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Anticoagulant Activity Test of Ethanol Extract *Coleus Scutellarioides* (L.) Benth

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ABSTRACT

Coleus scutellarioides (L.) Benth has been used as a traditional medicine as an antibacterial, anthelmintic, antidiabetic, and appetite enhancer and can treat various types of diseases such as coughs, hemorrhoids, boils, fever, diarrhea, and improves the menstrual cycle. *C. scutellarioides* plants grow in areas that have moist soil, such as rice fields, wild plants, or medicinal plants. This plant contains chemical compounds, including essential oils, flavonoids, phytosterols, alkaloids, and tannins. This study aims to determine the anticoagulant activity of the ethanol extract of *C. scutellarioides*. The method used in this research starts with sampling *C. scutellarioides*, making simplicia, extraction by maceration with ethanol solvent, and testing anticoagulant activity with two methods, namely the modified Lee-White method and the blood smear method. The modified Lee-White method was visually observed and tested on each type of human blood group, namely blood groups A, B, AB, and O, while the blood smear method was observed microscopically. The results of this research concluded that using the modified Lee-White method, *C. scutellarioides* extract with an extract concentration of 40 ppm had activity as an anticoagulant against various types of blood groups. In the directional smear method, preparations from blood samples were given. *C. scutellarioides* microscopically has anticoagulant activity because the blood cells were not related to each other, were still intact, and were separated from one another.

KEYWORDS: *Coleus s cutellarioides* (L.) Benth, Anticoagulants, Modified *Lee-White method*, Blood Smear

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INTRODUCTION

Indonesia is rich in various types of plants with medicinal properties [1], [2]. One of them is *C. scutellarioides*. This plant has efficacy for relieving pain, as an anti-inflammatory [3], antioxidant [4], antimalarial [5], TB [6], and antibacterial (*Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, and Salmonella typhimurium*), and can accelerate wound healing [7], [8].

Wounds were damage to the continuity of skin, mucosa, membranes, bones, or other body organs that can occur when the skin is exposed to temperature or pH, chemicals, friction, pressure trauma, and radiation [9]. Healing wounds were associated with cell regeneration until the function of the body's organs is restored, as indicated by signs and response sequences where the cells are together to interact, perform tasks, and function normally. Hemostasis or blood clotting disorders can cause complications from wound healing, necessitating the use of anticoagulants [10].

Anticoagulants prevent blood clotting by inhibiting several clotting factors' formation or function. Therefore, we need anticoagulants to stop the formation and spread of thrombus and embolism, as well as to prevent blood clots. In a thrombus that has already formed, anticoagulants only prevent it from enlarging and reduce the likelihood of embolism, but they do not reduce the thrombus [11], [12].

S. cutellarioides has various colors and brownish-red or blackish leaves with serrated edges, which can be used as medicine [13]. *C. scutellarioides* contains essential oils, phenols, tannins, fats, and phytosterols. Research that has been carried out states that *the C. scutellarioides* can be used as wound medicine. This was tested on rabbits by applying rub *C. scutellarioides* to the wound and the results of the phytochemicals containing it. *C. scutellarioides* extract contains essential oils, flavonoids, tannins, and 1-Octadecene.alpha.-Octadecene, Hexanedioic acid, dioctyl ester dioctyl adipate, Phenol, 3,5-bis (1,1-dimethyl ethyl)-3,5-Di-tert-butylphenol, and 1,4-diaza-2,5-dioxobicyclo[4.3.0] nonane [5], [8], [14], [15]. This chemical content can speed up the healing of wounds. Test of the anthelmintic activity of plant extract syrup against worms *in vitro*. The research results showed that *C. atropurpureus* extract syrup was proven to provide anthelmintic activity against *P. cervi*, which experienced lysis after 24 hours of observation. The dose used empirically has anthelmintic power with 100% worm death; at the 6th hour after treatment, the worm's body begins to stop not moving [15].

So this research aims to test the anticoagulant activity of ethanol extract *C. scutellarioides* using the Lee-White method and blood smear. We tested this research on samples of human blood types, specifically blood types A, B, AB, and O. To determine the blood clotting period, it was observed visually using the modified Lee-White method.

RESEARCH METHODS

We collected samples for *C. scutellarioides* in West Koya Village, Muara Tami District, Jayapura Regency, Papua Province.

Preparation simplicity

We picked, washed, drained, and air-dried the *C. scutellarioides* samples for one day. Next, we dried the samples in an oven at 40^{o}C for 1-2 days. Following the drying process, we ground the dried *C. scutellarioides* samples into a powder [16].

Extraction

C.scutellarioides simplicia powder was weighed at 200 grams and then soaked in 2 liters of 96% ethanol. The samples were soaked for 3×24 hours and stirred occasionally once a day. We filter the soaking results and then evaporate them using a rotary vacuum to obtain a thick extract of *C. scutellarioides* [17].

Preliminary testing of anticoagulant

We first conducted preliminary testing on *C. scutellarioides* leaf ethanol extract to determine the minimum concentration needed in 1 mL of blood. In this preliminary test, a test solution was made with a series of concentrations, namely 10 ppm, 20 ppm, 30 ppm, 35 ppm, 40 ppm, 45 ppm, 50 ppm, 55 ppm, 60 ppm, and 65 ppm, and then the blood clotting time was tested using the modified Lee-White method. The preliminary test results yielded an extract concentration of 40 ppm.

Anticoagulant activity

1. The Lee-White method

There were 5 clean vacuum tubes, and they were labeled from number 1 to number 5. 1 mL of blood is placed in vacuum tube number 1, and then *a stopwatch* is run to see the clotting period for control blood or without any treatment.

Using a micropipette, we put 1 mL of *C. scutellarioides* ethanol extract with a concentration of 40 ppm into vacuum tube number 2 and first evaporated it. First, use *a hairdryer* until dry. The next step involves placing 1 mL of blood into a vacuum tube and vortexing it. Simultaneously with the mixing process, a stopwatch measures the duration of the freezing period. The same treatment was also carried out for vacuum tubes 3 (blood + EDTA), 4, (blood + *C. scutellarioides* ethanol extract + EDTA), and 5 (blood + ethanol), with variations for each blood group (blood groups A, B, AB, and O) to be tested. If freezing has not occurred, then the vacuum tube is placed back, and every 30 seconds the tube is tilted to see if freezing has occurred. We continue this treatment until it reaches 120 minutes [18].

2. Blood smear method

Next, we used the blood smear method to see the effect of blood clotting microscopically. The May Grunwald-Giemsa mixed method used this method to see the condition of blood cells microscopically [19]–[21]. Samples used in This test select only one blood group that has the best anticoagulant activity. First, prepare five glass objects that were clean and without grease, and each is labeled from 1 to 5.

We took as much as 20 µl of blood from test tubes number 1 to number 5. The blood is spotted on glass objects from 1 to 5 in sequence, and a blood smear preparation shaped like a tongue is made. Next, we observed the preparations under a light microscope with a 400x magnification. The preparation was fixed in an ethanol solution until it covered the surface for 15 minutes and aired until dry. The preparation is then soaked in Giemsa solution for 30 minutes, rinsed with water, and air-dried until dry. We observe the results under a light microscope and document them with a camera.

RESULTS AND DISCUSSION

From the *C. scutellarioides* extract, *we* obtained an extract weight (Figure 1) of 17.90 grams, so the percent yield was 8.95%. The purpose of calculating the yield obtained is to determine the number of compounds attracted by the solvent so that, from determining the yield, we can know the amount of extract in simplicia with a certain weight. The higher the resulting yield value, the greater the value of the extract produced, but the higher the resulting yield value, the lower the quality obtained.

Figure 1. Leaves of *C. scutellarioides* (Left) and Simplicia powder (Right)

Preliminary Test Results

Preliminary tests were carried out to determine the minimum concentration range of *C. scutellarioides* extract to be used in 1 mL of a vacuum tube for 120 minutes. We chose 120 minutes as the time limit during which all blood clotting factors will not form, preventing the blood from clotting or coagulating. Usually, the time required for the blood not to clot and the action of anticoagulation is around 4-6 hours [22], [23].

Preliminary test results show that at a concentration of 10–35 ppm (Table 1), a coagulant effect occurs where the blood appears to thicken, but at a concentration of 40–65 ppm, an anticoagulant effect occurs. So it is concluded that the minimum concentration used is 40 ppm because, at this concentration, the blood looks liquid and does not clot.

Table 1. Preliminary test of anticoagulant activity

Concentration (ppm)	Time (minute)	Results
10	22	Coagulant
20	43	Coagulant
30	76	Coagulant
35	86	Coagulant
40	> 120 minutes	Anticoagulants
45	> 120 minutes	Anticoagulants
50	> 120 minutes	Anticoagulants
55	> 120 minutes	Anticoagulants
60	> 120 minutes	Anticoagulants
65	> 120 minutes	Anticoagulants

Anticoagulant Test Results Modified Lee-White method

An anticoagulant is a substance used to prevent blood clotting. The mechanism of action of anticoagulants is by inhibiting the function of one or several blood clotting factors, namely by binding calcium or by inhibiting the formation of thrombin, which is needed to convert fibrinogen into fibrin in the clotting process [12]. Examples of drugs commonly used as anticoagulants were dicomoral, warfarin, and heparin. We use these drugs to inhibit fibrin formation as a preventive measure to reduce the incidence of embolism, especially in veins.

In in vitro anticoagulant testing, only ethylene diamine tetraacetic acid (EDTA) is recommended as an anticoagulant because EDTA works by binding calcium so that the clotting process cannot occur[11]. EDTA has the following characteristics: it does not damage blood cells, does not cause changes in blood cell morphology, and is very suitable for hematological examination. The EDTA needed to bind calcium is 1 mg/mL of blood [12].

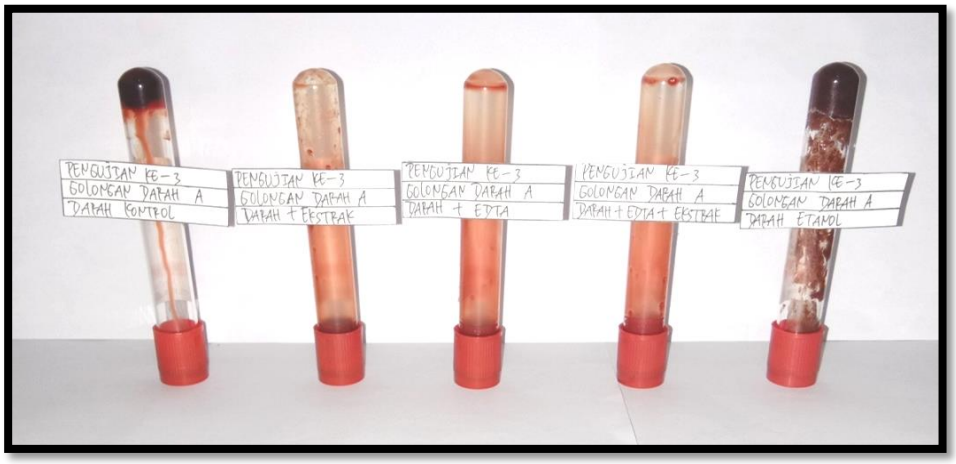

Figure 2. Lee-White Modification Method testing for blood type A

Testing using the modified Lee-White method aims to determine the blood clotting period, which is observed visually. Normal values for the modified Lee-White method were 9–15 minutes. Blood groups A, B, AB, and O have different control blood clotting periods, with an average ranging from 4 to 7 minutes. This shows that the control blood samples in each blood group clot within the normal blood clotting period (Figure 2).

Table 2. Average results of observations of anticoagulant activity using the modified Lee-White method

Tube	Treatment	Time (minute)			
		A	B	AB	O
1	Blood+control	5	4	7	4
2	Blood+ extract	>120	>120	>120	>120
3	Blood+ EDTA	>120	>120	>120	>120
4	Blood+ extract+ EDTA	>120	>120	>120	>120
5	Blood+etanol	29	28	27	27

In vacuum tube number 1 (control blood), the blood clots in 4–7 minutes. This time shows that the four blood group samples were still within the normal blood clotting period (3–18 minutes)). Blood clotting occurs when the plasma protein prothrombin changes to thrombin. Thrombin is an enzyme that catalyzes fibrinogen, which is a protein that dissolves into insoluble fibrin. The fibrin polymerizes into a mesh of long fibrin threads running in all directions within a few seconds. This mesh gathers blood elements in the form of clots, leading to the formation of a clot [24].

In vacuum tube number 2 (blood + extract 40 ppm), all blood types do not experience clotting. This indicates that there is anticoagulant activity occurring in that blood type. In vacuum tube number 3 (blood + EDTA), all blood types clot above 120 minutes. In vacuum tube number 4 (blood + EDTA + extract), each blood group does not show any damaging or detrimental effects; blood in vacuum tubes still shows anticoagulant activity. In vacuum tube number 5 (blood + ethanol), blood clotting occurred in the 27 to 30 minute. Apart from that, the blood in the vacuum tube deteriorated and turned black. This shows that ethanol is toxic and damages blood cells.

Test results Anticoagulant Method Blood Smear

The blood smear method to microscopically examine the condition of blood cells. In this test, blood group A samples were used because, based on the results obtained, blood group A showed the best anticoagulant activity [19]. In control of blood smear preparations, which can see blood cells that were not separated, related to each other, or damaged (Figure 3). In this picture, you can see the blood cells were still intact because they have been frozen, whereas the platelets in a blood smear preparation that experience clotting appear solid and in groups [18]. The direction is said to be normal if the red blood cell particles were round, stand-alone, and of the same size as each other, while frozen blood cells stick to each other.

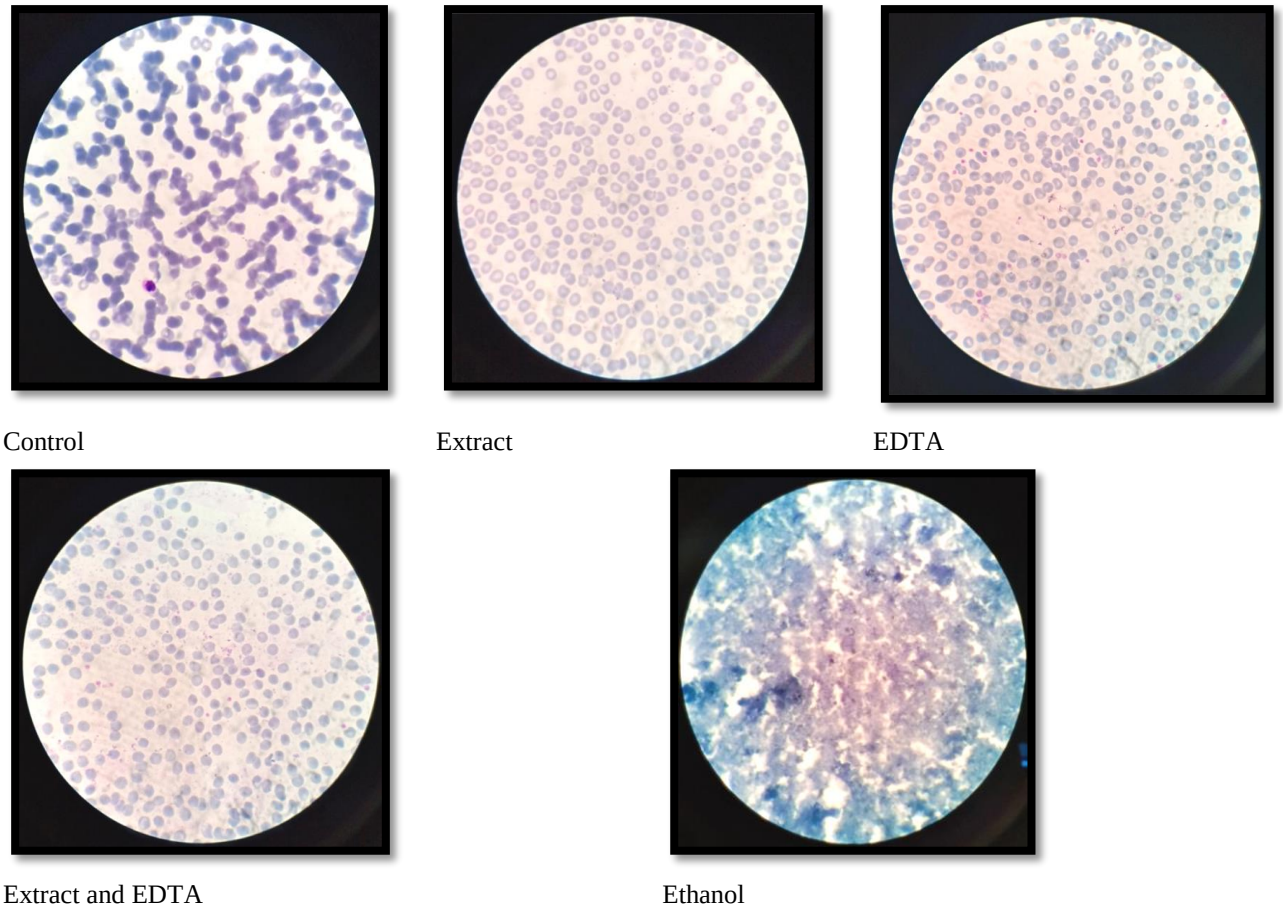

Figure 3. Result of blood smear with control, extract, EDTA, and ethanol.

The blood cells in tube 2's blood + extract smear were distinct, unrelated to one another, and perfectly round. This shows that C. scutellarioides extract has anticoagulant activity and does not damage blood cells. Blood cells that do not clot were generally round like a coin, yellowish in color, and do not have a nucleus. In this smear, the platelets appear round and not clustered, have the same size as each other, and have an empty nucleus [25].

In preparations from vacuum tube number 3 (blood + EDTA), no blood clotting occurred. This was because the presence of EDTA did not damage blood cells and did not cause morphological changes in blood cells. On observation, blood cells were seen that were not related to each other, and cells separate blood from each other. In preparations from vacuum tube number 4 (blood + EDTA + extract), it shows blood cells were separate, not related to each other, and have a complete round shape. No clumping occurs because blood is caused by this group of blood. In preparations from vacuum tube number 5 (blood + ethanol), it was found that the blood cells had been damaged and the erythrocyte cell walls had broken. This is because ethanol is toxic to the blood where it penetrates the membrane blood cells so that the membrane can no longer withstand external pressure, which causes the blood cells to burst or lyse [12].

CONCLUSION

Based on the results of this research conducted in vitro on various types of blood groups, it can be concluded that the modified Lee-White method produces *Coleus scutellarioides (L.) extract*. Benth extract with a concentration of 40 ppm had activity as an anticoagulant against various types of blood groups. In the directional smear method, preparations from blood samples were given. Microscopically, *C. scutellarioides* exhibits anticoagulant activity due to the unrelated, intact, and separated blood cells.

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