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Comparison of Extraction Methods, Antioxidant Activities, Total Phenol in Seeds and Seed Shells of Kabau (*Archidendron bubalinum* (Jack) I.C. Nielsen) From Lampung and South Sumatra

Hesti Riasari*, Sani Nurlaela Fitriansyah, Novi Irwan Fauzi

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2. Dewan Redaksi/Editorial Board
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4. Jurnal Published

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REVIEW ARTICLE(S)

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1-6

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ORIGINAL ARTICLE(S)

ANTI-DIABETIC ACTIVITY OF KABAU SEED POWDER SUSPENSION (ARCHIDENDRON BUBALINUM (JACK) I. C. NIELSEN) IN ALLOXAN-INDUCED DIABETIC RATS

HESTI RIASARI, SANI NURLAELA FITRIANSYAH, NOVI IRWAN FAUZI

7-12

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IN SILICO STUDY OF THE SYNERGISTIC INTERACTION OF 5-FLUOROURACIL AND CURCUMIN ANALOGUES AS INHIBITORS OF B-CELL LYMPHOMA 2 PROTEIN

LA ODE AMAN, ARFAN, AIYI ASNAWI

13-18

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<p>ANTI-ALOPECIA ACTIVITY OF MORINGA (MORINGA OLEIFERA LAMK.) SEED OIL AGAINST DIHYDROTESTOSTERONE-INDUCED RABBITS</p> <p>YULIUS BAKI KORASSA, NYI MEKAR SAPTARINI, RESMI MUSTARICHIE, RINI HENDRIANI, ANTONIUS R. B. OLA, YOAN NOVICADLITHA, LELY A V KAPITAN</p> <p>VIEW ABSTRACT PDF DOWNLOAD PDF</p> <p>10.22159/ijap.2023.v15s2.04</p>	19-24
<p>METHOTREXATE-INDUCED ORAL ERYTHEMA MULTIFORME</p> <p>ASTRID WIDHOWATY SANTOSO, AMIRA SHAFURIA, TENNY SETIANI DEWI</p> <p>VIEW ABSTRACT PDF DOWNLOAD PDF</p> <p>10.22159/ijap.2023.v15s2.05</p>	25-29
<p>MOTHERS KNOWLEDGE TOWARDS WOUND CARE IN THE GREATER BANDUNG AREA</p> <p>DEDE JIHAN OKTAVIANI, ADE ZUHROTUN, CECEP SAFA'ATUL BARKAH</p> <p>VIEW ABSTRACT PDF DOWNLOAD PDF</p> <p>10.22159/ijap.2023.v15s2.06</p>	30-36
<p>ANTIOXIDANT ACTIVITIES AND PHYTOCHEMICAL SCREENING OF ETHANOL EXTRACT FROM SURIAN LEAVES (TOONA SINENSIS)</p> <p>UCE LESTARI, MUHAIMIN MUHAIMIN, ANIS YOHANA CHAERUNISAA, WAWAN SUJARWO</p> <p>VIEW ABSTRACT PDF DOWNLOAD PDF</p> <p>10.22159/ijap.2023.v15s2.07</p>	37-43
<p>CYTOTOXIC ACTIVITY AND PHYTOCHEMICAL SCREENING OF ETANOL EXTRACT OF BAJAKAH TAMPALA (UNCARIA LANOSA VAR. FERREA (BLUME) RIDSDALE) STEM ON BREAST CANCER CELL LINES MCF-7</p> <p>MASHURI YUSUF, MUHAIMIN MUHAIMIN, RIEZKI AMALIA, YOPPI ISKANDAR</p> <p>VIEW ABSTRACT PDF DOWNLOAD PDF</p> <p>10.22159/ijap.2023.v15s2.08</p>	44-47
<p>ISOLATION OF CELLULOSE FROM SIWALAN FIBER (BORASSUS FLABELLIFER L.) USING RESPONSE SURFACE METHODOLOGY</p> <p>YUSUF IKROM NUR AZAMI, IDA MUSFIROH, MUCHTARIDI, RIMADANI PRATIWI, OKTA NAMA PUTRA</p> <p>VIEW ABSTRACT PDF DOWNLOAD PDF</p> <p>10.22159/ijap.2023.v15s2.09</p>	48-51
<p>EFFICACY OF CHLORINE DIOXIDE ON ORAL LESIONS IN ACUTE MYELOBLASTIC LEUKEMIA PATIENTS UNDERGOING CHEMOTHERAPY</p> <p>FAIZNUR RIDHO, NURI FITRIASARI, DEWI ZAKIAWATI</p> <p>VIEW ABSTRACT PDF DOWNLOAD PDF</p> <p>10.22159/ijap.2023.v15s2.10</p>	52-57



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□ 664

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□ 599

DERMATOLOGIC GELS SPREADABILITY MEASURING METHODS COMPARATIVE STUDY
□ 488

REGULATORY FRAMEWORK: VACCINE DEVELOPMENT IN US, INDIA AND EU
□ 444

ANTI-DIABETIC ACTIVITY OF KABAU SEED POWDER SUSPENSION (*ARCHIDENDRON BUBALINUM* (JACK) I. C. NIELSEN) IN ALLOXAN-INDUCED DIABETIC RATS

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ABSTRACT

Objective: The study was to optimize the method of test dosage form kabau seed simplicia suspension in alloxan-induced diabetic rat models.

Methods: Simplicia powder sizing was carried out using three sieve sizes through meshes of 80, 120, and 200. Making a suspension of kabau seed powder is carried out using two methods, with the addition of CMC, which has been developed first and the powder is added with CMC, which is then developed and then homogenized. The study consisted of six groups, dosage given of 250, 500, and 1000 mg/kg BW. Blood glucose levels were determined by the GOD-PAP, phytochemical screening and TLC tests were carried out on the test material. The data were tested statistically using One Way ANOVA.

Results: The powdered crude material with a particle size of 74 microns, obtained by sieving through a mesh size of 200, is utilized as the test material in the second method. An effective dose of 1000 mg/kg BW with a decrease in blood sugar levels by 132 ± 13 mg/dl. Phytochemical screening contained alkaloids, flavonoids, phenolics, monoterpenes and sesquiterpenes, steroids, quinones and saponins. TLC of the test material, mobile phase used in TLC was toluene: chloroform: ethyl acetate (4:5:1), obtained 6 spots which gives a specific spot after being sprayed with the chromogenic reagent.

Conclusion: powder with a particle size of 74 microns was used as the test material, using the second method. The effective dose as an antidiabetic is 1000 mg/kg BW with a reduction in blood sugar levels of 132.7 ± 13 mg/dl.

Keywords: Antidiabetic, GOD-PAP, Kabau, Suspension, Simplicia

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INTRODUCTION

Diabetes mellitus is one of the diseases that leads to an increased risk of death and a decrease in the quality of life of patients [1]. One of the traditional therapies widely used by the community in Indonesia is herbal therapy. Currently, more than 400 types of plants have been utilized as alternative treatments for diabetes, although only a few have been scientifically researched. One of the plants that has been traditionally used in several regions of Indonesia as a treatment for diabetes mellitus is *Archidendron bubalinum* [2].

Kabau (*Archidendron bubalinum* (Jack) I. C. Nielsen) is a close relative of jengkol (*Archidendron jiringa*) [3]. Kabau is a species endemic to Indonesia, especially on the island of Sumatra. This species has not been cultivated like its relative, namely jengkol (*A. jiringa*) [4]. The people of Sumatra in Indonesia often use kabau as fresh vegetables, therefore, this research looks at the anti-diabetic activity of simplicia powder from kabau seeds without any extraction method in order to obtain optimal anti-diabetic activity results according to people's habits. Histochemical results of kabau seed powder and incisions identified terpenoids, alkaloids, amino acids, phenolics and tannins [5], where these compounds can protect pancreatic beta cells that produce insulin [6, 7] and inhibition of glucose absorption in the intestine, increasing glucose transport in the blood, stimulating gluco-gen synthesis and inhibiting glucose synthesis by inhibiting the enzymes glucose 6-phosphatase, fructose 1,6-bisphosphatase, and increasing glucose 6-phosphate dehydrogenase [8] so that blood sugar levels become normal.

The aim and urgency of this research was to make medicinal preparations in the form of capsules or tea preparations that do not require extraction with expensive solvents, in addition to correlating with empirical use that has been used by local communities by consuming kabau seeds as fresh vegetables. The novelty of the research is using test materials that do not use extraction methods and optimizing the particle size of simplicia powder in order to optimize the form of the suspension made to be tested on test animals.

MATERIALS AND METHODS

Plant materials and preparation of kabau seed suspension

Kabau seeds (*Archidendron bubalinum* (Jack) Nielsen), Fabaceae were collected in January 2018 from Bumi Baru village, Blambangan Umpu District, Waykanan Regency, Lampung, Sumatra Island, Indonesia. The plant was determined at the Indonesian Institute of Sciences, Biological Research Center, LIPI Bogor, number: 408/IPH. I.01/If.07/II/2018 on February 13, 2018. The seeds were separated from the black seed coat, then washed with running water to remove dirt, cut into small pieces to speed up the drying process, dried at room temperature in an air oven then made into powder. The simplicia was mashed using a blender, then sieved using mesh 80 mesh, 120 mesh and 200 mesh, then a suspension preparation was made. Method I: Samples that have been mashed are made by weighing the test material according to the dosage used, then suspended with 0.5% CMC solution to the desired volume. Method II: In the second method, simplicia powder and 0.5% CMC were crushed together, then hot water was added, then crushed vigorously.

Animals

The experimental animals used in this study were Wistar strain male white rats with body weights of 150-250 grams obtained from PT. Biofarma, use and care for the experimental animals were approved by Ethic Committee of Universitas Padjadjaran (Approval no 114/UN6. KEP/EC/2019).

Phytochemical screening

All samples were analyzed using amyl alcohol reagent to detect flavonoid compounds [9], FeCl_3 10% for detecting phenolic compounds [10], gelatin reagent for tannin detection [11], Dragendorff and Mayer reagent for alkaloid detection [12, 13]. The quinone group can be identified using 5% KOH reagent [14]. Steroid and triterpenoid secondary metabolites can be identified using Lieberman-Buchard reagent, while volatile compounds in the monoterpene and sesquiterpenoid subgroups can be identified using vanillin sulfate

reagent (10% vanillin in H₂SO₄) [15-17]. Additionally, saponins, another type of secondary metabolite, were determined by observing continuous foaming after shaking the aqueous extracts for 10 min [18].

Qualitative analysis with thin-layer chromatography (TLC)

TLC was used to find out the secondary metabolite compounds in kabau seed simplicia. The mobile phase used is toluene: chloroform: ethyl acetate (4:5:1), where the mobile phase has been optimized first. Silica gel plat GF254 was used as the stationary phase, and FeCl₃ was used to detect phenolic compounds, ammonia vapor to detect flavonoids, and vanillin-sulfuric acid to detect saponins [19].

Antidiabetic testing in an alloxan-induced diabetic model

Antidiabetic activity testing employed the alloxan induction technique. The mouse test animal group was divided into six groups: Group I (normal control), Group II (negative control, received CMC-Na 0.5%), Group III (positive control, received glibenclamide 0.45 mg/kg), Group IV (dose 250 mg/kg BW), Group V (dose 500 mg/kg BW), VI (experimental dose 1000 mg/kg BW). Prior to administering the test substance/extract (excluding the control group), the group of animals underwent alloxan induction at 120 mg/kg for seven days. Mice used as diabetes models are mice that have fasting glucose levels >126 mg/dl and exhibit symptoms of increased urination and thirst. On day 15, mice were euthanized using CO₂. Subsequently, 3 ml of blood was collected from the heart [20].

Measurement of glucose levels by the enzymatic method of glucose oxidase-phenol aminophenazon (GOD-PAP)

Blood collected from the rat heart was spun in a centrifuge to separate the serum and plasma components. 10 µl of the serum was combined with 1000 µl of a reagent that liquefies glucose, and the mixture was left to incubate for 10 min at a temperature between 20-25°C. The absorbance of the resulting reaction was determined using a UV-Vis spectrophotometer, specifically at a wavelength of 510 nm. The collected data was subsequently analyzed using an analysis of variance method (ANOVA) [21].

Measurement of malondialdehyde (MDA) activity

From each mouse, 200 µl of blood plasma was collected and then 2000 µl of 20% TCA and 2000 µl of 0.067% TBA were added. The uniform mixture was warmed in a water bath for 10 min and, after cooling, spun at 3000 rpm for 10 min. The optical density of the resulting pigmented solution was determined using UV-Vis spectrophotometry at 532 nm [22].

Measurement of superoxyde dismutase (SOD) activity

The experimental material was created by introducing 2800 µl of sodium carbonate buffer into a 3 ml cuvette, along with 100 µl of either a sample or standard and 100 µl of an epinephrine solution. A control solution was also prepared, consisting of 2800 µl of carbonate buffer, 100 µl of ion-free water, and 100 µl of epinephrine. The absorption of the test material does not occur immediately upon the addition of epinephrine at a wavelength of 480 nm and a temperature of 30 °C. The measurements are taken at 1, 2, 3, and 4 min after the addition of epinephrine, and the change in absorption value (Δ absorption) should be 0.025/min. If the value exceeds this, the material is diluted with a buffer [23].

RESULTS AND DISCUSSION

Preparation of test material in the form of suspension

The preliminary tests are conducted in advance to determine the appropriate suspension preparations for testing materials. The initial technique settles more quickly than the alternate technique, as evidenced by the settling time of the suspension preparations obtained from each method. In the first method, particles of 80 mesh (177 microns), 120 mesh (125 microns), and 200 mesh (74 microns) settle in 38 seconds, while in the second method, each particle size settles in 1 min 22 sec. It is evident that the particle size of the simplicia impacts the settling speed; faster settling indicates poor distribution of the simplicia, raising concerns about uneven dosage during sampling.

Table 1: Results of speed of settling of suspension preparations

Method	Time					
	Method I			Method II		
	Sieve mesh size			Sieve mesh size		
	80	120	200	80	120	200
Speed of suspension preparation settling time	38 sec	38 sec	38 sec	1 min 7 sec	1 min 13 sec	1 min 22 sec
The overall settling time speed the suspension	1 min 15 sec	1 min 16 sec	1 min 21 sec	2 min 19 sec	2 min 22 sec	3 min 27 sec

Administration of suspension preparations to test animals was carried out by the second method, where simplicia powder and 0.5% CMC were crushed simultaneously, then hot water was added, then crushed vigorously. Simplicia powder for suspension preparation is good with sieve size no 200 mesh, because the larger the sieve size number, the finer the results of the simplicia powder size was 74 microns. Simplicia powder for suspension with a sieve size of 200 mesh which was developed first or directly, is well distributed compared to sieve sizes no. 80mesh and 120 mesh, so simplicia powder for administering suspension preparations to test animals is carried out with sieve size no. 200mesh using the second method where 0.5% CMC and simplicia powder were crushed simultaneously and then added hot water, then crushed vigorously, resulting in a better particle distribution in the CMC as seen from the longer settling time.

Phytochemical screening

Phytochemical screening revealed that it contained alkaloid compounds, flavonoids, phenols, tannins, terpenoids/steroids, mono/sesquiterpenes and quinones. The suspension and simplicia were tested for alkaloids, positive results were obtained, the color of the brown precipitate changed when dropped with Dragendorff. The previous sample was extracted by adding acid when it reacted to the reaction, forming precipitates and displacing ligands in the Dragendorff and Mayer reaction [24]. Flavonoids cause orange or

yellow color changes in the amyl alcohol layer. The addition of magnesium and hydrogen chloride powder causes the reduction of flavonoids to red [25]. Flavonoids are the largest naturally occurring phenolic compounds. Flavonoids can act as antioxidants as free radicals because their hydroxyl groups donate hydrogen to free radicals [26]. When testing the saponin compound, the results were positive; up to 1-1.5 cm high foam was noted. This foam is due to the presence of a hydrophilic group that binds to water, while a hydrophobic group binds to air. In addition, positive tannin test results are indicated by the presence of white deposits and appear cloudy in 1% gelatine drops that precipitate the gelatine protein [27]. Tannin compounds have astringent, antidiarrheal, antibacterial and antioxidant properties [28]. Positive phenol test results show a black-green change when it reacts with FeCl₃. The color change is due to the presence of hydroxyl groups in the compound. The terpenoid/steroid test gave a green color when reacted with Liebermann-Burchard, indicating positive steroid content. The results of the mono-and sesquiterpene tests show a purple color when the compounds react with vanillin sulfate. These chemical compounds act as a defense against nearby attacks, antibiotics and antioxidants [29].

Qualitative analysis with TLC on simplicia

The test procedure with TLC was carried out to further confirm the results obtained from the phytochemical screening. TLC was carried

out specifically to detect compound classes (phenolics, flavonoids, and saponins). This is because this group is thought to have antidiabetic activity. The mobile phase used in TLC was toluene: chloroform: ethyl acetate (4:5:1). Qualitative analysis of compounds using TLC obtained 6 spots with Rf 0.23, 0.30, 0.66, 0.68, 0.72, 0.76 which gives a specific spot after being sprayed with chromogenic reagent FeCl₃, ammonia vapor and vanillin sulfate. The results of qualitative analysis after spraying vanillin sulfate on the TLC plate showed a color change and was seen in visible light at RF 0.23 to a greenish-black color, suspected to be saponin glycoside [30]; Rf 0.68 turns green, presumably terpenoids [30]; while at Rf 0.72 it turns purple, presumably a steroid [31]. The TLC plate that had been

sprayed with FeCl₃ gave spot changes at Rf 0.30, which were seen in visible light suspected to be phenolic compounds [32]. While on the TLC plate that was steamed with ammonia and the spots were seen in the UV 366 lamp at Rf of 0.66. to fluoresce light blue and at Rf of 0.76 fluoresce bright blue, presumably a flavonoid compound [33].

Antidiabetic effect test results

Observation of clinical manifestations

Clinical manifestations of alloxan-induced diabetic rats can be seen from the profiles of polyuria and polydipsia. The following results of clinical manifestations of diabetic rats can be seen in fig. 1 and 2

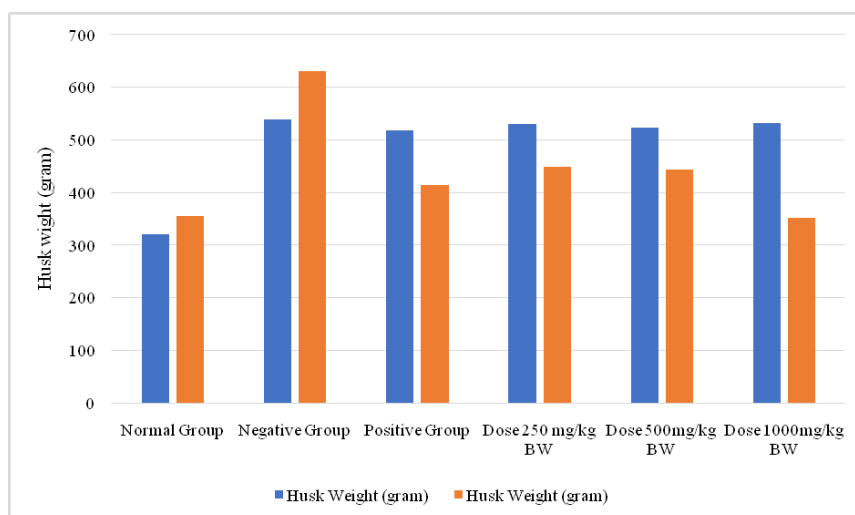


Fig. 1: Chart of polyuria clinical manifestation (n=6)

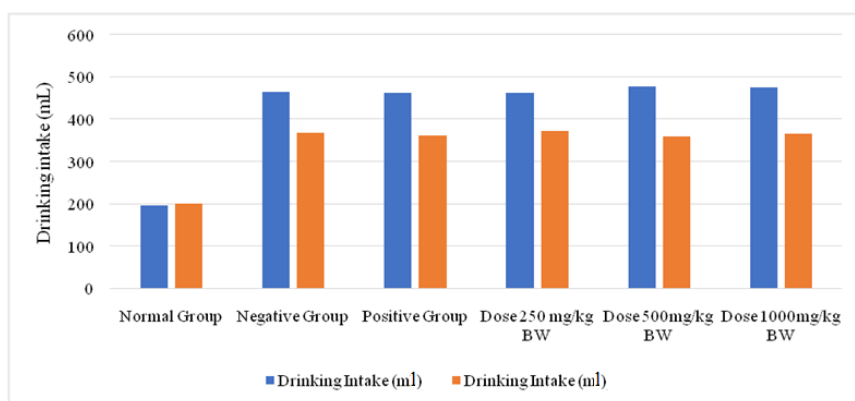


Fig. 2: Chart of polydipsia clinical manifestation (n=6)

Test animals after alloxan induction gave clinical symptoms such as polyuria and polydipsia, which means that all treatment groups except the normal group experienced clinical manifestations of diabetes. This is indicated by the higher number of husk weights and amount of drinking intake in the control and treatment groups compared to the normal group. Clinical manifestations can be confirmed that the control and treatment groups experienced diabetes before treatment and decreased to normal after being given the test material [34]. The test animals experienced a decrease in clinical symptoms to normal after administering the test material at various doses. Husk weight and water consumption returned to normal levels when measured after seven days of treatment (observation day 14).

Based on test findings, animals with elevated sugar levels experienced a decline in urine production and a decrease in clinical data for

excessive thirst after being administered the experimental substance. The obtained results can be considered favorable as the reduction in blood sugar levels closely aligns with the outcomes of the control group. Following a 14 d period of administering the extract to the test animals, blood sugar levels were assessed using the GOD-PAP technique. The GOD-PAP technique is an enzymatic method that employs a GOD-PAP reagent. The reaction involves the oxidation of glucose by the enzyme glucose oxidase (GOD) in the presence of O₂, resulting in the production of gluconic acid and H₂O₂. In the presence of the peroxidase enzyme (POD), H₂O₂ releases O₂, which then oxidizes the chromogen acceptor (4-aminoantipyrin) into chinonimin (a red-colored compound). The intensity of the color produced is directly proportional to the amount of glucose present. In this test, blood is drawn from the heart and subsequently centrifuged to separate plasma and serum, as the latter will be used for measuring glucose levels. Serum is preferred as it does not contain fibrinogen (blood

clots), whereas plasma may contain fibrinogen, which could potentially interfere with the measurement results. The results of the

measurements using GOD-PAP can be observed in the decreased administration of suspension, as shown in table 2.

Table 2: Results of measuring glucose levels using the GOD-PAP method

Test group	Blood sugar levels 7 d after alloxan induction, (mg/dl)	Blood sugar levels after 14 d treatment with test substances, (mg/dl)
Normal Group	121.62±6.04	119.11±7.21 ^b
Negative Group	376.31±18.21	527.42±9.06 ^{ac}
Positive Group	351.24±13.05	122.07±3.01 ^b
Dose 250 mg/kg BW	355.72±4.08	238.42±19.23 ^{abc}
Dose 500 mg/kg BW	368.33±10.12	159.06±18.41 ^{abc}
Dose 1000 mg/kg BW	382.52±15.07	132.72±13.05 ^{bc}

^a = significantly different from the normal group (P<0.05), ^b = significantly different from the negative group (P<0.05), ^c = significantly different from the positive group (P<0.05), Data was showed in mean and SD (n=6)

Based on the table 2, it is known that the highest average blood sugar level was the negative control group, which was 527 mg/dl, while the lowest average blood sugar level was the rat group at a dose of 1000 mg/kg BW, which was 132.7 mg/dl. It can be seen that the dose of simplicia powder of 1000 mg/kg BW can reduce fasting blood glucose more significantly compared to the dose of simplicia powder of 250 and 500 mg/kg BW. The results of the fasting blood glucose levels of the rats were analyzed using the Analysis of Variance (ANOVA) statistical test. Based on the results of the ANOVA analysis, the simplicia powder of the kabau seeds showed a significance value of 0.000 (P<0.05), which meant that there was a significant difference in each treatment. smallest among treatment groups. The LSD test showed that the simplex powder doses of 250 mg/kg BW, 500 mg/kg BW and 1000 mg/kg BW to the negative control group had a significant difference (P<0.05). It was proven in this study that a dose of 1000 mg/kg BW had better antidiabetic activity compared to doses of 250 mg/kg BW and 500 mg/kg BW.

The decrease in fasting blood glucose levels is thought to be caused by the mechanism of the active substances phenolics, flavonoids and saponins. In general, flavonoids are thought to be able to regenerate pancreatic beta cell damage due to alloxan induction [35] and can reduce blood glucose levels by stimulating pancreatic beta cells to produce insulin [36]. Flavonoids and phenolics can also act as antioxidants by binding to free radicals so as to reduce oxidative stress. If oxidative stress is reduced, it can reduce resistance to insulin action and can prevent the development of dysfunction and damage to pancreatic beta cells [37]. In addition, saponins function as antidiabetics as evidenced by [38]. After histological examination, it was found that saponins are able to regenerate the pancreas, which causes an increase in the number of pancreatic beta cells and islets of Langerhans so that insulin secretion will increase. Increased insulin secretion will help reduce blood glucose levels [39].

Effect of exposure to alloxan and kabau seed simplicia powder treatment on MDA levels

MDA is the end product of lipid oxidation [40] which forms when radical compounds attack lipid membranes rich in polyunsaturated fatty acids (PUFA). MDA is commonly used as an indicator of the presence of free radicals and oxidative damage [41]. Elevated levels of MDA are influenced by lipid oxidation, which indirectly indicates high levels of free radicals [42]. The measurement of free radical levels in this study was conducted by assessing serum MDA levels in Wistar rats. MDA levels were assessed using the TBA (thiobarbituric acid) assay, which serves as a marker for lipid oxidation. Prior to MDA measurement, the addition of TCA (trichloroacetate) is used to prevent protein precipitation in the sample after centrifugation at 3000 rpm. Protein precipitation is necessary as the protein content in the sample may interfere with MDA measurement. The mechanism of TCA as a precipitating agent involves the negative ions from TCA binding with proteins that are present as cations (at a solution pH in acidic conditions close to the isoelectric pH of the protein), resulting in the formation of protein salts. Some of these salts are insoluble, allowing for the separation of proteins from the sample [43].

The addition of a mixture of TCA and TBA compounds accompanied by incubation for 10 min can form a pink MDA-TBA complex

compound. The aim of this incubation is for the TBA to immediately react with the supernatant so that the color of the complex compound is more easily formed.

Table 3: Results average MDA levels

Test group	Average MDA levels (µl/ml)
Normal Group	0.048±0.43
Negative Group	0.154±0.41
Positive Group	0.046±0.38
Dose 250 mg/kg BW	0.093±0.33
Dose 500 mg/kg BW	0.080±0.51
Dose 1000 mg/kg BW	0.072±0.47

Data was showed in mean and SD (n=6)

Based on the MDA measurement results, table 3 showed that the MDA level in the negative group induced by alloxan 120 mg/kg BW had a higher MDA level, that is 0.154 µl/ml, while the average MDA level in the group that had been given the test substance could reduce MDA levels. The significant decrease in MDA levels was in the group of test animals given a dose of 1000 mg/kg BW, amounting to 0.072 µl/ml. This shows that giving kabau seed simplicia was able to reduce the MDA levels of test animals induced by alloxan. The data showed that the test groups had lower MDA levels compared to the negative group that was not given suspense. Indicating that kabau seed suspense decreased free radicals which correlated with lower blood glucose levels, among the suspense tested, dose 1000 mg/kg BW produced the lowest MDA level, indicating its potential. as an alternative for antidiabetic treatment and as an antioxidant [44].

Measurement of SOD content

The results of SOD measurements are presented in table 4. The data shows that the negative control showed a decrease in SOD activity compared to the positive control (ascorbic acid). The kabau seed suspension test substance at a dose of 1000 mg/kg BW showed high SOD measurement results, although it was still under positive control.

Table 4: Average results % inhibited SOD

Test group	Average
Normal Control	38.885±9 ^{bd}
Negative Control	5.461±2 ^{acd}
Positive Control (Glibenclamide)	47.232±3 ^{bc}
Positive Control (ascorbic acid)	92.372±1 ^{abc}
Dose 250 mg/kg BW	42.586±1 ^{bd}
Dose 500 mg/kg BW	64.812±11 ^{bd}
Dose 1000 mg/kg BW	73.463±5 ^{abcd}

Difference in significance; P<0.05, a: significantly different from the normal group, b: significantly different to the negative group, c: significantly different against the glibenclamide positive group, d: significantly different for the Vitamin C positive group, Data was showed in mean and SD (n=6)

SOD is a type of antioxidant enzyme found in the body. The activity of SOD is influenced by the level of oxidative activity. The presence of high levels of SOD is indicated by the low levels of lipid oxidation products [45]. Reactive Oxygen Species (ROS) in the body result in an imbalance between the amount of free radicals and antioxidants. Free radicals can interact with membrane lipids, nucleic acids, proteins, and enzymes, leading to cell damage commonly known as oxidative stress [46, 47]. Hyperglycemia leads to an increase in ROS production, which in turn causes dysfunction of pancreatic beta cells. Studies have shown that beta cell dysfunction is associated with decreased levels of antioxidant enzymes such as SOD, glutathione peroxidase (GPx), and Catalase (CAT), making them more susceptible to oxidative stress [48, 49].

CONCLUSION

The powdered crude material with a particle size of 74 microns, obtained by sieving through a mesh size of 200, is utilized as the test material in the second method. An effective dose as an antidiabetic is a dose of 1000 mg/kg BW with a decrease in blood sugar levels by 132±13. mg/dl The results of the phytochemical screening test showed that the simplicia powder of kabau seeds contained alkaloids, flavonoids, phenolics, monoterpenes and sesquiterpenes, steroids, quinones and saponins. In the TLC of the test material. The mobile phase used in TLC was toluene: chloroform: ethyl acetate (4:5:1). TLC obtained 6 spots with RF of 0.2, 0.30, 0.66, 0.68, 0.72, and 0.76 which gives a specific spot after being sprayed with chromogenic reagent FeCl₃, ammonia vapor and vanillin sulphate.

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CONFLICT OF INTERESTS

Declared none

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