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PHYTOCHEMICAL TEST OF SPRUCE ROOT ETHANOL EXTRACT

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ABSTRACT

Spruce root (Casuarinaceae) is known to be useful empirically used as a medicine for hemorrhoids. To determine Phytochemical activity of pine roots, it is necessary to conduct research on "Phytochemical activity of pine roots". The experimental research with posttest only design. Research site was in the Microbiology and Pharmacognosy Laboratory of the Pharmacy Study Program and the Biology Laboratory of Bangka Belitung University. Research object of Sea spruce roots (*Casuarinaceae equisetifolia*) were taken in Bangka Botanical Garden in fresh. It then was made into extracts. Research procedures were Plant determination and identification (2) Preparation (3) Standardization of extracts (*Casuarinaceae)* (4) Phytochemical test. (5) TLC. The results are Phytochemical test showed that the results of the content contained in the spruce root are Alkaloid, Flavonoid, Triterpenoid, Anthraquinone and Tannin.

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Introduction

Indonesia is rich in plants whose benefits are known or unknown (Hariana, 2007). Since long time ago, many plants have been used as traditional medicine, although their use is only spread from generation to generation or by word of mouth (Yuniarti, 2008).Traditional medicine has been integrated with society. It is used to treat various health problems. The ability of the community to self-medicate, regarding the symptoms of disease and to maintain health needs to be improved in order to maintain health. Therefore, traditional medicine by the community continues to increase. Not only their own concoctions, traditional medicinal products have also been available in the market (Sari Dewi et al., 2019). One of the plants that can be used as medicine is cypress (Casuarinaceae). Pine root (Casuarinaceae) is known to be useful empirically used as a medicine for

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hemorrhoids or hemorrhoids. Research results prove that pine roots (Casuarinaceae) contain saponins, flavonoids and tannins (Ernawati, 2014).

To determine the anticoagulant activity of spruce root, it was started to conduct research on "Phytochemical Test of Spruce root Ethanol Extract ".

Based on the problem above, the research problems are what is the content of the spruce root?

Materials and Methods

Research design and type used were experimental with post test only design. The research site was in the Microbiology Laboratory and Pharmacognosy Laboratory of the Pharmacy Study Program and the Biology Laboratory of the Faculty of Agriculture, Fisheries and Biology, Bangka Belitung University. Research object of beach pine/spruce root (*Casuarinaceae equisetifolia*) was taken in Bangka Botanical Garden (BBG) in fresh or brownish red state and it was made into extract.

This research used the following tools: knives, scales, ose needles, 5 ml injection syringes, micropipettes, glass objects, cover glass, tweezers, filter paper, rotary vacuum evaporator, blood tube, measuring cup, microscope, clean cloth, dry head oven, set the set, HCl; MERCK, 100 mesh sieve, blender. While the ingredients are pine root (Casuarinaceae), 96% ethanol; MERCK; EDTA; HCl; MERCK, dragendroff reagent, chloroform; MERCK, FeCl3; MERCK, mayer reagent , NaOH; MERCK, Liberman-Buchard reagent, Pb acetate; MERCK, AlCl3 solution; MERCK, KOH solution; MERCK, FeCl3, ethyl acetate; MERCK, methanol; MERCK, n-hexane; MERCK, butanol; MERCK, glacial acetic acid; MERCK, H2SO4; MERCK, tranexamic acid, TLC, aquadest;

1. Plant determination and identification of spruce root plants *(*Casuarinaceae*)*. The first stage of this research was the determination of the spruce root (Casuarinaceae) related to the macroscopic features of the spruce root (Casuarinaceae) by matching the morphological characteristics of the plant. Determination was conducted at the University of Bangka Belitung.
2. Making spruce root (Casuarinaceae*)* extract. The brownish red spruce roots (Casuarinaceae) were first cleaned with running water, then cut into small pieces. The 2.750 g cut simplicial was then put into a prepared vessel then poured with 96% ethanol until it was submerged and then covered and coated with paper and tied with a rubber band. Thus, it did not spill, left for 5 days, protected from sunlight. Then it was filtered using filter paper to maserate. The maserate obtained with 96% ethanol solvent was then evaporated with a rotary vacuum evaporator at a temperature of 70ºC, then evaporated in a water bath until a thick extract was obtained.
3. Extract Standardization.
   a. Standardization of total ash content
      2g of spruce root ethanol extract that had been crushed and weighed carefully, was put into a silicate crucible or platinum crucible that has been annealed and tared, then the powder was spread out. The crucible slowly annealed to a temperature of 500-600^{0}C until the charcoal runed out, cooled and weighed. If the charcoal couldn't be lost, hot water was added, filtered through an ash-free filter paper. The remaining charcoal was incandescent and filter paper in a crucible. The filtrate was put into a crucible, evaporated and annealed, then weighed to a fixed weight. Ash content is calculated in percent of simplicia that has been dried in air (Depkes RI, 2000).

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b. Determination of specific gravity was carried out using 1% extract. The empty vial was filled with 2 mL of water and marked. The empty vial (V0) was weighted and the vial containing 2 mL of 1% ethanol extract (V1) solution. The specific gravity of the extract was calculated by comparing the weight of the 1% solution of ethanol extract to the weight of water, assuming that the specific gravity of the water is equal to 1.

c. Determination of drying shrinkage was by carefully weighing 1 gram of ethanol extract of spruce roots in a closed porcelain crucible that had previously been heated at 105 ° C for 30 minutes and had been tared. Flatten by shaking until a layer was 5-10 mm thick. It was dried in the oven at 105° C until the weight remained with the lid open. Next, the crucible in the closed state was removed from the oven and cooled in a desiccator to room temperature and then recorded. Then put it back in the oven at 105 ° C for 1 hour. The procedure was repeated until the difference in the results of the weighing was no more than 0.5 mg per gram of sample after drying for 1 hour (Depkes RI, 2000).

d. Determination of drying shrinkage was by carefully weighing 1 gram of ethanol extract of spruce root in a closed porcelain crucible that had previously been heated at 105°C for 30 minutes and had been tared. Flatten by shaking until a layer was 5-10 mm thick. It was dried in the oven at 105°C until the weight remains with the lid open. Furthermore, the crucible in the closed state was removed from the oven and cooled in a desiccator to room temperature and then recorded. Then, put it back in the oven at 105 ° C for 1 hour. The procedure was repeated until the difference in the results of the weighing was no more than 0.5 mg per gram of sample after drying for 1 hour (Depkes RI, 2000).

e. Determination of water soluble extract and ethanol content. A total of 5 grams of ethanol extract of spruce root was macerated for 24 hours with 100 mL of water-chloroform (for water-soluble extract content) and 100 mL of 96% ethanol (for ethanol-soluble extract content) using a clogged flask while shaking it repeatedly for the first 6 hours. Then left for 18 hours and filtered. Furthermore, 20 mL of the filtrate was evaporated to dryness in a shallow, flat bottom dish that has been tared, the residue was heated at 105^0C until the weight remains.

4. Phytochemical test

Preparation of the test solution for phytochemical screening was presented by dissolving 500 mg of the extract in 50 mL of the appropriate solvent.

a. AlkaloidTest

2 ml of the test solution was evaporated on a porcelain plate until a residue was obtained. Furthermore, residue was dissolved with 5 mL of 2N HCl. After cool, the solution was filtered. The solution obtained was divided into 3 test tubes. The first tube served as a control. The second tube was added 3 drops of dragendorff reagent and the third tube was added 3 drops of mayer reagent (through the tube wall).

The formation of orange deposits in the second tube and yellow deposits in the third tube indicates the presence of alkaloids (Farnsworth, 1966 in Putri *et al*., 2015).

b. Flavonoid Test

Each 1 ml of test solution was inserted into 3 test tubes. Tube 1 was

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as a control, tube 2 was added with 1 mL of 10% Pb acetate (lead acetate) solution, positive for flavonoids if there is a yellow precipitate (Raphael, 2012). Tube 3 was added with a few drops of 20% NaOH forms a yellow color if it contains flavonoids (Ugochukwu *et al*., 2013).

c. Tanin

A total of 2 mL of the test solution was put into 2 test tubes, tube 1 as a control and tube 2 were added a few drops of 5% FeCl3 or 10% FeCl3 solution, a positive sign of tannins. formed a dark green/blue color (Robinson, 1911 in Putri *et al*., 2015).

d. Triterpenoid/Steroid

2 mL of the test solution was evaporated in the evaporator cup. The residue was dissolved with 0.5 mL chloroform, transferred to a test tube, 0.5 mL anhydrous acetic acid and 2 mL concentrated sulfuric acid was added through the tube wall. The formation of a brown or violet ring on the border of the solution indicated the presence of triterpenoids, whereas if a blue-green ring appeared, it indicates the presence of steroids (Ciulei, 1984 in Putri *et al*., 2015).

e. Anthraquinones

A total of 50 mg of extract was added 10 mL of water then heated for 5 minutes and filtered. A total of 3 mL of solution was put into 2 test tubes, tube 1 was added a few drops of 1 N NaOH solution. If positive, a red solution was formed and tube 2 as a control (Putri *et al*., 2015).

f. Saponins

4 mL of the test solution were added to 5 mL of distilled water, shake, see that there was a stable foam. A little extract was added to 5 mL of water, shake it in a test tube, a stable foam was formed (1 cm high foam and stable for 30 minutes). 4 mL of the test solution was put into a test tube as a control (MOH, 1995 in Putri *et al*., 2015).

5. Thin-layer chromatography

Silica gel G60 F254 was prepared stationary phase/TLC platewith a length of 8 cm and a width of 2 cm, then washed with methanol, then activated in an oven at 100^0C for 10 minutes. A total of 10 mg of the extract were dissolved in 1 ml of ethanol and then put on the stationary phase

a. Identification of Flavonoid Compounds

Mobile phase glacial acetic acid: butanol: water (1: 4: 5), with the appearance of ammonia vapor stains. A positive reaction was indicated by the formation of yellow-brown stains after being evaporated by ammonia in visible light and blue at 366 nm UV which confirmed the presence of flavonoids (Marliana, 2005).

b. Identification of Steroid Compounds

The mobile phase used was Chloroform - methanol (9: 1), with Liberman-Buchard reagent stains appearing accompanied by heating at 1050C for 5 minutes. Positive steroid reactions are indicated by the presence of green and blue stains (Kristanti *et al*., 2008).

c. Identification of Tannin Compounds

Methanol-water mobile phase (6: 4), with 5% FeCl3 reagent stains visible. A positive reaction is indicated by the formation of black stains (Banu and Nagarajan, 2014).

d. Anthraquinone Compound Identification

The mobile phase used was n-hexane-ethylacetate (3: 7), with the appearance of stains of 10% KOH solution in methanol. A positive reaction is indicated by the formation of yellow, brown, red, purple stains (Banu, 2014).

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Results

1. Identification result pf Spruce (Casuarinaceae).
   Robika (2023) stated that the results of plant identification conducted at the Bangka Belitung University based on the herbarium collection and garden collection as well as scientific references that the plant has the Latin name *Casuarina equisetifolia* J.R. and G. Forst. The classification is as follows:
   Kingdom : Plantae
   Divisio : Magnoliophyta
   Clasis : Magnoliopsida
   Subclasis : Hamamelidae
   Ordo : Casuarinales
   Familia : Casuarinaceae
   Genus : *Casuarina*
   Spesies :*Casuarina equisetifolia* J.R. dan G. Forst.

2. The simplicia result of the root of *Casuarina equisetifolia* J.R. and G. Forst.
   The result of comparison of the wet weight of the roots of *Casuarina equisetifolia* J.R. and G. Forst..with *Casuarina equisetifolia* roots J.R. and G. Forst. dry of 20.6% is presented in Table 3.

3. Rendement Results of *Casuarina equisetifolia* J.R. and G. Forst root extract
   Calculation of the result of 556,5 g of *Casuarina equisetifolia* root simplicia powder J.R. and G. Forst soaked in 5556 mL of 96% ethanol as a solvent, the rendement result of the extract was 36.65%. The rendement result of the root of *Casuarina equisetifolia* J.R. and G. Forst extract is presented in Table 4.

Table 3. Results of Comparison of Wet Weight and Dry Weight of Roots of Casuarina equisetifolia J.R. and G. Forst.

Root of *Casuarina equisetifolia* J.R. and G. Forst. **Wet (g)**	**Root** of *Casuarina equisetifolia* J.R. dan G. Forst. **Dry (g)**	**Persentage (%)**
2.750	566,5	20,6

(Source: Processed primary data)

Table 4. Rendement Result of *Casuarina equisetifolia* J.R. dan G. Forst root extract

Root powder of *Casuarina equisetifolia* J.R. dan G. Forst. (g)	**Solvent (mL)**	**liquid extract (mL)**	**Thick extract (g)**	**Rendement of root extract *Casuarina equisetifolia* J.R. dan G.Forst. (%)**
556,5	5.565	204	204	36,65

(Source: Processed primary data)

Table 5. Results of Phytochemical Screening of *Casuarina equisetifolia* J.R. and G. Forst Root Ethanol Extract

Compound	**Reactor**	**Test Result**			**Information**
		U_1	**U_2**	**U_3**	
Alkaloid	HCl, P.Dragendorf, and	+	+	+	Orange and yellow

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	P.Mayer				
Flavonoid	Pb acetate and NaOH	+	+	+	Yellow
Tannin	$FeCl_3$ 10%	+	+	+	Blackish green
Triterpenoid	Chloroform, $C_4H_6O_3$ and concentrated H_2SO_4	+	+	+	Brownish red
Steroid	Chloroform, $C_4H_6O_3$ and concentrated H_2SO_4	-	-	-	There was no change in color
Anthraquinones	NaOH 2 N	+	+	+	Red
Saponin	*Aquadest*	+	+	+	Foam

(Source: Processed primary data)

Information:
U_1 : First repetition; U_2 : second repetition; U_3 : third repetition
(+) : positive test result
(-) : negative test result

4. Extract Standardization Results

Standardization of medicinal plant extracts in Indonesia is an important steps in the development of native Indonesian medicines. Total ash content, specific gravity, drying loss, water soluble extract content and ethanol are non-specific parameters of the extract. The objective of this test was a simple initial introduction to possible subjective use of the senses by describing shape, color, smell and taste (Anonymous, 2000; Anonymous 2008).

a. Standardization of total ash content
The determination of the ash content couldn't be carried out at UBB because the oven used to glow powder until a temperature of 500-600^{0}C. Ash contened 0.00 g/mL

b. Determination of specific gravity
Determining specific gravity was conducted by using a pycnometer. As a result, the extract specific gravity was obtained of 0.96 g /mL.

c. Determination of drying shrinkage
The results of this drying shrinkage test obtained a percentage of 13.48%

a. Determination of water-soluble extract and ethanol content
The determination of the concentration of extracts that dissolved in ethanol aimed to determine the number of compounds that could be extracted with ethanol from a simplicia (Fauzi, 2013). In this research, it was found that the water soluble extract content was 18.11% and the ethanol soluble extract content was 23.22%.

5. Phytochemical screening results of *Casuarina equisetifolia* J.R. and G. Forst root ethanol extract.

Table 5 shows that the root extract of Casuarina equisetifolia J.R. and G. Forst. positive contains secondary metabolites, such as flavonoids, alkaloids, tannins, anthraquinones, and triterpenoids. The results of phytochemical screening research can be seen in Table 5.

6. Thin-layer chromatography

a. Identification of Flavonoid Compounds.

TLC analysis on the extract was conducted by spotting the extract with the help of a capillary tube on the TLC plate. The mobile phase used was glacial acetic acid: butanol: water (1: 4: 5). The results obtained were then evaporated with ammonia vapor. The results showed that there were 4 spots, at Rf 0.5; 0.625; 0.75;

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and 0.93. Reddish yellow spots was appeared after steaming. This indicates the presence of flavonoids in the extract (Marliana, 2005). Based on the Rf value of 0.93 (close to 0.95) in the TBA, this compound is a compound of the flavone, flavonol group, biflavonoid, calvon and auron (Aminah, 2004). According to Robinson (1995) flavonoids are a group of phenolic compounds that are acidic, causing a distinctive color change in the presence of ammonia. Flavonoids will give a distinctive color when mixed with ammonia. Reddish yellow color indicates the presence of the flavone and flavonol groups.

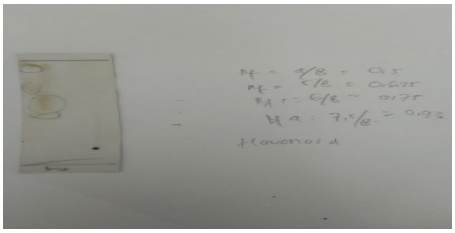

Figure 3. Flavonoid compounds

b. Identification of Steroid Compounds.
The mobile phase used was Chloroform - methanol (9: 1), with Liberman-Buchard reagent stains appearing accompanied by heating at 105^0C for 5 minutes. In this research, the results were negative.

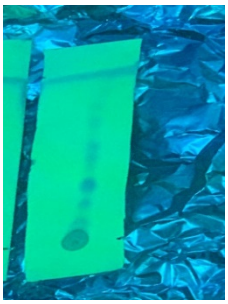

Figure 4. Steroid

c. Identification of Tannin Compound.
Methanol-water mobile phase (6: 4), with 5% FeCl3 reagent stains visible. Before adding FeCl3, a brick red color was formed. When FeCl3 was added, it formed a black color. The stain was namely at Rf 0.75 (Banu and Nagarajan, 2014).
The phytochemical test with FeCl3 gave positive results, it was possible that in the sample, there were phenolic compounds and it was possible that one of them was tannins because tannins are polyphenol compounds (Harborne, 1987). The formation of a blackish green or blue ink in the extract was after adding FeCl3 because tannins would form complex compounds with Fe3 + ions.

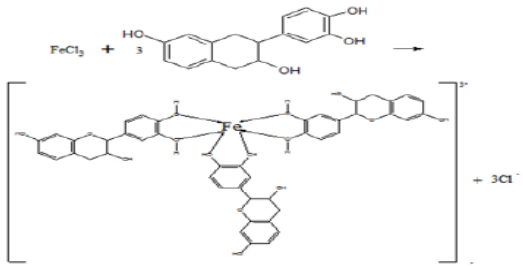

Figure 5.Tannin Compounds

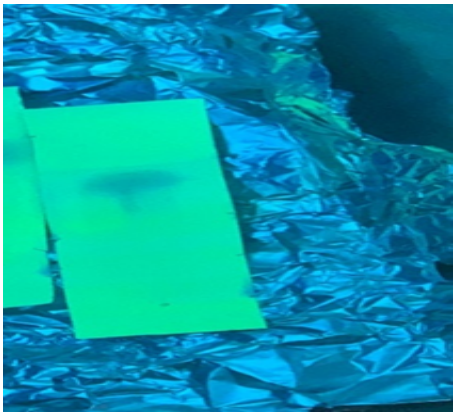

Figure 6.TLC Test Results for Tannin Compounds

d. Anthraquinone Compound Identification.
The mobile phase used was n-hexane-ethylacetate (3:7), with the appearance of stains of 10% KOH solution in methanol. The stain was namely at Rf 0.83

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Figure 8.Anthraquinone Compound TLC Test Results

Based on Banu (2014), a positive reaction is shown by the formation of yellow, brown, red, purple stains. The results of this research showed a brown color, thus, the extract was positive for anthraquinone.

Discussion

1. **Simplicia preparation of** *Casuarina equisetifolia* **J.R. dan G. Forst root.**

Simplicia is a natural ingredient used as medicine. It has not undergone any processing and generally in the form of a dried material (Depkes RI, 2000). This research used simplicia of *Casuarina equisetifolia* J.R. and G. Forst root in the Bangka Botanical Garden Bangka Belitung of 2,360 g. Roots *of Casuarina equisetifolia* J.R. and G. Forst. were wet sorted to separate the roots from impurities or foreign objects then cleaned with running water, then drained and chopped which aims to facilitate the drying process (Depkes RI, 1985). Furthermore, *Casuarina equisetifolia* J.R. and G. Forst.root was dried using oven to obtain simplicia that is not easily damaged (Depkes RI, 1985). The simplicia results obtained after drying were 566,5 g with a water content percentage of 20.6%.

2. **The ethanol extract of *Casuarina equisetifolia* J.R. and G. Forst root**

The method used in making of the ethanol extract of the roots of Casuarina equisetifolia J.R. and G. Forst. was a maceration method. Maceration is a process of withdrawing secondary metabolite compounds contained in simplicia powders with the appropriate solvent. Maceration was carried out for 5 days and stirred occasionally every day (Depkes RI, 2000). Stirring aimed to ensure a faster balance of extracted concentrations in the solvent. The stationary state during maceration can cause a decrease in the transfer of active substances (Voigt, 1994 cit. Sholikhah, 2016).

In this research, the simpliciaofof *Casuarina equisetifolia* J.R. and G. Forst root was macerated of 500 g using a 96% ethanol solvent of 5000 mL with a ratio of 1:10, 1 part for the simplicia mass of Casuarina equisetifolia J.R. and G. Fors roots and 10 parts for volume 96% ethanol solvent. After the maceration process, the maceration results are filtered then the pulp is squeezed to obtain macerate. Maserate is a solution obtained from the maceration process (Ansel, 2008). The yield obtained was 5,565 mL of macerate, evaporated with a rotary vacuum evaporator at a temperature of 70ºC to obtain a thick extract. The use of a rotary vacuum evaporator aimed to accelerate the evaporation of 96% ethanol solvent below the boiling point of 78ºC and a speed of 175 rpm. Therefore, the compounds contained in the solvent were not damaged by high temperatures and did not evaporate 20.6

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g of *Casuarina equisetifolia* J.R. and G. Forst was obtained after evaporation with a yield percentage of 36.65%.

3. Phytochemical screening of *Casuarina equisetifolia* J.R. dan G. Forst root ethanol extract

Phytochemical screening is a qualitative analysis that aims to determine the secondary metabolite components contained in the ethanol extract of Casuarina equisetifolia J.R. and G. Forst root. Based on the results of phytochemical screening in this research, it showed the presence of secondary metabolite compounds, namely alkaloids, flavonoids, tannins, anthraquinones, and triterpenoids.

a. Alkaloid Identification
Identification of alkaloids in *Casuarina equisetifolia* J.R. and G. Forst.root ethanol extract by adding HCl and Dragendorf reagentobtained positive results with a change in color to orange and a change in color to yellow when reacted with Mayer's reagent (Hanani, 2015).

b. Flavonoid Identification
Identification of Flavonoids in the root extract of *Casuarina equisetifolia* J.R. and G. Forst obtained positive results by changing the color to yellow when reacted with Pb acetate and NaOH (Hanani, 2015).

c. Tannin Identification
Tannins identification in the ethanol extract of *Litsea oppositifolia* Gibbs root by adding 10% FeCl3 reagent into the test tube,obtained positive result by changing the color to blackish green. The formation of a green-black color is due to the reaction between tannins and Fe^{3+} ions to form complex compounds (Wijaya *et al*., 2015).

d. Anthraquinone identification
Anthraquinone identification in the ethanol extract of *Litseaoppositifolia* Gibbs root. by adding 2 N NaOH reagent into the test tube obtained positive results. The addition of NaOH causes Na to react with phenol in the quinonet, thus, the sample changes color to red (Wijaya et al., 2015).

e. Triterpenoids Identification
Identification of triterpenoids in the ethanol extract of the root of *Litseaoppositifolia* Gibbs root. by adding concentrated C4H6O3 and H2SO4 reagents into the test tube obtained positive results. The formation of a red color is due to the addition of C4H6O3 and H2SO4 reagents an acetyl derivative compound is formed so that the color changes to red (Wijaya *et al*., 2015).

Conclusion and Suggestion

Based on the result of the research and data analysis that have been conducted, it can be drawn the conclusion that: Phytochemical test showed the results of the content contained in the spruce root (Casuarinaceae), such as alkaloids, flavonoids, triterpenoids, anthraquinones and tannins.

Suggestions

1. For Health Polytechnic of Health Ministry Pangkalpinang.

It should be conducted further research on spruce root as an anticoagulant because the calculation of blood clotting time between the treatment group and the positive control group and the negative group is significantly different.

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2. For researcher

To provide information that spruce root can't be used as a coagulant but as an anticoagulant.

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