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Anticoagulant Activity of Ethanol Extract Arcangelisia Flava (L.) Merr

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ABSTRACT

Many types of plants can be used as medicinal plants, one of which is the Arcangelisia flava plant (L.) Merr **(**A. flava). According to the people of Maribu-Sentani-Papua, A. flava is used as medicine for antimalarial, jaundice, and diabetes. This study aims to determine the anticoagulant activity of A. flava ethanol extract using the Lee-White method. This research uses human blood with blood groups A, B, O, and AB who do not have blood coagulation disorders. Tests were carried out each with 5 treatments with two replications. The first treatment was carried out on blood as a control, the second treatment was on blood treated with ethanol, the third treatment was on blood treated with EDTA, the fourth treatment on blood given EDTA and A. flava extract, and the fifth treatment on blood given A. flava test extract. The results of the study showed that A. flava extract using a concentration of 150 ppm did not have an anticoagulant effect on human blood groups A, B, O, and AB.

KEYWORDS: Human blood, Maribu, Kayu kuning, Lee-White method.

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INTRODUCTION

One of the Indonesian medicinal plants has been using as traditional medicine which is well known as katola (1,2) (3); tali kuning (4); akar kuning (5,6); kayu kuning (7–9) [Arcangelisia flava plant (L.) Merr]. This plant is used as an antimalarial, and antidiabetic, reduces fever, treats canker sores, dysentery, hepatitis, water fleas, and as a stomachic and others. The way to use this traditional medicine is by boiling the stems plant until the color turns yellow and drinking it (1,4,9). There were many studies continuously that prove this plant has many advantages. For example, A. flava has good antimicrobial capabilities (10), antifungal (9), and antibacterial (11)(3), used antibacterial for tooth (12), anti-cancer (13), and diarrhea. Other studies proved the antioxidant activity and anticytotoxic activity (14) (15), anti-inflammatory (16), anti-depressant(9,17), aphrodisiac (18), etc. In advanced research, tried to make some formulation tea and beverage products.

The secondary metabolite that is found in A. flava contains saponin, flavonoid, polyphenolic substance, glycoside, and alkaloid. Isolate from this plant comes from the secondary metabolite, ie berberine, jatrorrhizin, palmatine, kolumbamin (14), and pacybasin (19). Berberine, a group of alkaloids, was tested as effective for antioxidants and toxicity, and effective for anti-protozoa like plasmodium (20,21) as antimalarial. In medical practice most alkaloids have their value, Due to their prominent pharmacological properties and physiological activities, it is widely used in the field of medicine. The benefits of alkaloids in the health sector include stimulating the nervous system, raising or lowering blood pressure, and fighting microbial infections. Also, terpenoids in A. flava have been tested as antifungal and antimalarial (13,22). Flavonoids of A. flava have been tested for antibacterial, in vitro antioxidant in various methods. Flavonoids have various functions in medicine, including functioning as an antioxidant, antimicrobial, anticoagulant, antihypertensive, antiviral, anti-inflammatory, and anti-mouth ulcer. The role of flavonoids is to improve blood circulation throughout the body prevent blockages in blood vessels, anti-inflammatory. (anti-inflammatory), anti-oxidant, and helps reduce pain (15,16,23,24).

This plant is positive for containing flavonoids and phenols (25). Flavonoids and phenol (2) have functions as anticoagulants. However, anticoagulant activities have never been attempted for this plant. Anticoagulants are substances used to prevent blood clots which are generally used in clinics

and laboratories. Anticoagulants are used to prevent blood clotting by inhibiting the function of several blood clotting factors. Anticoagulants are needed to prevent the formation and spread of thrombus and embolism and to prevent in vitro blood clots during laboratory tests or transfusions (26,27).

In vitro, anticoagulant compounds are used to prevent or reduce blood clots, such as EDTA (Ethylene Diamine Tetraacetic acid), sodium citrate, and heparin (26). Apart from being used in hemorrhagic examinations, EDTA anticoagulant is also used in examining hemoglobin levels, blood cell counts, reticulocytes, Westergren's ESR, blood typing, and blood smear preparations. So this research aims to find out whether A. flava can be used as an anticoagulant or coagulant which was tested on human blood samples, namely using A, B, O, and AB blood using the Lee-White method.

RESEARCH METHODS

Materials and tools

3.1.1 Material

The material studied was A. flava obtained from Maribu Sentani Papua and blood samples were obtained from several volunteers who had blood types A, B, O, and AB. The chemicals used are ethanol, distilled water, Giemsa solution, and EDTA.

3.1.2 Tool

The tools used are extraction equipment, titration equipment, measuring cups, test tubes, stir rods, Erlemeyer, beakers, funnels, micropipettes, filter paper, test tube racks, 5ml/cc disposable syringes, sterile 22 G needles, glass objects, paper label, Rotary Vaccum Evaporator, stopwatch, Vortex, and Microscope.

Method

Extraction

The roots of A. flava are peeled, pounded, crushed into fibers, blended, and dried in hot heat then after being half dry they are put back in the oven at a temperature of 50ºC. After drying, the root samples were ground again into powder and sieved. Sample extraction was carried out using the maceration method. The root simplicia of A. flava was weighed as much as 300 grams and then soaked in 900 mL of ethanol. Samples that have been soaked in ethanol for 3 days and stirred occasionally during the 3 days of soaking are done 3 times. The results of the soaking are collected then put together and filtered using filter paper. The resulting supernatant was evaporated using a rotary vacuum evaporator to evaporate the ethanol and A.flava root extract was obtained .

Preparation of blood test samples

The blood samples in this study were whole blood taken using a 22 G syringe at 5 mL/cc taken from the number of blood samples from volunteers with blood type A, blood samples from volunteers with blood type B, blood samples from volunteers O, and blood samples from volunteers AB aged 22-26 years, physically healthy and with no history of prolonged bleeding disease was taken from volunteers who did not have hemostasis disorders.

Sample preparation for testing

Before testing the ethanol extract of the A. flava plant on blood samples, a preliminary test was carried out. This aims to determine the minimum concentration range of the extract that will be used in 1 mL of blood. The minimum concentration of the extract obtained was 150ppm.

Testing of A. flava extract

The method used in this test is the modified Lee-White method (Gandasoebrata 1992). This method is used to determine the blood clotting period which is observed visually. The normal blood clotting period in humans generally occurs between 3-18 minutes. Based on the normal blood clotting period (Bithell 1993).

The working procedure of the Lee-White method is carried out by preparing 5 test tubes with a diameter of 8 mm, which are clean and labeled from number 1 to number 5. 1 mL of blood is put into test tube number 1, the stopwatch is run to see the blood clotting period. In test tube number 2, 1 mL of blood and 150 µL of A. flava dry extract were added using a micropipette and mixed using a vortex. At the same time as mixing, a stopwatch was run to determine the freezing period that occurred. In test tube number 3, 1 mL of dried A. flava and EDTA were added, then 1 mL of blood was added and then mixed with a vortex. At the same time, time is calculated using a stopwatch. In test tube number 4, 1 mL of blood was added and 1 mL of EDTA was added. EDTA is an anticoagulant that can bind calcium so that it cannot play a role in the clotting process. In test tube number 5, put 1 mL of blood and add ethanol then mix with a vortex. At the same time, calculate the blood clotting time using a stopwatch. After 3 minutes the tube was removed and each test tube was tilted to see the changes that occurred.

Analysis of data

The results of an anticoagulant examination of human blood cells were processed manually and presented in table form. The concentration levels of A. flava extract in various blood groups were analyzed qualitatively and the data collected to describe the blood clotting effect was qualitative data.

RESULTS AND DISCUSSION

In Indonesia, the roots of A. flava (Figure 1) are widely used to reduce fever and treat canker sores. Meanwhile, in several countries such as Thailand, this plant is used as a gastrointestinal medicine and the flowers are used as a medicine for dysentery. In Malaysia, the shoots from the stem of A. flava are used as a fever-reducing medicine, worm medicine, and gastrointestinal medicine. In the Philippines, A. flava is used as an antiseptic, expectorant, tonic, and medicine for stomach irritation and other gastrointestinal diseases (28). After carrying out several tests, it was proven that the ArcangelisiA extract. flava (L.) Merrini has quite good antimicrobial abilities. Apart from that, this extract is also able to provide quite good antioxidant activity and has

good anticytotoxic activity as well. This plant also has been developed as herbal tea and other products (7) and cosmetics (29).

Figure 1. A. flava plant

Extraction

In preparation of A. flava extract using the maceration method using ethanol because the equipment and workmanship are very simple, the simplicia is soaked for 3 x 24 hours, stirring occasionally and then the red chocolate extract is obtained. With this maceration method, it is hoped that thermolabile compounds will not be easily damaged. With this method, it is also hoped that the diffusion of the solute with the solvent will be better, where the solvent will enter and penetrate the cell walls that have been damaged during the manufacture of simplicia powder, then enter the cell cavity containing the active substance and it will dissolve. With a difference in concentration in the cell, the active substance will come out. Therefore, the maceration process is influenced by the size of the simplicia particles, where the smaller the extraction simplicia, the better because the smaller the particle size, the greater the surface area of the simplicia whose particles are too small, which will affect the filtering process (30).

Figure 2. A. flava (left) and simplicia (right)

A. flava powder (Figure 2) is macerated using ethanol (universal solvent). This solvent is used because of its excellent ability to extract polar and non-polar solvents. This solvent was also chosen because it can prevent microbial growth in the extract. Apart from that, ethanol is better than other solvents because it is safe and not toxic, such as methanol, ethyl acetate, etc. From the maceration results of 300 grams of A. flava, 5.58 grams of extract was obtained with a recovery percentage of 1.86 %.

A. flava, which is a member of the Menispermaceae family, is known to contain a mixture of benzylisoquinoline alkaloids, which are biosynthetically obtained from the amino acids phenylalanine or tyrosine (14). The plant stem contains six types of quaternary alkaloid compounds, namely thalifedine, dehydrocorydalim, jatrorrhizine, berberine, pcynarrhin, and palmatine. Apart from that, it also contains three types of tertiary alkaloid compounds, namely hydroxy-berberberine, limesin, and homoarmulin (5,31). Seven furanoditerpene compounds have been isolated from the stems of A. flava which include 6-hyrdoxyarcangelisin, 2-dehydroarcangelisinol, tinophylol, 6-hydroxyfibleucin, 6-hydroxyfibraurin and fibrin (32).

Preliminary Test

Ethanol extract of the A. flava plant was obtained before being tested on blood samples, first for 120 minutes. This test aims to determine the minimum concentration range of the extract to be used in 1 ml of blood. The minimum concentration of anticoagulant effect can be seen in Table 1 where the minimum concentration of the extract obtained is 50 ppm.

Table 1. Preliminary test of anticoagulant activity

Concentration (ppm)	Time (minutes)
25	30'45
50	43'49
75	48'19
100	50'33
125	>20
150	>20
175	>20
200	>20
225	>20
250	>20

Activity test of A. flava ethanol extract

The period of anticoagulant activity or blood clotting A, B, O, and AB for each volunteer is shown in Table 2. Test tube number contains 1 ml of blood (control), test tube number 2 contains 1 ml of blood given 1 ml of ethanol, tube reaction number 3 contains 1 ml of blood given EDTA, test tube number 4 contains 1 ml of blood given EDTA and A. flava extract at the minimum concentration obtained from the preliminary test, namely 150 ppm and test tube number 5 contains 1 ml of blood given 150 ppm A. flava extract.

Based on Table 2, it can be seen that the control blood clotting period in test tube number 1 experienced blood clotting (coagulation) from each donor who had a different blood type. The clotting (coagulation) time of control blood from each donor appeared to experience differences in time. Control blood samples from each donor had a period of blood coagulation activity with an average clotting time ranging from 2 minutes to 3 minutes. This shows that blood samples from volunteers clotted were not above normal limits.

Control blood is blood that has not received any treatment that normal blood clotting occurs in the range of 3-18 minutes. Control blood samples taken are still within the normal blood clotting period (33). Blood clotting occurs by changing the plasma protein prothrombin into thrombin, which is an enzyme that catalyzes fibrinogen, which is a protein that dissolves into insoluble fibrin, within a few seconds the fibrin polymerizes into a mesh made of threads. Long fibrin threads run in all directions, this mesh catches blood elements that form a clot (34).

Table 2. Average blood clotting time

Blood group	Control (minute)	Ethanol (minute)	EDTA (minute)	EA (minute)	Extract (minute)
	1	2	3	4	5
A	41.3	56	125.3	95	53.3
B	45.3	54.6	77.3	55	62.3
O	57.6	56.6	300	66	65
AB	64	57.3	79.6	151.6	53.3

MPD: Blood clotting period

EA: EDTA and A. flava extract

In the 5th test tube, the blood added to the blood extract of the A. flava sample was of blood groups A, B, O, and AB. For test tube number 2, which contained blood and was given ethanol, it was seen that blood clotting had occurred with an average clotting time of 54 to 57 minutes for each donor. These results show that ethanol is freezing, even though the freezing period has passed the normal freezing period.

The occurrence of the blood anticoagulant process was also seen in blood samples added with EDTA in test tube number 3 and blood samples added with EDTA and A. flava extract (test tube number 4). In test tubes number 3 and number 4 which can be seen in Table 2, the clotting time for blood groups A, B, O, and AB in blood to which 1 mg of dry EDTA was added and blood samples to which 1 mg of dry EDTA and A. flava extract were added. Extract clots occur with an observed clotting period of more than 120 minutes (>120). Apart from being used to check hematocrit, EDTA anticoagulant is also used to check hemoglobin levels, blood

cell counts, reticulocytes, blood groups, and blood smears. EDTA anticoagulant is also used in two forms, namely in the form of a solution or in the form of a dry substance or solid substance. The use of EDTA anticoagulant as an anticoagulant that binds Ca^{2+} so that the blood clotting process does not occur (34–36).

Meanwhile, in the 5th test tube, blood was added to the extract of plant samples in groups A, B, O, and AB, and the anticoagulant activity or prevention of blood clotting was observed for 120 minutes (2 hours). The results from the 5th tube showed that blood clots occurred and did not have blood clotting activity. The time required to observe the prevention of blood clotting is 2 hours because 120 minutes is the time limit during which all coagulation or non-coagulation factors occur. It will take 2 hours for the desired effect to occur, namely the blood will not clot and the action of the anticoagulant will last around 4-6 hours. From the results of testing anticoagulant activity on A. flava, it was observed that there was no activity effect

CONCLUSION

From the results of in vitro testing research on various types of blood groups, it can be concluded that Arcangelisi A. flava extract (L.) Merr) using a concentration of 150 ppm does not have an anticoagulant effect on several human blood groups A, B, O, and AB.

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