anggur adalah asam hidroksinamat, stilbene, flavonoid termasuk antosianin dan proantosianidin. Quercetin yang merupakan flavonoid dan resveratrol yang termasuk ke dalam stilbene merupakan antioksidan kuat, dan diduga berperan dalam perlindungan terhadap penyakit kardiovaskular. Tannin terkondensasi bernama proantosianidin juga dilaporkan memiliki sifat penurun kolesterol dan menurunkan tekanan darah. Anggur juga kaya akan pitosterol dan asam lemak yang sebagian dapat menghambat penyerapan usus dari kolesterol makanan dan empedu yang diproduksi secara endogen, menurunkan tingkat sirkulasi dan mengerahkan efek anti-aterogenik dan kardio-protektif Kumar and Goel, (2019); Chen et al., (2020); Sabra, Netticadan and Wijekoon, (2021); Dewi et al., (2023).

Senyawa resveratrol banyak ditemukan pada kulit anggur. Sedangkan biji anggur mengandung banyak senyawa proantosianidin Szkudelska, Nogowski and Szkudelski, (2009). Beberapa penelitian menunjukkan bahwa buah anggur dapat menurunkan kadar trigliserida. Kandungan proantosianidin dan resveratrol dalam buah anggur dapat menghambat pencernaan dan penyerapan lemak Orbaniyah and Permana, (2011); Saputra, Sutrisna and Nurhayani, (2016). Oleh karena itu peneliti tertarik melakukan penelitian terhadap ekstrak buah anggur hijau terhadap kadar trigliserida serum pada tikus.

METODE

Penelitian ini menggunakan desain eksperimental murni dengan menggunakan pre-test post-test control group design. Dosis ekstrak anggur hijau yang diberikan pada penelitian ini ditentukan berdasarkan penelitian yang dilakukan sebelumnya. Penelitian ini dilakukan pada tikus dengan menggunakan ekstrak etanol anggur hijau terhadap kadar trigliserida.

Penelitian dilaksanakan di beberapa tempat yaitu untuk perlakuan dengan hewan percobaan dilakukan di Animal Laboratorium kemudian dilanjutkan dengan analisis kadar serum trigliserida di Laboratorium kampus Teknik Laboratorium Medik Poltekkes Denpasar. Waktu penelitian dilakukan pada bulan Maret hingga Oktober 2022.

Populasi dalam penelitian ini adalah seluruh tikus Wistar Jantan. Populasi terjangkau meliputi tikus putih Wistar Jantan dengan berat 100-150 gram, yang berumur 8-12 minggu.

Kriteria inklusi sampel adalah tikus Wistar Jantan berumur 8-12 minggu dengan berat badan 100-150 gram. Kriteria eksklusi sampel adalah tikus Wistar Jantan dengan kondisi sakit. Tikus yang sakit ini dikeluarkan dari sampel sebelum diberi perlakuan. Kriteris drop out sampel adalah tikus Wistar Jantan yang mati selama penelitian.

Besar sampel yang dipakai dalam penelitian ini dihitung menggunakan rumus Federer yaitu $(t-1)(r-1) \geq 15$. Dimana t adalah banyaknya perlakuan yaitu 5 dan r adalah banyaknya pengulangan. Dari rumus tersebut diperoleh jumlah sampel yang digunakan untuk setiap kelompok perlakuan adalah 5 ekor sehingga banyaknya sampel yang digunakan dalam penelitian ini adalah 25 ekor.

Kelompok perlakuan terdiri dari: (1) P0 = kelompok kontrol yang diberi pakan standar, (2) P1 = kelompok yang diberikan pakan tinggi kolesterol, lalu diberikan pakan standar, (3) P2 = kelompok yang diberikan pakan tinggi

kolesterol, lalu diberikan simvastatin 0,2mg/200mgBB/hari, (4) P3 = kelompok yang diberikan pakan tinggi kolesterol, lalu diberikan ekstrak buah anggur hijau 500mg/200mgBB/hari, (5) P4 = kelompok yang diberikan pakan tinggi kolesterol, lalu diberikan ekstrak buah anggur hijau 250mg/200mgBB/hari.

Preparasi Ekstrak Anggur Hijau. Buah anggur dicuci dengan air mengalir lalu diiris tipis. Kemudian dijemur di tempat teduh hingga menjadi kering. Buah anggur yang telah kering kemudian dihancurkan hingga menjadi serbuk. Serbuk kemudian disaring hingga didapatkan serbuk halus. Serbuk halus direndam dalam etanol 96% selama 24 jam untuk menarik zat aktif yang ada dalam anggur hijau. Rendaman tersebut kemudian disaring menggunakan corong gelas yang dilapisi kertas saring sampai didapatkan ekstrak anggur cair. Ekstrak cair tersebut kemudian diuapkan menggunakan rotary evaporator hingga didapatkan ekstrak kental.

Pembuatan pakan tinggi kolesterol dibuat berdasarkan penelitian sebelumnya yang terdiri dari 50% pakan standar, 31,8% terigu, 1% kolesterol, 0,2% asam kolat, 10% minyak babi, 2% otak babi, dan 5% kuning telur. Semua bahan dicampur dan digiling kemudian dibentuk butiran-butiran kecil dan dikeringkan. Untuk persiapan hewan coba, tikus Wistar Jantan yang memenuhi kriteria dipilih secara acak sebanyak 25 ekor kemudian dilakukan aklimitisasi selama satu minggu dengan pemberian pakan standar dan air minum. Pengambilan darah dilakukan pada akhir minggu keempat pemberian perlakuan. Tikus dianestesi kemudian diambil darahnya dari sinus orbitalis untuk pemeriksaan kadar trigliserida.

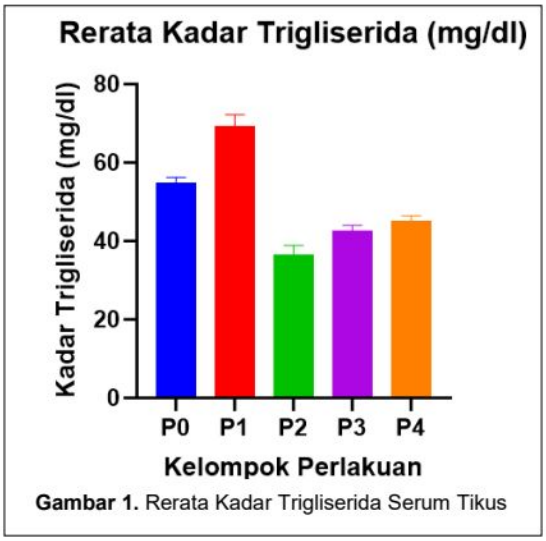
Pemeriksaan kadar trigliserida dilakukan menggunakan metode enzimatis kolorimetri yaitu Glyserol Peroxidase Phosphat Acid (GPO-PAP). Trigliserida yang ada dalam darah akan dihidrolisis secara enzimatis menjadi gliserol dan asam lemak bebas menjadi lipase khusus yang akan membentuk kompleks warna sehingga dapat diukur menggunakan spektrofotometer. Data yang diperoleh disajikan dalam bentuk grafik dan narasi. Kadar trigliserida sebelum dan sesudah perlakuan dianalisis menggunakan SPSS dengan uji ANOVA yang dilanjutkan dengan uji Bonferroni.

HASIL DAN PEMBAHASAN

Hasil pemeriksaan kadar trigliserida pada tikus setelah perlakuan diperoleh rerata yang dapat dilihat pada Gambar 1.

Sampel Dari hasil pemeriksaan diperoleh kadar trigliserida tertinggi didapatkan pada kelompok P1 sedangkan kadar terendah didapatkan pada kelompok P2 sebagai kontrol positif. Pada kelompok perlakuan dengan ekstrak buah anggur hijau, kadar trigliserida lebih rendah ditemukan pada kelompok P3.

Setelah dilakukan analisis data menggunakan SPSS dengan uji ANOVA, didapatkan nilai sig. < 0,05 yang menunjukkan bahwa terdapat perbedaan antara kelompok perlakuan. Sehingga analisis data dilanjutkan menggunakan uji Bonferroni.



Gambar 1. Rerata Kadar Trigliserida Serum Tikus

Uji Bonferroni untuk melihat perbedaan antara kelompok perlakuan, didapatkan perbedaan antara kelompok P0 dengan P1, P0 dengan P2, P0 dengan P3, P0 dengan P4, P1 dengan P2, P1 dengan P3, P1 dengan P4, P2 dengan P3, dan P2 dengan P4.

Dari hasil penelitian didapatkan bahwa terdapat perbedaan bermakna antara tiap kelompok perlakuan. Hal ini sejalan dengan penelitian yang dilakukan oleh Orbaniyah dan Permana (2011) bahwa pemberian ekstrak anggur merah memiliki pengaruh yang signifikan terhadap penurunan kadar trigliserida darah tikus. Hal serupa juga ditunjukkan oleh Saputra, Sutrisna, dan Nurhayani (2016) Orbaniyah and Permana (2011); Saputra, Sutrisna and Nurhayani (2016).

Anggur (Vitis spp.) adalah salah satu tanaman buah yang paling banyak diproduksi di dunia. Sekitar 75 ton diproduksi setiap tahun, dimana 41% ditanam di Eropa, 29% di Asia, dan 21% di Amerika Serikat. Mereka dipanen di daerah beriklim sedang, dimana musim panas yang hangat dan musim dingin yang agak sejuk membentuk pola iklim yang khas. Sekitar 50% buah anggur digunakan untuk membuat wine, sepertiga digunakan sebagai buah segar, dan sisanya disuling untuk menghasilkan makanan seperti selai, jus, ekstrak biji anggur, jeli, minyak biji anggur, anggur kering (kismis), dan cuka. Anggur termasuk buah yang paling kaya karbohidrat (17g/100g), memiliki kandungan kalori yang tinggi (65kkal/100g), dan indeks glikemik yang relatif rendah. Selain menjadi sumber mangan dan potassium yang luar biasa, anggur juga merupakan sumber vitamin B6, C, tiamin, yang merupakan salah satu sumber polifenol terkaya Unusan (2020).

Ekstrak anggur memiliki kandungan proantosianidin dan resveratrol yang diketahui dapat menurunkan kadar trigliserida. Proantosianidin adalah kelas senyawa polifenol yang merupakan salah satu kelompok metabolit sekunder dari tanaman yang paling banyak tersebar dimana-mana sehingga banyak dimanfaatkan oleh manusia. Proantosianidin dianggap sebagai senyawa bioaktif karena mempengaruhi proses fisiologis dan seluler yang dapat berpengaruh terhadap kesehatan. Proantosianidin telah

digambarkan sebagai senyawa antimikroba, antioksidan, agen anti kanker, dan agen anti inflamasi dengan sifat kardioprotektif Bladé, Arola and Salvadó, (2010). Proantosianidin juga dikenal sebagai tannin terkondensasi. Proantosianidin diketahui dapat mengurangi resiko penyakit kardiovaskuler, kanker, tekanan darah, tinggi, hiperlipidemia, dan diabetes Qi et al., (2022). Salah satu mekanisme yang digunakan proantosianidin untuk memberikan perlindungan kardiovaskular adalah dengan meningkatkan homeostasis lipid. Penelitian pada hewan menunjukkan bahwa proantosianidin mengurangi kadar apolipoprotein B yang ada di trigliserida dan kolesterol Low Density Lipoprotein (LDL) tetapi meningkatkan High Density Lipoprotein (HDL) Unusan, (2020). Penelitian lain menunjukkan bahwa proantosianidin menyebabkan hipotrigliseridemia dengan menghambat sekresi lipoprotein Quesada et al., (2012). Varietas anggur, kondisi geografis dan iklim, pemupukan, tanah, praktik budidaya, dan tingkat kematangan semuanya mempengaruhi kandungan proantosinanidin. Biji anggur memiliki konsentrasi molekul bioaktif paling tinggi. Sekitar 30% dari total proantosianidin disimpan dalam biji anggur dan 15% dalam kulit, meskipun dinding sel perlu dipecah agar proantosianidin dapat diekstraksi dari kulit dan biji Unusan (2020).

Resveratrol merupakan senyawa difenolik alami yang memberikan banyak efek menguntungkan Szkudelska, Nogowski and Szkudelski (2009). Recveratrol diakui sebagai faktor utama yang bertanggungjawab atas sifat kardioprotektif dari buah anggur. Resveratrol menunjukkan efek perlindungan pada beberapa penyakit degeneratif dan kardiovaskular termasuk aterosklerosis, hipertensi, penyakit iskemik, serta diabetes, obesitas, dan penuaan Carrizzo et al., (2013). Resveratrol juga diketahui dapat menurunkan kadar

LDL dan trigliserida, secara bersamaan meningkatkan kadar HDL. Perubahan profil lipid dengan pemberian resveratrol diyakini dimediasi melalui regulasi enzim 3-hydroxy-3-methylglutaryl Coenzyme A (HMG-CoA), yang memiliki peran kunci dalam biosintesis kolesterol. Peningkatan ekspresi kolesterol hati 7α-hydroxylase (CIP7A1) yang mengarah pada peningkatan sintesis dan sekresi asam empedu dan penurun kolesterol total dan LDL dalam plasma juga diduga bertanggungjawab atas sifat penurun lipid dari resveratrol. Penelitian lain menunjukkan bahwa resveratrol dapat meningkatkan ekspresi reseptor LDL pada hepatosit dan meningkatkan serapan LDL hati melalui mekanisma dependen adenosin monofosfat protein kinase (AMPK) Chen et al., (2012); Yashiro et al., (2012); Rašković et al., (2019).

KESIMPULAN

Berdasarkan uji Bonferroni didapatkan hasil bahwa terdapat perbedaan yang bermakna antara tiap kelompok perlakuan dalam penelitian ini. Pada kelompok yang diberi dosis ekstrak anggur 500mg/200mgBB/hari memiliki rerata kadar trigliserida yang lebih rendah dibandingkan dengan kelompok yang diberi dosis ekstrak anggur 250mg/200mgBB/hari. Akan tetapi memiliki rerata yang lebih rendah dibandingkan dengan kelompok yang memperoleh simvastatin dengan dosis 0,2mg/200mgBB/hari. Meskipun demikian hal ini menunjukkan bahwa pemberian ekstrak anggur hijau pada tikus putih dapat menurunkan kadar trigliserida dalam serum sehingga dapat dijadikan sebagai pengobatan alternatif untuk hipertrigliseridemia. Untuk peneliti selanjutnya perlu dilakukan penelitian lebih lanjut berapa dosis yang efektif dalam menurunkan kadar trigliserida yang setara dengan pengobatan yang ada saat ini serta perlu dilakukan penelitian lebih lanjut tentang efek samping dari pemberian ekstrak anggur hijau dalam menurunkan kadar trigliserida dalam serum.

KONTRIBUSI PENULIS

Penulis berperan dalam pengumpulan data dan penyusunan artikel.

PENDANAAN

Dana penelitian berasal dari dana mandiri peneliti

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An Overview of Serologic Test Results in Covid-19 Patients

Gambaran Hasil Pemeriksaan Serologi pada Pasien Covid-19

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ABSTRACT
Coronavirus Disease 2019 (Covid-19) is an infectious disease caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). SARS-CoV-2 protein and antibody/serology assays can be used for sero-surveillance studies and analyze the epidemiology and virology of SARS-CoV-2. This study aims to review the results of serological tests in Covid-19 patients with the parameters of IgM and IgG antibodies, C-Reactive Protein, procalcitonin, and serum ferritin through literacy studies. This research is descriptive. The study was conducted by collecting and reviewing data on the results of serological examinations in Covid-19 patients contained in articles and journals. After collecting data, 10 journals were obtained that matched the inclusion criteria set by the researcher. The conclusion of this study was that there was an increase in c-reactive protein and procalcitonin levels in Covid-19 patients, serum ferritin levels were also found to tend to increase. IgM antibodies were found to increase earlier than IgG antibodies, but their levels decreased more quickly. An increase in IgG antibodies occurs around the second week after the onset of symptoms.

Keywords: Covid-19, C-Reactive Protein, Ferritin, Procalcitonin, SARS-CoV-2

ABSTRAK
Coronavirus Disease 2019 (Covid-19) adalah penyakit menular yang disebabkan oleh Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). Pemeriksaan protein dan antibodi/ serologi SARS-CoV-2 dapat digunakan untuk studi sero surveilans dan menganalisis epidemiologi dan virologi SARS-CoV-2. Penelitian ini bertujuan untuk mereview hasil pemeriksaan serologi pada pasien Covid-19 dengan parameter pemeriksaan antibodi IgM dan IgG, C-Reactive Protein, prokalsitonin, dan serum ferritin melalui studi literasi. Penelitian ini bersifat deskriptif. Penelitian ini mengumpulkan dan mengkaji data hasil pemeriksaan serologi pada pasien Covid-19 yang terdapat pada artikel maupun jurnal. Setelah dilakukan pengumpulan data didapatkan 10 jurnal yang sesuai dengan kriteria inklusi yang telah ditetapkan peneliti. Kesimpulan penelitian ini yaitu terjadi peningkatan kadar c-reactive protein dan prokalsitonin pada pasien Covid-19, kadar ferritin serum juga ditemukan cenderung mengalami peningkatan. Antibodi IgM ditemukan lebih dulu mengalami peningkatan dibandingkan dengan antibodi IgG, namun kadarnya lebih cepat mengalami penurunan. Peningkatan antibodi IgG terjadi sekitar minggu kedua setelah timbulnya gejala.

Kata Kunci: Covid-19, C-Reactive Protein, Ferritin, Prokalsitonin, SARS-CoV-2

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PENDAHULUAN

Coronavirus Disease 2019 (Covid-19) adalah penyakit menular yang disebabkan oleh Virus *Severe Acute Respiratory Syndrome Coronavirus* 2 (SARS-CoV-2). SARS-CoV-2 merupakan *coronavirus* jenis baru yang belum pernah diidentifikasi sebelumnya pada manusia. Virus ini muncul pertama kali di kota Wuhan, Hubei, China pada akhir tahun 2019 yang menyebabkan kasus pneumonia. etiologinya Susilo et al., (2020).

Diagnosis Covid-19 dilakukan dengan pemeriksaan molekuler, metode yang dianjurkan adalah metode deteksi molekuler/ NAAT (*Nucleic Acid Amplification Test*) seperti pemeriksaan RT-PCR (WHO, 2020). Selain pemeriksaan molekuler, klinis pasien, dan riwayat kontak atau terpapar dengan orang yang terkonfirmasi positif Covid-19 juga perlu diperhatikan Kemenkes RI, (2020).

Pemeriksaan imunoserologi berperan dalam mengidentifikasi terbentuknya antibodi sebagai respon terhadap masuknya antigen, dan untuk mengetahui kadar antibodi dalam serum yang melibatkan antigen yang tidak larut (Elfidasari dan Puspitasari, 2013). Pemeriksaan antibodi/ serologi SARS-CoV-2 bertujuan untuk mendeteksi antibodi yang diproduksi oleh tubuh manusia sebagai respons terhadap infeksi alamiah oleh SARS-CoV-2 dan terhadap vaksinasi, sehingga hasil pemeriksaan serologi pada pasien Covid-19 dapat digunakan untuk studi sero surveilans dan menganalisis epidemiologi dan virologi SARS-CoV-2 Isnaeni, (2020).

Saat pasien dalam tahap pemulihan genom virus tidak lagi terdeteksi, maka untuk menilai status kekebalan dan terkait kemampuan untuk melawan infeksi akan didasarkan pada hasil pemeriksaan antibodi Jacofsky et al., (2020). Karena SARS-CoV-2 adalah patogen baru, maka pemahaman mengenai respons antibodi yang ditimbulkannya masih terus dipelajari dan oleh karena itu tes deteksi antibodi harus digunakan dengan hati-hati, dan tidak dapat digunakan untuk menentukan infeksi akut.

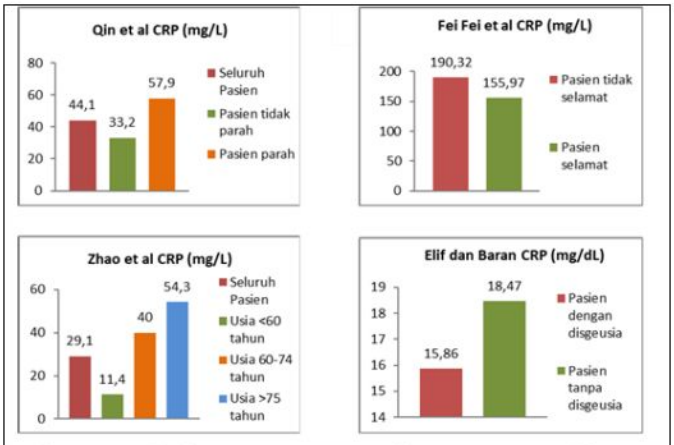
Pada kasus Covid-19 dengan penanda klinis berat, ditemukan peningkatan pada penanda infeksi seperti prokalsitonin, ferritin dan C-*reactive protein* (CRP) Qin et al., (2020). Berdasarkan penelitian yang dilakukan oleh Sahu et al., (2020) pasien yang meninggal akibat Covid-19 memiliki konsentrasi C-*Reactive Protein* (CRP) yang lebih tinggi secara signifikan dibandingkan dengan pasien yang selamat, sehingga CRP dinilai dapat menjadi biomarker yang menjanjikan untuk menilai kematian akibat penyakit. CRP juga dinilai dapat berfungsi sebagai prediktor kuat pasien yang membutuhkan dukungan ventilator mekanis akibat kegagalan pernapasan Herold et al., (2020); Sahu et al., (2020).

Penelitian yang dilakukan oleh Hu et al., (2020) menunjukkan adanya peningkatan kadar prokalsitonin serum seiring dengan semakin memburuknya penyakit, sehingga prokalsitonin dapat digunakan sebagai indikator tingkat keparahan pasien Covid-19. Selain itu, pengukuran prokalsitonin secara serial mungkin berguna dalam memprediksi prognosis pasien Covid-19 Hu et al., (2020) Selain *C-reactive protein* dan prokalsitonin, serum ferritin juga dapat digunakan sebagai biomarker prognostik dan stratifikasi Kappert et al., (2020). Ferritin merupakan mediator utama dari gangguan sistem imun, terutama pada hiperferritinemia ekstrim melalui penekanan sistem imun dan efek pro inflamasi yang dapat mengakibatkan badai sitokin (*cytokine storm*). Oleh karena itu, apabila penderita terinfeksi SARS-CoV-2 dan disertai sindrom badai sitokin, maka akan mempengaruhi tingkat keparahan dari Covid-19 Vargas-Vargas & Cortés-Rojo, (2020). Tujuan penelitian ini yaitu mereview hasil pemeriksaan serologi pasien Covid-19 parameter *C-Reactive Protein* (CRP), Feritin, Prokalsitonin, Antibodi IgG dan IgM SARS-COV-2.

METODE

Jenis penelitian yang digunakan pada penelitian ini adalah penelitian deskriptif, Penelitian ini mengumpulkan dan mengkaji data hasil pemeriksaan serologi pada pasien Covid-19 dengan parameter pemeriksaan antibodi IgM dan IgG, *C-reactive protein*, prokalsitonin, dan serum ferritin yang terdapat pada artikel maupun jurnal. Penelusuran artikel/ jurnal dilakukan secara online. Kriteria jurnal yang dijadikan data adalah jurnal teratas yang dicari menggunakan *searching engine google*. Setelah dilakukan pengumpulan data didapatkan 10 jurnal yang sesuai dengan kriteria inklusi yang telah ditetapkan peneliti yaitu terindeks scopus.

HASIL DAN PEMBAHASAN



Gambar 1. Grafik Hasil Pemeriksaan CRP pada Pasien COVID-19. (Elibol & Baran, (2021); Fei et al., (2021); Mengmeng Zhao et al., (2020); Qin et al., (2020)).

Berdasarkan hasil penelitian mengenai data gambaran hasil pemeriksaan imunoserologi pada pasien Covid-19 yang diambil dari artikel maupun jurnal dapat diketahui bahwa karakteristik pasien yang terbanyak berada pada rentang usia 48-61 tahun dengan dominasi pasien berjenis kelamin laki-laki. Berdasarkan Gambar **1** pada penelitian yang dilakukan oleh Qin et al., (2020) dengan

membandingkan hasil pemeriksaan CRP pada pasien Covid-19 dengan kondisi klinis yang parah dan tidak parah, ditemukan peningkatan kadar CRP yang cukup signifikan pada pasien dengan kondisi klinis yang parah dengan rata-rata kadar CRP 57,9 mg/L.

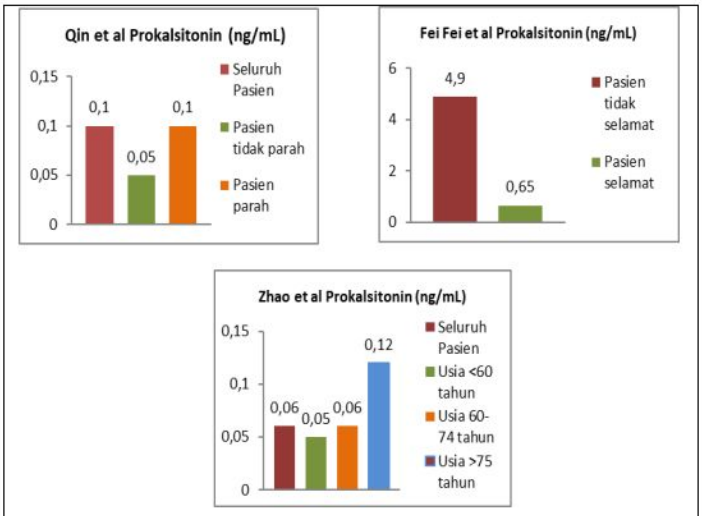
Pada penelitian lainnya yang dilakukan oleh Fei et al., (2021), ditemukan hasil pemeriksaan CRP pada pasien Covid-19 yang meninggal mengalami peningkatan dengan rata-rata 190,32 mg/L. Sedangkan rata-rata kadar CRP pada pasien Covid-19 yang selamat adalah 155,97 mg/L. Pada Gambar 1 pada penelitian yang dilakukan oleh Mengmeng Zhao et al., (2020), pasien digolongkan menjadi 3 kategori yaitu pasien dengan rentang usia <60 tahun, pasien usia 60-74 tahun, dan pasien usia >75 tahun. Ditemukan terjadi peningkatan kadar CRP seiring dengan peningkatan usia, yaitu rata-rata kadar CRP tertinggi ditemukan pada pasien dengan kategori usia >75 tahun dengan kadar CRP 54,3 mg/L.

Berdasarkan Gambar **1** pada penelitian yang dilakukan oleh Elibol & Baran, (2021) kadar CRP juga ditemukan meningkat pada pasien tanpa gejala disgeusia dengan rata-rata CRP 18,47 mg/dL sedangkan pada pasien Covid-19 dengan gejala disgeusia didapatkan rata-rata kadar CRP 15,86 mg/dL. Kadar CRP yang meningkat menunjukkan adanya proses inflamasi selama terinfeksi Covid-19.

Pada penderita Covid-19 peningkatan CRP berkorelasi dengan terjadinya kerusakan di paru, sehingga CRP dapat digunakan sebagai indikator penting untuk memprediksi keparahan dan mortalitas pada penderita Covid-19 yang di rawat di rumah sakit Mus et al., (2021). Adanya kerusakan ginjal akut dan tingkat cedera jantung juga telah dikaitkan langsung dengan konsentrasi CRP. Sistem kekebalan tubuh diduga merespons lebih kuat dengan memproduksi berbagai molekul kekebalan, dan salah satunya dengan produksi CRP untuk pembersihan infeksi virus. Beberapa faktor yang dapat mempengaruhi tingginya kadar CRP serum yaitu usia, jenis kelamin, perokok aktif, berat badan, kadar lipid, tekanan darah, hingga kerusakan hati Sahu et al., (2020).

Berdasarkan Gambar **2** pada penelitian yang dilakukan oleh Qin et al., (2020), ditemukan rata-rata kadar prokalsitonin lebih tinggi pada pasien Covid-19 yang parah yaitu 0,1 ng/mL. Sedangkan pada pasien Covid-19 dengan kondisi klinis yang tidak parah, didapatkan kadar prokalsitonin berada pada rentang nilai normal.

Pada penelitian lainnya yang dilakukan oleh Fei et al., (2021), pada pasien Covid-19 yang meninggal dan pasien Covid-19 yang selamat, didapatkan kadar prokalsitonin pada pasien yang meninggal mengalami peningkatan dengan rata-rata prokalsitonin 4,9 ng/mL.



Gambar 2 Grafik Pemeriksaan Prokalsitonin pada Pasien Covid-19 (Fei et al., (2021); Mengmeng Zhao et al., (2020); Qin et al., (2020)).

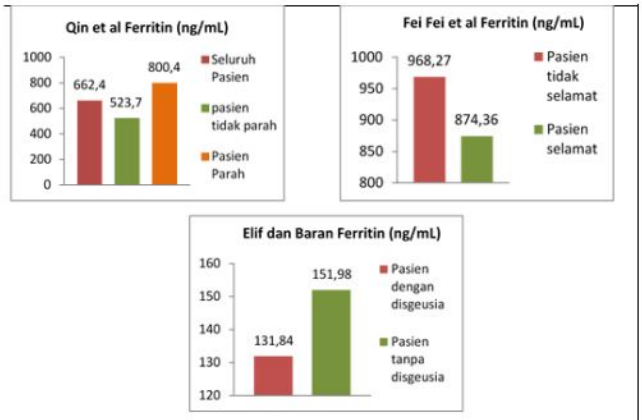
Berdasarkan Gambar **2** pada penelitian yang dilakukan oleh Mengmeng Zhao et al., (2020) juga ditemukan adanya peningkatan kadar prokalsitonin seiring dengan peningkatan usia. Didapatkan kadar prokalsitonin tertinggi yaitu pada pasien dengan kategori usia >75 tahun dengan rata-rata kadar prokalsitonin 0,12 ng/mL.

Pada pasien kritis dapat terjadi peningkatan kadar prokalsitonin hingga delapan kali lipat dari kadar normal yaitu <0,05 ng/ml dan kadar prokalsitonin akan menurun selama pasien dalam proses pemulihan (Hu et al., 2020). Pasien rawat inap dengan kadar prokalsitonin yang tinggi memiliki resiko perburukan yang lebih parah hingga resiko kematian yang tinggi. Pemeriksaan prokalsitonin secara teratur dapat berfungsi sebagai penanda infeksi bakteri sekunder yang sering ditemukan pada pasien yang tidak selamat. Temuan ini dapat memberikan panduan untuk meningkatkan prognosis pasien dengan Covid-19 yang parah Liu et al., (2020).

Berdasarkan Gambar **3** pada penelitian yang dilakukan oleh Qin et al., (2020), ditemukan adanya peningkatan kadar ferritin serum pada pasien Covid-19. Kadarnya diketahui lebih tinggi pada pasien dengan gejala klinis yang parah dengan rata-rata ferritin serum mencapai 800,4 ng/mL, dan pada pasien dengan gejala klinis yang tidak parah mengalami peningkatan ferritin serum dengan rata-rata 523,7 ng/mL.

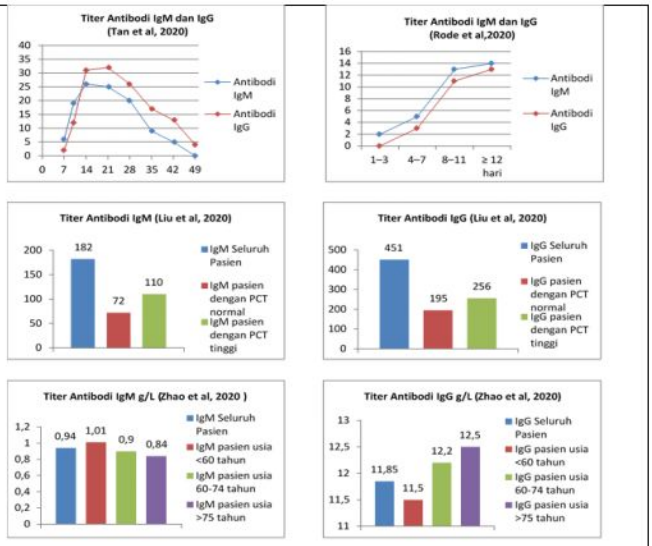
Berdasarkan Gambar **3** pada penelitian lainnya yang dilakukan oleh Fei et al., (2021), pada pasien Covid-19 yang meninggal ditemukan peningkatan kadar ferritin serum mencapai 968,27 ng/mL. Sedangkan pada pasien Covid-19 yang selamat ditemukan peningkatan ferritin serum dengan rata-rata 874,36 ng/mL. Pada penelitian lainnya yang dilakukan oleh Elibol & Baran, (2021), didapatkan kadar ferritin serum pada pasien dengan gejala

disgeusia dan tanpa gejala disgeusia berada pada rentang nilai normal yaitu <200µg/dL.



Gambar 3 Grafik Kadar Feritin pada Pasien Covid-19 (Elibol & Baran, (2021); Fei et al., (2021); Qin et al., (2020))

Peningkatan kadar ferritin serum bersama dengan peningkatan CRP pada pasien Covid-19 dapat menunjukkan terjadinya perkembangan *Systemic Inflammatory Response Syndrome* (SIRS) pada pasien dengan kondisi yang buruk. Mekanisme hubungan hiperferritinemia dan keparahan penyakit pada pasien dengan Covid-19 masih belum jelas, namun kemungkinan disebabkan oleh hal-hal berikut, sitokin proinflamasi seperti interleukin-Iβ (IL-lβ), *Tumor Necrosis Factor-a* (TNF-α), dan IL-6 dapat meningkatkan sintesis ferritin, kerusakan selular karena inflamasi dapat menyebabkan kebocoran feritin intraselular sehingga menaikkan feritin serum, Pada asidosis, lingkungan mikrovaskular dan peningkatan *reactive oxygen species* (ROS) dapat membebaskan besi dari feritin dan membentuk radikal hidroksil yang menyebabkan kerusakan jaringan yang lebih berat sehingga menciptakan lingkaran setan inflamasi Elibol & Baran, (2021).



Gambar 4 Grafik Titer Antibodi IgM dan IgG pada Pasien Covid-19 (Liu et al., (2020); Mengmeng Zhao et al., (2020); Rode et al., (2021); Tan et al., (2020))

Berdasarkan Gambar **4** pada penelitian yang dilakukan oleh Qin et al., (2020), diketahui rata-rata titer antibodi IgM yang terbentuk pada pasien Covid-19 dengan kondisi klinis yang tidak parah adalah 1,02 g/L dan rata-rata titer antibodi IgG 11,85 g/L. Sedangkan pada pasien Covid-19 dengan kondisi klinis yang parah, didapatkan hasil rata-rata titer antibodi IgM dan IgG yang terbentuk lebih rendah yaitu 0,9 g/L untuk antibodi IgM dan 11,7 g/L untuk antibodi IgG.

Pada penelitian lainnya yang dilakukan oleh Tan et al., (2020), pemeriksaan titer antibodi pada pasien Covid-19 dilakukan secara serial sebanyak 8 kali dimulai pada hari ke-7 dan berakhir pada hari ke-49. Data yang terdapat pada jurnal menunjukkan bahwa antibodi IgM muncul lebih dulu yaitu pada 7 hari pertama setelah timbulnya gejala dan kadarnya terus mengalami peningkatan hingga hari ke-14. Pada hari terakhir pemantauan, yaitu pada hari ke-49 diketahui antibodi IgM yang terbentuk menghilang. Sedangkan pada hari ke-7 pemantauan, antibodi IgG yang terbentuk masih rendah yaitu 2 g/L. Namun titernya terus mengalami peningkatan dan memuncak pada hari ke-28, kemudian diketahui mengalami penurunan hingga 4 g/L di hari ke-49.

Berdasarkan Gambar **4** pada penelitian yang dilakukan oleh Rode et al., (2021), pemeriksaan titer antibodi IgM dan IgG dilakukan secara serial dengan metode *Immunochromatographic Assay* (ICA). Pada pemeriksaan hari ke 1-3 diketahui antibodi IgM muncul lebih dulu dari antibodi IgG dan titernya terus meningkat. Terbentuknya antibodi IgG dimulai pada hari ke 4-7 dan titernya terus mengalami peningkatan, hingga hari terakhir pemantauan didapatkan titer antibodi IgM yang terbentuk tetap lebih tinggi dibandingkan antibodi IgG.

Berdasarkan Gambar **4** pada penelitian yang dilakukan oleh Liu et al., (2020), didapatkan rata-rata titer antibodi IgM dan IgG yang terbentuk lebih tinggi pada pasien Covid-19 dengan kadar prokalsitonin yang tinggi dibandingkan dengan pasien dengan kadar prokalsitonin normal. Pada penelitian lainnya yang dilakukan oleh Mengmeng Zhao et al., (2020) dengan membagi pasien berdasarkan kategori usia <60 tahun, usia 60-74 tahun dan usia >75 tahun didapatkan hasil rata-rata titer antibodi IgM yang terbentuk lebih tinggi pada pasien yang berusia <60 tahun dengan kadar antibodi 1,01 g/L. Sedangkan rata-rata antibodi IgG ditemukan lebih tinggi pada pasien dengan kategori usia >75 tahun dengan kadar antibodi 12,5 g/L.

Rentang lamanya antibodi SARS-CoV-2 akan bertahan dalam tubuh masih belum diketahui dengan pasti. Pada mayoritas individu (penyintas maupun pasca vaksin) akan memiliki IgG (baik terhadap protein S maupun N) yang dapat bertahan hingga beberapa bulan. Hingga saat ini pemeriksaan antibodi belum dapat digunakan untuk menilai efektivitas vaksin maupun untuk menentukan apakah seorang

individu membutuhkan vaksin, dikarenakan masih terbatasnya hasil penelitian terkait hal tersebut (Aryati, 2020)

Respon imun yang lambat terbentuk dapat disebabkan oleh imunosupresi atau *viral load* yang rendah. Peningkatan titer antibodi tidak selalu disertai dengan pembersihan virus, hal ini menunjukkan bahwa dengan adanya antibodi saja tidak cukup untuk membunuh virus Rode et al., (2021). Pemeriksaan antibodi SARS-CoV-2 dapat digunakan untuk mendukung diagnosis Covid-19 atau komplikasinya.

KESIMPULAN

Kesimpulan penelitian ini yaitu terjadi peningkatan kadar c-reactive protein dan prokalsitonin pada pasien Covid-19, kadar ferritin serum juga ditemukan cenderung mengalami peningkatan. Antibodi IgM ditemukan lebih dulu mengalami peningkatan dibandingkan dengan antibodi IgG, namun kadarnya lebih cepat mengalami penurunan. Peningkatan antibodi IgG terjadi sekitar minggu kedua setelah timbulnya gejala.

KONTRIBUSI PENULIS

Penulis berperan dalam pengumpulan data dan penyusunan artikel.

PENDANAAN

Dana penelitian berasal dari dana mandiri peneliti.

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RESEARCH ARTICLE
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Acute Toxicity Test Against Mice (*Mus Musculus*) On The Lempuyang Gajah Extract (*Zingiber Zerumbet (L.) Roscoe Ex Sm.*) With Sgot Sgpt Parameters

Uji Toksisitas Akut Terhadap Mencit (*Mus Musculus*) Pada Ekstrak Lempuyang Gajah (*Zingiber Zerumbet (L.) Roscoe Ex Sm.*) Dengan Parameter SGOT SGPT

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ABTRACT

Lempuyang Gajah (*Zingiber zerumbet (L.) Roscoe ex Sm*) is a medicinal plant that has many benefits in the medicinal industry. Useful as a tonic, external medicine, anti-seizure and appetite enhancer. This study aims to determine the toxicity of the lempuyang gajah. This research uses laboratory experimental methods. The lempuyang gajah plant was obtained in the village of Terungkulon, Krian. The initial stage was the administration of lempuyang gajah extract with various doses of 4000, 5000 and 6000 mg/kg BB orally, consisting of 5 treatment groups. Parameters observed, changes in toxic symptoms, macroscopic examination and parameters of SGOT, SGPT. The results were analyzed using the One Way ANOVA test. The results of the study after giving the extract did not cause death so it was included in the non-toxic category, there were several symptoms such as weakness and seizures. The levels of SGOT, SGPT showed normal average results, statistically there was no effect ($p > 0.05$). Macroscopic results showed no changes or abnormalities in the liver. So that the ethanol extract of lempuyang gajah can be said to be safe for humans.

Keywords: Acute Toxicity Test, Lempuyang Gajah, Liver, SGOT, SGPT

ABSTRAK

Lempuyang gajah (*Zingiber zerumbet (L.) Roscoe ex Sm*) merupakan tanaman obat yang memiliki banyak manfaat dalam industri obat. Bermanfaat sebagai tonikum, obat luar, anti kejang dan penambah nafsu makan. penelitian ini bertujuan untuk mengetahui toksisitas pada lempuyang gajah. Penelitian ini menggunakan metode eksperimental laboratorium. Tanaman lempuyang gajah diperoleh di desa Terungkulon, Krian. Tahap awal adalah pemberian ekstrak lempuyang gajah dengan variasi dosis 4000, 5000 dan 6000 mg/kg BB secara peroral, terdiri dari 5 kelompok perlakuan. Parameter yang diamati, perubahan gejala toksik,

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pemeriksaan makroskopis dan parameter SGOT, SGPT. Hasil dianalisis menggunakan uji *One Way* ANOVA. Hasil penelitian setelah pemberian ekstrak tidak menimbulkan kematian sehingga termasuk dalam kategori tidak toksik, terdapat beberapa gejala seperti lemas dan kejang. Kadar SGOT, SGPT menunjukan hasil rata-rata normal secara statistik tidak ada pengaruh ($p > 0,05$). Hasil makroskopis tidak ada perubahan dan kelainan pada organ hati. Sehingga ekstrak etanol lempuyang gajah dapat dikatakan aman bagi manusia.

Kata Kunci: Hati, Lempuyang Gajah, SGOT, SGPT, Uji Toksisitas Akut

PENDAHULUAN

Obat – obatan tradisional telah lama dikenal dan digunakan di Indonesia. Obat tradisional lebih mudah diterima oleh masyarakat karena obat tradisional selain murah juga mudah untuk didapatkan. Sudah banyak beraneka ragam obat tradisional yang berasal dari tanaman dan telah diteliti kandungan kimianya serta khasiat yang ada didalamnya, tetapi masih banyak tanaman yang belum diketahui kadar toksisitasnya, sehingga perlu diteliti lebih lanjut Cahyadi (2009).

Tanaman Lempuyang dari zaman dulu sudah dikenal sebagai bahan untuk jamu dan obat tradisional, selain itu untuk bagian rimpang dari tanaman lempuyang terutama lempuyang gajah dapat dimanfaatkan sebagai lalapan, rimpang lempuyang gajah memiliki rasa yang pedas, tajam dan bersifat hangat. Tanaman lempuyang gajah pada umumnya mengandung alkaloid, saponin, flavonoid, polifenol dan minyak atsiri terutama pada bagian rimpang. Pada rimpang lempuyang gajah juga dapat bermanfaat sebagai tonikum, obat luar, anti kejang dan sebagai penambah nafsu makan S. Rejeki & Priyandari (2017).

Uji toksisitas merupakan suatu uji yang digunakan untuk mendeteksi efek toksik suatu zat terhadap sistem biologi serta untuk mendapatkan sebuah data dosis-respon yang khas dari sediaan uji. Data yang diperoleh, digunakan untuk mengetahui informasi tentang besarnya bahaya dari sediaan uji tersebut bila terpapar pada manusia, sehingga dapat ditentukan penggunaan dosis yang sesuai untuk keamanan manusia BPOM (2014). Uji toksisitas awal yang dapat dilakukan dalam uji toksisitas akut. Uji toksisitas akut dilakukan dalam kurun waktu 24 jam dengan memberikan perlakuan pada suatu sediaan atau zat kimia, menggunakan dosis tunggal ataupun berulang. Uji ini dapat dilakukan dengan menggunakan hewan uji To'bungan et al., (2021).

Mencit (*Mus musculus*) adalah hewan yang biasa digunakan sebagai bahan uji karena penanganan yang relatif mudah, harga yang murah, jumlah peranakan yang banyak, mencit dalam sekali melahirkan bisa mencapai 16-18 ekor, memiliki ukuran yang kecil, serta fisiologis yang hampir sama dengan manusia. Mencit banyak digunakan sebagai penelitian obat, karena memiliki katup di lambung, sehingga tidak mempunyai kemampuan untuk muntah Marbawati & Ikawati (2009).

Hati merupakan organ yang memiliki beberapa fungsi, dengan fungsi utamanya adalah memproduksi empedu yang disalurkan ke dalam pencernaan, organ hati terlibat dalam berbagai aktivitas metabolik yang berkaitan dengan metabolisme karbohidrat, lemak, protein, filtrasi darah, kemampuan dalam eliminasi bakteri dan partikel asing lain yang masuk ke dalam darah. Hati memiliki peran dalam sistem pencernaan sebagai kelenjar yang mensekresikan getah empedu serta berperan dalam digesti dan absorbsi lemak. Hati juga berfungsi dalam metabolisme nutrien seperti karbohidrat, protein, dan lipid setelah diabsorbsi oleh saluran pencernaan Lestari et al., (2019).

SGOT dan SGPT adalah enzim yang ditemukan pada sel hati. Kadar SGPT tertinggi terdapat pada bagian sel hati yang terletak di sitoplasma. Sedangkan untuk enzim SGOT terdapat pada bagian dalam sel hati, jantung, ginjal, otot rangka, otak, pankreas, limpa dan paru.

Peningkatan kadar SGOT dan SGPT dapat disebabkan karena adanya perubahan permiabilitas atau terdapat kerusakan pada dinding sel hati sehingga digunakan sebagai penanda adanya gangguan fungsi hati. Nilai kadar SGOT dan SGPT yang meningkat hingga 300 U/L tidak spesifik untuk kelainan hati saja, tetapi jika nilai kadar mengalami peningkatan lebih dari 1000 U/L maka dapat ditemukan adanya penyakit hati akibat virus, iskemik hati yang disebabkan hipotesi lama atau gagal jantung akut, serta adanya kerusakan hati akibat obat atau zat toksik Rosida (2016).

METODE

Alat yang digunakan adalah sebagai berikut. Seperangkat alat gelas, nampan, ayakan mesh, penggilingan, *rotatory vaccum evaporator*, neraca analitik, plat, hot plate, toples kaca, fotometer, sentrifus, sonde oral, spuit 3 cc, dan peralatan bedah. Bahan yang digunakan adalah rimpang lempuyang gajah, hewan uji mencit putih jantan berat 25-35 gram, pelarut etanol 70%, klorofrom, amoniak, asam sulfat 2N, sebuk magnesium, HCl pekat, asam sulfat pekat, asam asetat anhidrat, FeCl3 1%, NaCl 1%, larutan gelatin 10%, pereaksi mayer, wager, dragendrof, etil asetat, reagen SGOT dan SGPT.

Penanganan sampel menggunakan etika penelitian. Ethical Clearance penelitian ini diperoleh dari STIKES Ngudia Husada Madura. Setelah mendapatkan perizinan dari proses kelayakan etik, akan didapatkan bukti sertifikat uji kelayakan penelitian Ethical Clearance dengan nomor 1309/KEPK/STIKES-NHM/EC/Vl/2022.

A. Pembuatan Simplisia

Rimpang lempuyang gajah yang sudah dipisahkan dari akarnya dicuci bersih dengan air mengalir, dikeringkan di bawah sinar matahari selama beberapa hari dan dihaluskan Makalalag et al., (2010). selanjutnya ditimbang lalu diayak sehingga diperoleh serbuk lempuyang gajah. Serbuk ditimbang dan disimpan dalam wadah yang tertutup Rohmah et al., (2020).

B. Ekstraksi Maserasi

Serbuk rimpang lempuyang gajah ditimbang sebanyak 150 g, lalu direndam dengan pelarut etanol 70% sebanyak 600 ml dengan perbandingan 1:4 lalu diaduk menggunakan batang pengaduk. Campuran yang sudah diaduk didiamkan selama 24 jam didalam toples kaca dan ditutup rapat. hasil maserasi disaring didapatkan filtrat dan residu. Residu direndam kembali dengan etanol hingga filtrat tidak berwarna dan disaring kembali. Filtrat yang dihasilkan lalu dikentalkan atau dievaporasi dengan menggunakan *rotatory vaccum evaporator*

C. Uji Fitokimia

1) Uji Alkaloid

Sampel sebanyak ± 1 mL ekstrak etanol lempuyang gajah dicampur dengan 1 mL klorofrom dan 1 mL amoniak, dipanaskan diatas hot plate, lalu dikocok dan disaring. Filtrat yang diperoleh dibagi tiga bagian yang sama lalu masing-masing ditambahkan 3 tetes asam sulfat 2 N, kocok dan diamkan selama beberapa menit hingga terpisah. Bagian atas dari filtrat diambil dan diuji dengan pereaksi meyer, wager dan dragendof.

2) Uji Flavonoid

Sampel sebanyak ± 1 mL ekstrak etanol lempuyang gajah dicampur dengan 3 mL etanol 70%, kemudian dikocok dan dipanaskan, setelah dipanaskan dikocok lagi dan disaring. Hasil filtrat yang diperoleh ditambahkan bubuk magnesium 0,1 gram dan 2 tetes HCl pekat.

3) Uji Saponin

Sampel sebanyak ± 1 mL ekstrak etanol lempuyang gajah dipanaskan dengan 10 mL air dengan *hot plate*. Filtrat dikocok dan didiamkan selama 15 menit.

4) Uji Steroid

Sampel sebanyak ± 1 mL ekstrak etanol lempuyang gajah dicampur dengan 3 mL etanol 70% dan ditambahkan 2 mL asam sulfat pekat dan 2 mL asam asetat anhidrat.

5) Uji Triterpenoid

Sampel sebanyak ± 1 mL ekstrak etanol lempuyang gajah dicampur dengan 2 mL kloroform dan 3 mL asam sulfat pekat.

6) Uji Tanin

Sampel sebanyak ± 1 mL ekstrak etanol lempuyang gajah dididihkan dengan 20 mL air di atas hot plate, kemudian disaring. Hasil filtrat yang diperoleh ditambahkan 2-3 tetes FeCl3 1%.

7). Uji Fenolik

Sampel sebanyak ± 1 mL ekstrak etanol lempuyang gajah ditambahkan 1 ml larutan NaCl 1% dan larutan gelatin 10% sebanyak 1 mL.

D. Prosedur Adaptasi dan Uji Toksisitas Pada Mencit

Mencit dengan berat 25-35 gram sebayak 30 ekor yang diambil secara acak dan dikelompokkan menjadi 5 kelompok dengan satu kandang berisi 5 ekor mencit. Setelah itu mencit akan diadaptasi dengan lingkungan selama 1 minggu sebelum diberi perlakuan, selama masa adaptasi mencit diberi pakan standart dan minum secara *ad libitum* Rafita (2015).

Pada kelompok perlakuan K1, K2 dan K3 akan diberikan ekstrak lempuyang gajah dengan variasi dosis 4.000, 5.000, dan 6.000 mg/kg BB yang disuspensi dalam Na CMC 1% sebanyak 1 kali. Pengamatan akan dilakukan selama 24 jam setelah pemberian sediaan uji, apabila tidak ada kematian pada hewan uji, pengamatan akan dilakukan selama 14 hari Nurfatwa (2018).

E. Pengambilan Darah Mencit

Pengambilan darah pada hewan uji mencit (*Mus musculus*) dilakukan melalui intrakardial di jantung. Langkah pertama mencit dianastesi menggunakan kloroform lalu lakukan pengambilan darah dengan cara memasukkan langsung jarum suntik ke jantung dan disedot perlahan. Setelah darah keluar, ditampung dan dibiarkan dalam suhu ruang setelah itu disentrifus dengan kecepatan 3000 rpm selama 15 menit Nugroho (2018).

F. Pengukuran Kadar SGOT danSGPT

Sampel darah mencit yang sudah disentrifus lalu diambil serumnya. Siapkan masing – masing tabung reaksi yang akan diisi dengan campuran reagen R1 400 µl + R2 100 µl dan sampel serum mencit sebanyak 25 µl, setelah itu campur dan inkubasi selama 1 menit. Lalu baca pada fotometer dengan panjang gelombang 340 nm dan suhu 37ºC.

G. Pemeriksaan Makroskopis

Pemeriksaan makroskopis mencit (*Mus musculus*) dilakukan dengan mengukur volume organ dengan cara memasukkan ke dalam gelas ukur yang berisi akuades. Volume organ akan diukur dari selisih kenaikan volume akuades pada gelas ukur, lalu mengamati perubahan warna yang terjadi pada organ hati dan menimbang berat organ hati Sutomo et al., (2019).

HASIL DAN PEMBAHASAN

A. Pembuatan Simplisia

Pembuatan simplisia terdapat beberapa tahapan yaitu, pengumpulan sampel, pencucian sampel, pengeringan dan penghalusan sampel. Pada proses pencucian sampel bertujuan untuk menghilangkan kotoran yang menempel pada rimpang lempuyang gajah yang terkena tanah, setelah proses pencucian sampel yang sudah bersih dikeringkan. Proses pengeringan bertujuan untuk menghilangkan kadar air dalam sampel, sehingga sampel dapat terhindar dari perkembangbiakan bakteri Maulana (2018). Pada proses pengeringan dilakukan dengan cara diangin-anginkan dan tidak terkena sinar matahari secara langsung, karena dapat menyebabkan berkurangnya kandungan senyawa metabolit.

Tabel 1. Hasil berat sampel rimpang lempuyang gajah (*Zingiber zerumbet (L.) Roscoe ex Sm*).

Parameter	Berat Sampel
Berat basah	1000 gram
Berat kering	500 gram
Berat serbuk	150 gram

Berdasarkan Tabel 1 didapatkan berat basah sebanyak 1000 gram, untuk berat kering sampel didapatkan sebesar 500 gram, pada sampel kering mengalami penyusutan dikarenakan hilangnya kadar air karena proses pengeringan. Setelah itu sampel dibuat serbuk dengan cara dihaluskan untuk mempermudah proses ekstraksi, untuk berat serbuknya didapatkan sebesar 150 gram. Penyusutan berat serbuk dari berat kering disebabkan karena adanya perubahan ukuran simplisia.

B. Ekstrasi Maserasi

Ekstraksi maserasi menggunakan pelarut alkohol 70% adalah karena etanol merupakan salah satu jenis pelarut yang aman dan tidak memiliki sifat racun apabila dikonsumsi karena tingkat toksisitas yang rendah dibandingkan dengan pelarut yang lainnya. Etanol juga dapat lebih banyak menarik senyawa aktif dibandingkan dengan pelarut organik yang lainnya Hasanah & Novian (2020).

Hasil dari ekstraksi maserasi pada rimpang lempuyang gajah didapatkan ekstrak sebanyak 1000 ml, kemudian hasil ekstraksi akan dipisahkan dengan pelarut atau dikentalkan dengan menggunakan alat *rotatory vacuum evaporator*. Hasil ekstrak pekat yang didapatkan yaitu sebesar 24 gram dengan warna kecoklatan dan memiliki bau seperti jamu.

Ekstrak pekat yang sudah didapat selanjutnya dihitung presentase rendemen. Perhitungan rendemen bertujuan untuk mengetahui nilai presentase hasil perolehan ekstrak, sehingga dapat diketahui jumlah simplisia yang dibutuhkan untuk membuat ekstrak kental tertentu Samudra (2014). Hasil rendemen dari ekstraksi maserasi adalah 16%. Dari hasil tersebut menunjukkan bahwa banyak zat berkhasiat yang terkandung dalam rimpang lempuyang gajah. Semakin tinggi nilai rendemen maka ekstrak yang dihasilkan akan semakin besar Nahor et al., (2020).

C. Uji Fitokimia

Uji fitokimia merupakan tahap awal dalam penelitian yang bertujuan untuk mengetahui golongan senyawa metabolit sekunder yang terkandung dalam tanaman. Metode fitokimia dilakukan dengan melihat adanya reaksi perubahan warna dengan menggnakan suatu pereaksi Saragih & Arsita (2018).

Tabel 2. Hasil Uji Fitokimia

Ekstrak	Uji Fitokimia	Pereaksi	Hasil	Keterangan (+/-)
Lempuyang gajah (*Zingiber zerumbet* (L.) Roscoe ex Sm)	Alkaloid	Mayer	Endapan putih	++
		Wagner	Endapan coklat	+++
		Dragendorf	Endapan jingga	-
	Flavonoid	Mg + HCl_{pekat} + etanol	Warna merah	+++
	Saponin	-	Adanya busa stabil	+++
	Steroid	Libermann-Burchard	Ungu kebiru/hijau	+++
	Titerpenoid	Kloroform + $H_2SO_{4\ pekat}$	Merah kecoklatan	++
	Fenolik	NaCL 10% + Gelatin 1%	Endapan putih	+++
	Tannin	$FeCl_3$ 1%	Coklat kehijauan	+++

Berdasarkan Tabel 2 hasil uji fitokimia pada ekstraksi maserasi rimpang lempuyang gajah mengandung senyawa alkaloid, flavonoid, saponin, steroid, triterpenoid, fenolik dan tannin. Tetapi pada senyawa alkaloid dengan pereaksi dragendorf terdapat hasil negatif.

Menurut penelitian yang telah dilakukan oleh Rohmah, et al., (2022) yang menyatakan pada uji fitokimia ekstrak lempuyang gajah positif mengandung adanya senyawa alkaloid, flavonoid, saponin, steroid, triterpenoid, fenolik dan tannin. Pada penelitian ini, uji fitokimia ekstrak lempuyang gajah juga positif mengandung senyawa alkaloid, flavonoid, saponin, steroid, triterpenoid, fenolik dan tannin. Namun terdapat perbedaan pada intensitas kadar yang terkandung di dalam senyawa metabolit. Perbedaan intensitas kadar tersebut dapat terjadi dikarenakan adanya perbedaan daerah asal tanaman yang didapat.

D. Uji Toksisitas Ekstrak Lempuyang Gajah

Pengamatan dilakukan dengan mengamati terjadinya gejala yang muncul setelah pemberian ekstrak secara peroral selama 14 hari. Pengamatan dilakukan pada setiap kelompok dengan mengamati adanya perubahan tingkah laku ataupun gejala tertentu. Pengamatan pada gejala toksik dapat terjadi seperti lemas, nafsu makan berkurang, kehilangan arah, resah, ataksia, cegukan dapat ditemukan pada perlakuan 1, 2 dan 3. Sementara pada kelompok normal dan kontol negatif tidak terdapat gejala yang spesifik, dapat dilihat dari hasil Tabel 3 berikut:

Tabel 3. Hasil gejala Klinis Mencit

Kelompok	Mencit	Gejala klinis
Normal	1	Tidak ada gejala
	2	Tidak ada gejala
	3	Tidak ada gejala
	4	Tidak ada gejala
	5	Tidak ada gejala
	6	Tidak ada gejala
Na CMC 1%	1	Tidak ada gejala
	2	Tidak ada gejala
	3	Tidak ada gejala
	4	Tidak ada gejala
	5	Tidak ada gejala
	6	Tidak ada gejala
4000 mg/kg BB	1	Tidak nafsu makan
	2	Tidak ada gejala
	3	Tidak ada gejala
	4	Tidak ada gejala
	5	Lemas, nafsu makan berkurang,
	6	Tidak ada gejala
5000 mg/kg BB	1	Lemas
	2	Tidak ada gejala
	3	Kejang
	4	Tidak ada gejala
	5	Tidak ada gejala
	6	Tidak ada gejala
6000 mg/kg BB	1	Resah, cegukan, detak jantung cepat
	2	Cegukan
	3	Tidak ada gejala
	4	Ekor terdapat benjolan ungu
	5	Tidak ada gejala
	6	Kejang

Berdasarkan Tabel 3 pada kelompok normal dan kelompok kontrol tidak terdapat gejala klinis serta tidak ada tanda-tanda perubahan prilaku. Hal itu dikarenakan pada kelompok normal tidak diberi ekstrak dan hanya diberi makan minum, sementara untuk kelompok kontrol negatif diberi NaCMC 1%. Sedangkan hasil pengamatan pada kelompok perlakuan 1 yaitu dosis 4000 mg/kg BB ditemukan pada beberapa mencit mengalami gejala seperti lemas dan nafsu makan berkurang. Berkurangnya nafsu makan dapat diakibatkan karena stress, sehingga memungkinkan mencit tidak mau makan dan minum.

Pengamatan pada kelompok perlakuan 2 yaitu dosis 5000 mg/kg BB juga ditemukan beberapa gejala pada mencit seperti lemas dan kejang, kejang pada mencit disebabkan karena pada saat proses pemberian ekstrak mengenai paru-paru. Hal itu menyebabkan terjadinya penumpukan ekstrak pada paru-paru sehingga menyebabkan mencit mengalami kejang tetapi tidak sampai mengalami kematian. Pada kelompok perlakuan 3 dengan dosis 6000 mg/kg terdapat beberapa gejala berat seperti kejang, resah dan cegukan. Pengamatan dilanjutkan dengan mengamati adanya jumlah kematian dari hari pertama hingga hari ke 14. Terdapat jumlah kematian mencit setelah pemberian ekstrak lempuyang gajah adalah 1 ekor dari 30 ekor mencit yang diberi perlakuan. Dapat dilihat dari hasil Tabel 4 berikut:

Tabel 4. Jumlah kematian hewan uji 14 hari setelah pemberian ekstrak lempuyang gajah (*zingiber zerumbet* (L.) Roscoe ex Sm.

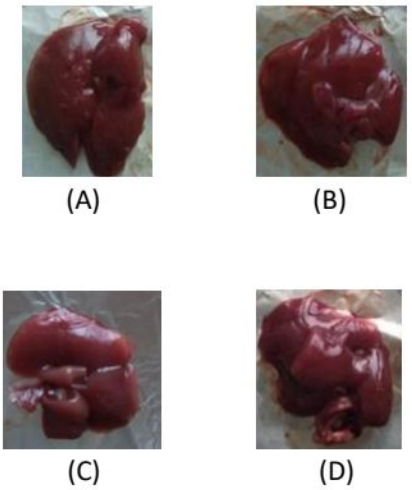
Dosis (mg/kg BB)	Jumlah mencit yang mati	Jumlah mencit yang hidup
Normal	0	6
kontrol negatif	0	6
4000 mg/kg BB	0	6
5000 mg/kg BB	0	6
6000 mg/kg BB	1	5

Berdasarkan Tabel 4 pengamatan terhadap jumlah kematian selama 14 hari terhadap mencit setelah pemberian ekstrak etanol lempuyang gajah menunjukkan adanya kematian pada dosis 3 yaitu 6000 mg/kg BB yaitu sebanyak 1 ekor. Sebelum mengalami kematian mencit ditandai dengan adanya gejala seperti detak jantung cepat dan resah. Gejala seperti detak jantung cepat dapat disebabkan karena mencit panik setelah diperikan perlakuan, sehingga belum dapat dikatakan karena akibat dari pemberian ekstrak lempuyang. Pada dosis 4000 mg/kg BB tidak terdapat mencit yang mati, tetapi terdapat gejala klinis seperti nafsu makan berkurang. Pada dosis yang leih besar yaitu 5000 mg/kg BB menunjukkan gejala toksik seperti lemas dan kejang.

Berdasarkan hasil pengamatan tersebut menunjukkan bahwa semakin tingi konsentrasi ekstrak akan semakin tinggi efek yang ditimbulkan. Hal tersebut dapat terjadi karena dipengaruhi oleh konsentrasi dosis yang semakin tinggi sehingga semakin banyak kandungan zat aktif yang terdapat pada ekstrak. Sehingga dapat diketahui bahwa dosis merupakan hal utama yang menentukan apakah suatu zat kimia bersifat racun Makalalag et al., (2011).

E. Pengamatan Makroskopis Organ hati

Hasil pengamatan makroskopis meliputi volume, berat dan Hasil pengamatan pada makroskopis hati mencit dapat dilihat pada Gambar 1 yang menunjukan hasil makroskopis warna organ hati. Pada kontrol normal, kontrol negatif, perlakuan 1, perlakuan 2 dan perlakuan 3 didapatkan rata-rata memiliki warna merah gelap kecoklatan dengan permukaan yang halus. Menurut Sutomo et al., (2019) hati yang normal akan berwarna merah kecoklatan dengan permukaan yang rata dan halus, sedangkan pada hati yang tidak normal akan mengalami perubahan warna dan permukaan berbintik.



Gambar 1. Organ hati mencit setelah diberikan ekstrak lempuyang gajah selama 14 hari. (A) Organ hati kelompok normal. (B) Organ hati perlakuan 1. (C) Organ hati perlakuan 2. (D) Organ hati perlakuan 3.

Kelompok normal, kontrol negatif, perlakuan 1 dan 2 memiliki organ hati yang normal setelah pemberian ekstrak, hal ini juga dapat dilihat dari hasil kadar SGOT dan SGPT yang normal dan tidak terdapat gejala klinis. sedangkan pada perlakuan 3 meskipun terdapat beberapa gejala, tetapi nilai kadar SGOT SGPT yang normal dan hasil maksroskopis normal serta tidak ada perubahan warna pada organ, sehingga dapat dikatakan organ hati tidak mengalami kerusakan.

Tabel 5. Berat dan Volume Organ Hati

Kelompok Perlakuan	Berat Organ Hat X ± SD	Volume organ
Kn (Kontrol normal)	1,620 ± 1,324	41
K- (Kontrol negatif)	1,172 ± 3,796	40
PI (4000 mg/kg BB)	1,265 ± 2,558	41
PII (5000 mg/kg BB)	1,261 ± 2,117	41
PIII (6000 mg/kg BB)	1,469 ± 2,705	41

Hasil pada Tabel 5 menunjukan rata-rata berat organ hati pada mencit. Pada kelompok normal memiliki rata-rata dengan berat 1,620 gram. Pada kelompok kontrol negatif memiliki rata-rata berat 1,172 gram, sedangkan pada kelompok perlakuan 2 dan 3 memiliki berat rata-rata yang hampir sama yaitu 1,265 gram dan 1,261 gram. Pada kelompok perlakuan 3 memiliki berat rata-rata yaitu 1,469 gram. Hasil rata-rata bobot tersebut masih dalam kisaran normal, karena berat hati normal mencit dewasa berkisar antara 1,2 – 1,6 gram Hasana et al., (2019).

Volume hati mencit diukur menggunakan gelas ukur volume 50 ml yang diisi akuades sebanyak 40 ml, untuk menentukan volume, organ dimasukkan kedalam gelas ukur dan dilihat adanya penambahan volume atau tidak. Hasil volume organ hati pada mencit kelompok normal, kelompok perlakuan 1, 2 dan 3 memiliki rata-rata volume yang sama yaitu 41. Sedangkan pada kelompok kontrol negatif memiliki rata-rata volume yang lebih kecil, yaitu 40.

F. Pemeriksaan Kadar SGOT dan SGPT

Tabel 6. Hasil Kadar SGOT dan SGPT Mencit

Kelompok Perlakuan	Kadar SGOT (U/L) X ± SD	Kadar SGPT (U/L) X ± SD
Kn (Kontrol normal)	36.00 ± 6.229	38.50 ± 7.688
K- (Kontrol negatif)	36.83 ± 3.125	34.83 ± 6.735
PI (4000 mg/kg BB)	35.67 ± 15.319	39.60 ± 5.771
PII (5000 mg/kg BB)	39.33 ± 4.546	37.50 ± 3.507
PIII (6000 mg/kg BB)	42.83 ± 16.142	42.67 ± 10.309

Hasil uji *one way* ANOVA pada kadar SGOT diperoleh nilai signifikan sebesar 0,710 ($p > 0,05$) maka Ha ditolak dan H0 diterima. Pada kadar SGPT diperoleh nilai signifikan sebesar 0,477 ($p > 0,05$) maka Ha ditolak dan H0 diterima. Yang artinya tidak terdapat pengaruh terhadap kadar SGOT dan SGPT sehingga tidak dapat dilanjutkan analisis ke uji *Post Hoc*.

Berdasarkan hasil Tabel 6, pada kelompok normal menunjukkan rata-rata kadar SGOT dan SGPT adalah normal. Kadar SGOT pada kelompok kontrol normal didapatkan hasil rata-rata sebesar 36.00 U/L. Pada kelompok kontrol negatif didapat hasil rata-rata 36.83 U/L. Untuk perlakuan 1 didapatkan rata-rata 35.67, pada perlakuan 2 rata-rata 39.33 U/L dan pada perlakuan 3 rata-rata 42.83 U/L. Hasil tersebut menyatakan tidak ada kenaikan pada kadar SGOT dan rata-rata hasil kadarnya normal. Namun pada perlakuan 3 jika dilihat dari Tabel 3, muncul gejala klinis pada mencit seperti kejang, resah dan cegukan. Gejala yang muncul pada perlakuan 3 diakibatkan karena mencit mengalami stress dan cemas, tetapi pada keadaan tersebut tidak mempengaruhi organ hati sehingga kadar SGOT dan SGPT pada kelompok perlakuan 3 rata-rata hasilnya normal.

KESIMPULAN

Berdasarkan pada penelitian yang telah dilakukan dapat disimpulkan bahwa ekstrak lempuyang gajah didapatkan nilai LD50 lebih besar dari 6000 mg/kg BB, serta didapatkan nilai sig dari kadar SGOT sebesar 0,710 ($p > 0,05$) dan SGPT sebesar 0,477 ($p > 0,05$) yang artinya tidak terdapat pengaruh terhadap kadar SGOT dan SGPT. Tidak terdapat perubahan gejala klinis mencit dan makroskopis pada kelompok perlakuan setelah pemberian ekstrak lempuyang gajah hingga dosis 6000 mg/kg BB.

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KONTRIBUSI PENULIS

Penulis utama pertama berperan dalam penyusunan artikel dan penulis kedua berperan untuk membimbing jalannya penelitian dari awal hingga pengumpulan data.

PENDANAAN

Penelitian ini menggunakan dana pribadi dari peneliti.

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Formulation, Evaluation and Physical Stability Test of Aloe Vera (*Aloe vera L.*) Extract Gel Preparations with the Addition of Sodium Metabisulfite

Formulasi, Evaluasi dan Uji Stabilitas Fisik Sediaan Gel Ekstrak Lidah Buaya (Aloe vera L.) dengan Penambahan Sodium Meta

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ABTRACT

Aloe vera (*Aloe vera L.*) is a plant that has many benefits, such as anti-inflammatory, anti-fungal, antibacterial, and moisturizing the skin. Gel preparations are easily contaminated by bacteria and fungi, so it is necessary to consider adding sodium metabisulfite as a preservative. The aim of this research is to prove the effectiveness of using sodium metabisulfite as a preservative for aloe vera extract gel preparations. The aloe vera plant (*Aloe vera L.*) was extracted using the maceration method for 3x24 hours using methanol solvent. This research used 5% aloe vera (*Aloe vera L.*) extract, and in the evaluation and stability test used 4 variation formulations using a carbomer base, only gel base (F1), gel base with the addition of sodium metabisulfite (F2), gel base with 5% aloe vera extract (F3), and gel base with 5% aloe vera extract and sodium metabisulfite (F4). The evaluations carried out were organoleptic tests, pH tests, viscosity tests, and ALT (Total Plate Number) tests and AKK (Yeast Mold Number) tests. The physical stability test is carried out for 3-14 days. The extract yield test results obtained were 4.892%. Organoleptic tests on F1, F2, F3 and F4 did not show changes in color, odor and texture, whereas on day 14 changes in color, odor and texture began to occur in the F3 preparation. The pH test showed pH results of 4.67 (F1), 4.9 (F2), 5 (F3) and 5.1 (F4). The viscosity test shows the gel has a viscosity of 2760 cps (F1), 2650 cps (F2), 2590 cps (F3), and 2575 cps (F4). The ALT and AKK tests showed that there was an increase in the value of the F3 preparation. So based on these results it can be concluded that the best gel preparation is F4

Keywords: Aloe vera, Formulated, Sodium Metabisulfite, Stability test

ABSTRAK

Lidah buaya (*Aloe vera* L.) merupakan tanamanan yang mempunyai banyak khasiat, seperti anti inflamasi, anti jamur, antibakteri, dan melembabkan kulit. Sediaan gel mudah mengalami kontaminasi baik oleh bakteri maupun jamur sehingga perlu dipertimbangkan penambahan *sodium metabisulfite* sebagai pengawet. Tujuan dari penelitian ini untuk membuktikan efektivitas penggunaan *sodium metabisulfite* sebagai pengawet untuk sediaan gel ekstrak lidah buaya. Tumbuhan lidah buaya (*Aloe vera L.*) diekstrak dengan metode maserasi selama

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3x24 jam menggunakan pelarut metanol. Penelitian ini menggunakan ekstrak lidah buaya (*Aloe vera L.*) sebanyak 5%, dan dalam evaluasi dan uji stabilitasnya menggunakan 4 formulasi variasi dengan menggunakan basis carbomer, yaitu basis gel (F1), basis gel dengan penambahan *sodium metabisulfite* (F2), basis gel dengan ekstrak lidah buaya 5% (F3), dan basis gel dengan ekstrak lidah buaya 5% dan *sodium metabisulfite* (F4). Evaluasi yang dilakukan yaitu uji organoleptis, uji pH, uji viskositas, dan uji ALT (Angka Lempeng Total) serta uji AKK (Angka Kapang Khamir). Uji stabilitas fisik dilakukan selama 3-14 hari. Hasil pengujian randemen ekstrak yang diperoleh adalah 4, 892%. Uji organoleptis pada F1, F2, F3 dan F4 tidak menunjukkan perubahan warna, bau dan tekstur sedangkan di hari ke-14 mulai terjadi perubahan warna, bau dan tekstur pada sediaan F3. Uji pH menunjukan hasil pH 4,67 (F1), 4,9 (F2), 5 (F3) dan 5,1 (F4). Uji viskositas menunjukan gel memiliki viskositas 2760 cps (F1), 2650 cps (F2), 2590 cps (F3), dan 2575 cps (F4). Uji ALT dan AKK menunjukkan terdapat peningkatan nilai pada sediaan F3. Sehingga berdasarkan hasil tersebut dapat disimpulkan sediaan gel terbaik adalah F4.

Kata Kunci: Formulasi, Lidah Buaya (*Aloe veera L.*), Sodium Metabisulfite, Uji stabilitas

PENDAHULUAN

Lidah buaya (*Aloe vera L.*) merupakan tanaman suku Liliaceae asli Afrika yang dapat tumbuh dengan mudah di daerah tropis dengan lahan berpasir dan sedikit air serta memiliki pertumbuhan yang mudah dan cepat Sanchez M. et.al., (2020). Lidah buaya memiliki ciri seperti, bunga merah cerah dengan akar pendek. Bunga dari lidah bermekaran dengan masa hidup sekitar 1-2 minggu sebelum akhirnya rontok dan batangnya mengering. Lidah buaya memiliki batang yang tidak besar dan pendek berukuran 10 cm. Daun lidah buaya letaknya berhadapan dengan bentuk memanjang Hes M. et al., (2019). Di Indonesia tanaman ini mulai dibudidayakan pada abad 17. Tanaman ini dijuluki "*miracle plant*" karena kandungannya seperti aloin, emodin, resin gum dan minyak atsiri Aulia and Pane (2022). Dalam dunia kecantikan lidah buaya seringkali digunakan dalam bentuk gel dengan tujuan regenerasi sel kulit. Berbagai penelitian telah melaporkan aktivitas *Aloe vera L.* dalam mempercepat proses penyembuhan luka insisi, di antaranya penelitian Mustaqim et al., (2018) yang melaporkan bahwa pemberian gel *Aloe vera L.* pada luka mencit menunjukkan perbaikan dalam penyembuhan luka dilihat dari parameter tebal epitel dan jumlah rata-rata fibroblas. Penelitian lainnya menyebutkan bahwa efek penyembuhan luka menggunakan ekstrak *Aloe vera L.* menjadi lebih cepat dan lebih baik, khususnya pada konsentrasi 5% Apriyasa et al., (2022).

Sediaan gel rentan mengalami kontaminasi bakteri maupun jamur. Pengawet merupakan komponen penting dalam sediaan farmasi yang ditambahkan dengan tujuan meningkatkan stabilitas/absorpsi, konsumsi, pemberian, penampilan, dan sebagainya. Tanpa adanya pengawet sediaan farmasi atau obat dapat rusak karena bakteri tumbuh. Adanya bakteri/jamur tumbuh mengakibatkan risiko adanya racun yang timbul Mubarok, (2019). Salah satu bahan pengawet yang sering digunakan dalam industri kecantikan adalah sodium metabisulfit.

Sodium metabisulfit merupakan golongan garam-garam anorganik berbentuk serbuk, berwarna putih, larut dalam air, sedikit larut dalam etanol, dan berbau khas seperti gas sulfur dioksida sifatnya asam dan mempunyai rasa asin. Dalam formulasi obat, sodium metabisulfit digunakan sebagai agen pembawa obat topikal, dan sebagai pelindung agen obat. Selain itu, sodium metabisulfit merupakan preservatif berbasis antioksidan yang berfungsi untuk mencegah adanya kontaminasi oleh bakteri dan jamur Ilie-Mihai et al., (2022). Sebagai antioksidan sodium metabisulfit juga dapat berfungsi dalam memperpanjang masa simpan produk. Stabilitas sediaan dalam formulasi obat sangat penting untuk menjamin kualitas dan kuantitas dari zat aktif yang terkandung. Ekstrak aloe vera berbasis gel memiliki kerentanan pada stabilitas karena sensitif terhadap cahaya dan panas. Hal ini menyebabkan urgensi dari penelitian ini untuk mendapatkan formula gel terbaik dengan penambahan sodium metabisulfit yang diharapkan dapat menjaga stabilitas dan efektivitas dari sediaan gel ekstrak aloe vera Avis T.J. et al., (2007).

METODE

Alat yang digunakan dalam penelitian ini berupa *rotary elevator, thinky mixer, homogenizer*, gelas kimia, wadah penempataan gel, timbangan digital, pH meter, *viscometer rheosys, colony counter* dan erlenmeyer. Sedangkan untuk bahan yang digunakan pada penelitian ini meliputi tumbuhan lidah buaya (*Aloe vera L.*), Gel, sodium metabisulfit, NaCl 0.85 % dan etanol. Bahan utama yang digunakan dalam penelitian ini adalah tanaman lidah buaya. Lidah buaya sebelumnya dicuci bersih, dipisahkan gel daun dari kulitnya, kemudian dirajang kecil dan dimaserasi menggunakan etanol selama semalam. Setelah satu malam, filtrat dipisahkan kemudian diuapkan pada suhu ±40°C menggunakan *rotary evaporator* sampai diperoleh ekstrak etanol. Selanjutnya hasil ektraksi dicampur dengan basis gel dengan konsentrasi 5%. Pencampuran dilakukan menggunakan *thinky mixer* dengan kecepatan 2000 rpm selama 3 menit hingga homogen. Sedangkan, untuk sediaan dengan penambahan sodium metabisulfit ditambahkan sebanyak 0,2% dan dicampur pada tahap akhir.

Tabel 1. Rancangan Formula Sediaan Gel

Bahan	Formula (%)			
	Formula 1	Formula 2	Formula 3	Formula 4
Aquademin	93.60	93.60	93.60	93.60
TEA	0.40	0.40	0.40	0.40
Aloe vera water	0	0	5.00	5.00
Sodium metabisulfite	0	0.20	0	0.20
Carbomer	6.00	5.80	1.00	0.80

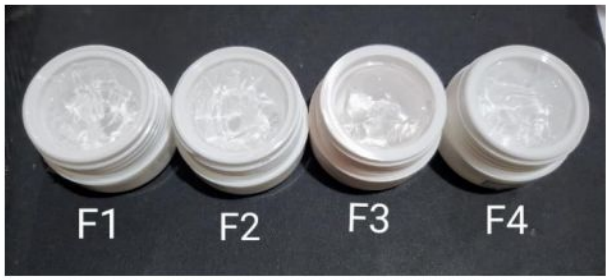
Pada tabel 1 merupakan rancangan formula sediaan gel dengan variasi pemberian sodium metabisulfit dan aloe vera. Setiap sediaan mengandung aquademin 93,60 %, dan TEA 0,40 %. Penamabahan ektraksi *aloe vera L.* pada sediaan sebanyak 5 %. Penambahan sodium metabisulfit pada sediaan sebanyak 0.20 %. Penambahan carbormer pada setiap formulasi sebanyak *ad* 100 %. Setiap bahan kemudian dicampur dan di homogenkan menggunakan *thinky mixer* dengan kecepatan 2000 rpm selama 3 menit. Setiap formulasi yang sudah homogen kemudia di evaluasi. Pertama kali evaluasi yang dilakukan adalah pemeriksaan organoleptis. Pemerikasaan organoleptis dilakukan menggunakan panca indra peneliti dan meliputi aspek warna, bau dan bentuk. Gusnadi et al., (2021). Selanjutnya peneliti melakukan evaluasi derajat keasaman dengan menggunakan pH. Sebelum evaluasi derajat keasaman, alat pH meter kalibrasi terlebih dahulu dengan menggunakan larutan pH standar netral (pH 7,00), larutan pH basa (pH 9,00), dan larutan pH asam (pH 4,00). Kemudian elektroda dicuci dengan air suling, lalu dikeringkan dengan kertas tisu. Selanjutnya elektroda dicelupkan kedalam basis gel, sampai alat menunjukkan harga pH yang konstan, pH kulit wajah manusia berkisar antara 4,5−6 Setiawan R. et al., (2023). Selanjutnya dilakukan uji stabilitas dan evaulasi dari sediaan gel dengan menggunakan pengujian viskositas

dan pengujian angka lempeng total (ALT) dab Angka Khapang Khamir (AKK). Uji Viskositas dilakukan dengan menggunakan sampel basis gel sebanyak 1 gram. Sampel basis gel diletakan pada permukaan slinder kemudian diukur menggunakan viscometer yang dilengkapi spindle dengan kecepatan 1 rpm. Uji angka lempeng total (ALT) dan angka kapang khamir (AKK). Uji ALT dilakukan bertujuan untuk mengetahui jumlah bakteri mesofil yang terkandung dalam basis gel, Sedangkan uji AKK dilakukan untuk mengetahui cemaran mikroba yang terkandung dalam basis gel. Uji ALT dan uji AKK dilakukan dengan cara mencampur 5 mL sampel kedalam tabung erlenmeyer yang berisi NaCl 0,85 % bervolume 45 %, kemudian dihomogenkan. Setelah homogen dilakukan pengenceran sebanyak tiga kali. Selanjutnya, dilakukan penaburan menggunakan *inoculum* 1.0 mL, sedangkan untuk mengetahui sterilitas medium dilakukan sebaran 28 0.1 mL. Sampel di inkubasi selama 24 jam pada suhu 37°C lalu hasil sampe di hitung menggunakan *colony counter* (Hasanah et al., 2023).

HASIL DAN PEMBAHASAN

Ektraksi tanaman lidah buaya dipekatkan dengan menggunakan *rotary evaporator*. Ektraksi tanaman lidah buaya (*aloe vera L.*) berwarna putih, berbau khas dan memiliki konsitensi kental. Persentase uji rendemen didapatkan berat simplisia sebelum dan sesudah di ekstrak adalah 4,892 %. Kemudian rendemen diformulasi dan didapatkan hasil pemeriksaan lain yang sesuai dengan acuan.

Hasil dari uji homogenitas pada sediaan formulasi menunjukan semua sediaan formulasi homogen. Hal ini diperkuat dengan bukti tidak adanya butiran kasar ketika melakukan pengamatan secara visual.



Gambar 1. Hasil sediaan gel dengan empat formula, sediaan basis gel (F1), sediaan basis gel dengan sodium metabisulfit (F2), sediaan gel esktrak Aloe vera 5% (F3), dan sediaan gel ekstrak Aloe vera 5% dengan sodium metabisulfit (F4), keempatnya menunjukan hasil yang homogen di hari awal pembuatan dan belum terdapat cemaran mikroba.

Berdasarkan hasil pengamatan uji organoleptis pada formulasi sediaan gel ekstrak aloe vera pada hari 1 dinyatakan, F1, F2, F3 dan F4 tidak menunjukkan perubahan warna, bau dan tekstur hal ini dikarenakan sediaan gel yang baru dibentuk belum terkontaminasi oleh agen mikroba. sedangkan pengamatan ulang pada hari ke-14 mulai terjadi perubahan warna, bau dan tekstur pada sediaan F3, sedangkan pada sediaan F1, F2, dan F4 tidak mengalami perubahan. Hal ini dikarekan sediaan F3 hanya berisi aloe vera L. dan basis gel. Tidak adanya penambahan sodium metabisulfit terbukti mengurangi masa simpan dari formulasi ektraksi aloe vera L. basis gel. Hal ini sesuai dengan penelitian yang menyatakan sodium metabisulfit dapat berperan sebagai pengawet apabila di tambahkan pada formulasi obat, hal ini dikarenakan sodium metabisulfit memiliki fungsi mencegah perkembangan mikroba Wahlanto et al., (2020).

Gambar 2. Hasil sediaan gel dengan empat formula, sediaan basis gel (F1), sediaan basis gel dengan sodium metabisulfit (F2), sediaan gel esktrak Aloe vera 5% (F3), dan sediaan gel ekstrak Aloe vera 5% dengan sodium metabisulfit (F4), keempatnya menunjukan hasil di hari ke-14 terdapat cemaran mikroba pada sediaan F3.

Bedasarkan hasil pengamatan uji pH pada formulasi sediaan gel ekstrak aloe vera pada hari ke-1 dinyatakan F1, F2, F3, dan F4 memiliki pH masing masing 4,67 (F1), 4,9 (F2), 5 (F3) dan 5,1 (F4) sedangkan pengamatan ulang pada hari ke-14 menunjukan hasil yang sama. Hal ini menunjukan bahwa penambahan sodium metabisulfit tidak mempengaruhi tingkat pH pada sediaan. Hal ini sesuai dengan penelitian yang dilakukan oleh Khan dkk tahun 2017 Khan et al., (2017).

Gambar 3. pH diukur dengan menggunakan pHmeter. Uji viskositas merupakan uji untuk menentukan nilai kekentalan suatu zat.

Nilai viskositas yang tinggi menunjukan semakin kental suatu formulasi (). Bedasarkan hasil pengujian menunjukan nilai viskositas 2760 cps (F1), 2650 cps (F2), 2590 cps (F3), dan 2575 cps (F4). Hal ini menujukan bahwa penambahan sodium metabisulfit memiliki nilai yang lebih rendah. Hal ini menandakan bahwa pencampuran sodium metabisulfit mengecerkan formulasi. Pengenceran dari formulasi terjadi karena berat molekul dari sodium mataabisulfit dan aloe vera yang kecil sehingga ketika dicampur bersama pada sediaan formulasi menyebabkan penurunan konsitensi keseluruhan dari sediaan

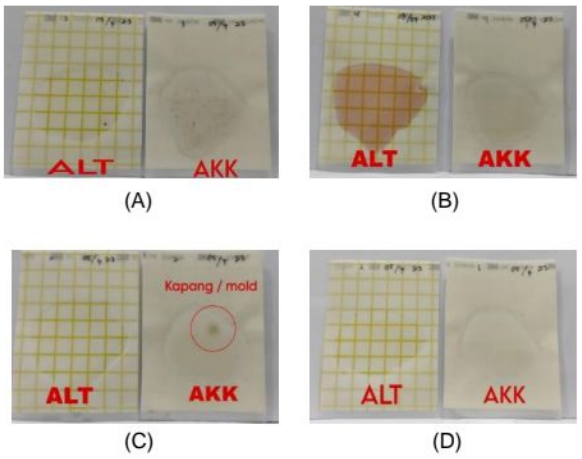
Gambar 4. Alat yang digunakan untuk memeriksa viscositas yaitu viscometer Brookfield

Bedasarkan hasil pengamatan pada uji di hari ke-14 nilai dari ALT dan AKK pada setiap formulasi sediaan ekstrak *aloe vera* menunjukkan angka seperti pada Tabel 2.

Tabel 2. nilai dari ALT dan AKK pada setiap formulasi sediaan ekstrak *aloe vera*

Uji	Formulasi 1	Formulasi 2	Formulasi 3	Formulasi 4
ALT	4,5 x 10^1 cfu/g	< 10^1 cfu/g	TBUD	< 10^1 cfu/g
AKK	1,21 x 10^3 cfu/g	< 10^1 cfu/g	< 10^1 cfu/g	1 x < 10^ cfu/g

Pada formulasi satu dan tiga memiliki angka ALT yang tinggi. Sedangkan pada formulasi dua dan empat memiliki angka ALT yang kecil. Hal ini terjadi akibat penambahan sodium metabisulfite pada formulasi dua dan empat yang menyebabkan pencegahan perkembangan anti mikroba pada sediaan. Hal ini sesuai dengan penelitian Kristantri et al., (2022), yang menunjukkan perkembangan agen mikroba lebih cepat terjadi pada sediaan tanpa sodium metabisulfit, hal ini dikarenakan fungsi sodium metabisulfit selain sebagai anti oksidan dapat juga berperan sebagai penghambat agen mikroba pada suatu sediaan Kristantri et al., (2022).



Gambar 5. Hasil Uji ALT dan AKK; Formula 1 (A); Formula 2 (B); Formula 3 (C) dan Formula 4 (D).

KESIMPULAN

Bedasarkan hasil penelitian ini disimpulkan bahwa sediaan yang mengandung sodium metabisulfite memiliki hasil evaluasi yang baik. Hal ini dikarenakan penambahan sodium metabisulfite pada formulasi dapat menambah masa simpan dari formulasi. Hal ini disebakan karena sodium metabisulfite dalam dunia farmasi berfungsi sebagai pengawet sediaan dengan cara menahan perkembangan dari agen mikroba pada sediaan.

KONTRIBUSI PENULIS

YWP, J, NAR berkontribusi pada desain, analisis data dan menulis draft manuskrip untuk submit

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Dana penelitian berasal dari dana mandiri peneliti

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