Phytochemical Screening and Test of Mucolytic Activity of Nira Stem Sente (Allocaasia Macrorrhizos) by in Vitro

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Abstract. Nira stem sente (Allocaasia macrorrhizos) is one of the natural riches of Indonesia who have not much utilized, but since the age of the first communities in the Ciamis area using nira stem sente this as an alternative to relieve the symptoms of cough. As for the research on assay activity mucolytic nira stem sente (Allocaasia macrorrhizos) there has never been, thus providing the inspiration for researchers to conduct research that aims to find out the chemical compounds and activity mucolytic from nira stem sente (Allocaasia macrorrhizos). Nira stem sente (Allocaasia macrorrhizos) accommodated overnight, then conducted screening phytochemical compounds, flavonoids, saponins, alkaloids, tannins, Quinones, triterpenoid. Next mucolytic activity testing nira stem sente (Allocaasia Macrorrhizos) with variations in concentrations of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%. Artificial mucus using analog white duck egg. Raw comparison mucolytic using asetilsistein 0.1% and the negative control using 10 ml aqua dest. Sample test consists of 10 ml of water from different concentration nira stem sente (Allocaasia Macrorrhizos) in 100 ml of analog artificial mucus, then incubated at a temperature of 37°C for an hour and do a test mucolytic each interval of 15 minutes viskometer stormer. Testing conducted by as much as 3 times the replication. From the results obtained it was concluded that nira stem sente (Allocaasia macrorrhizos) positive contain chemical compounds, alkaloids, flavonoids, saponins, tannins, Quinones, triterpenoid and all test groups have mucolytic activity. At all a concentration have mucolytic activity

1. Introduction

Cough is a physiological mechanism that is useful for removing and cleaning the respiratory tract of phlegm, foreign stimulating substances and elements of infection [8]. Thick phlegm will affect the difficulty of expectoration of sputum, so that in this condition often used drugs to thin phlegm (mucolytic). Acetylcysteine is one example of mucolytic drugs that are often used in therapy, but acetylcysteine adverse reactions such as skin rashes, nausea, vomiting, diarrhea and anaphylactoid reactions that sometimes become passive complaints [4].

Nira stem sente is one of Indonesia’s natural wealth that has not been utilized, but from the time it first has many societies especially in Ciamis, which use nira (water) stem sente this as an alternative to relieve symptoms cough.
Several research of sentimental (Alocasia macrorrhizos) parts of leaves, stems and rhizomes explain that the sentiment plant (Alocasia macrorrhizos) contains flavonoids, cyanogenetic glycosides, citric acid, ascorbic acid, polyphenols, proteins, ash, crude fiber, carbohydrates, starch, oxalate, proteases, nitrate and tannin, besides centrifuge (Alocasia macrorrhizos) has biological activity as antioxidant, antimicrobial, antidiarrheal, diuretic, antihyperglycemic and others [2]. As of now there has been no research that focuses on other parts of tamanan sente (Alocasia macrorrhizos), which is sente stem juice as a mild coughing symptom.

This research aims to determine the mucolytic activity of sente stem juice (Alocasia macrorrhizos). So that it can be a preliminary test in the search for alternative cough treatment options in the future.

2. Experimental
This type of research is experimental research that aims to find out the chemical compounds and activity of mucolytic from nira stem sente. Method of with withdrawal of nira from plants using traditional methods by means of accommodating the nira stem sente overnight. Phytochemical screening of nira stem sente and to test the of mucolytic activity created 10 concentration, i.e. 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% 100%. Artificial mucous using analog white duck egg, a positive control using asetilsistein 0.1% and the negative control using 10 ml aquadest.

2.1 Phytochemical Screening
2.1.1 Alkaloids. The simplicia powder was weighed as much as 0.5 g and then added 1 ml of 2 N hydrochloric acid and 9 ml of distilled water, heated on a coke of water for 2 minutes, then filtered. Filtrate is used for the following experiments:

- Taken 3 drops of filtrate, then added 2 drops of Dragendorf reagent to produce brick red sediment (Marjoni, 2016)
- Taken 3 drops of the filtrate, and then added 2 drops of reagents Bouchardat produce deposits of dark chocolate (Marjoni, 2016).

Description: in relation to sampling used nira (water) directly inserted, then the molasses into two test tubes each 2 ml.
- Tube 1: Added 3 drops of Dragendorf reagents generate deposits of red brick.
- Tube 2: Added 3 drops of reagents Bouchardat produce deposits of dark chocolate.

2.1.2 Flavonoids
a. Method 1
As many as 10 g of simplicia powder were added with 100 ml of hot water. The mixture is then boiled for about 5 minutes, then filtered when it's hot. A total of 5 ml of filtrate were obtained, 0.1 g of Mg powder, 1 ml of concentrated HCl and 2 ml of amyl alcohol were added, then shaken, and left to separate. Positive flavonoids occur in red, yellow, orange, in the amyl alcohol layer (Marjoni, 2016).

Description: in relation to the sample used nira (water) , then directly enter into tube 1 and 2 as much as 5 ml tubes, each tube added 0.1 g powder Mg, 1 ml concentrated HCl and 2 ml of Amyl alcohol, then beaten, and left to split. Flavonoids are positive if there were red, yellow, Orange, on a layer of Amyl alcohol.

b. Method 2
Performed according to Simes et al. Method (2016) where 4 grams of finely ground, lumped samples were boiled in ethanol for 15 minutes, filtered in heat, the filtrate obtained was dried. The dry extract obtained was added with 5 ml of distilled and chloroform water, then shaken vigorously and left for a while until two layers were formed, namely the water layer and chloroform layer. A layer of water is added with 0.1 ml HCl and a few grains of magnesium metal, a positive reaction if there is a pink to red color [6]. Description: In relation to the sample used nira (water), 4 grams of the sample is boiled in 25 ethanol for 15 minutes, filtered in heat, the filtrate obtained is added with 5 ml of distilled water and chloroform, then shaken vigorously and left for a while until two layers are formed, namely the
water layer and chloroform layer. A layer of water is added with 0.1 ml HCl and a few grains of magnesium metal, a positive reaction if pink to red occurs.

2.1.3 Saponins
a. Forth Reagent
As many as 10 ml of water puts in the test tube and is shaken vigorously for 10 seconds, forming a foam or scum that is for no less than 10 minutes as high as 1-10 cm. In addition 1 drop of 2 N hydrochloric acid solution, the foam function does not disappear indicating the presence of saponins [6].
b. Liebermann-Burchard Reagent
Solvent/Solution of reactant Liebermann-Bouchard prepared by mixing 20 parts of anhydrous acetic acid with one part concentrated sulfuric acid and 50 parts of chloroform. This reactant solution should be made new.
As much as 5 ml nira (water) included into test tubes, and then added 5 drops of reactant Liebermann-Bouchard. Positives arise in white with a red background formed immediately or after some time [1].
The results of the research data were obtained based on the observation of color changes, the formation of sediments and the appearance of foam from the samples used.

2.1.4 Tannins. A total of 0.5 g of the sample was extracted using 10 ml of aquadest. The extraction results were filtered then the obtained filtrate was diluted with distilled water until it was colorless. The results of this dilution were taken as much as 2 ml, then added with 1-2 drops of iron (III) chloride. The occurrence of black or blue green indicates tannins [6].
The sample used was nira (water) stem sente, then immediately taken 2 ml of juice and put in 3 test tubes 2 ml each, then added with 1-2 drops of iron (III) chloride will occur blue or blackish green indicating tannins.

2.1.5 Quinone. A total of 5 g of simplicia powder, then added 100 ml of hot water, boil for 15 minutes then filtered, as much as 5 ml. Then the results of the simplicia powder filter, dripped with 1N NaOH solvent/solution, formed a yellow to red color indicating the presence of quinone compounds [3].
The sample used was nira (water) stem sente, then immediately taken 2 ml of juice and put in 3 test tubes 2 ml each, then dripped with 1N NaOH solution to taste. The formation of yellow to red indicates the presence of quinone compounds.

2.1.6 Triterpenoid. As many as 1g of the sample was macerated with 20 ml of n-hexane for 2 hours, then filtered. The filtrate is evaporated in a vaporizer cup. When added 2 drops of anhydrous acetic acid and 1 drop of concentrated sulfuric acid. Purple or red color then turns into blue green indicating the presence of steroid triterpenoids [6].
The sample used was nira (water) stem sente, taken a sample of 10 ml nira (water) stem sente.
The results of the research data were obtained based on the observation of color changes, the formation of sediment and the appearance of foam from the sample used.

2.2 Mucolytic Activity Test
Consisted of 10 ml of nira water from various concentrations (10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%) in 100 ml of artificial mucus analogs, then incubated at a temperature of 370C, the test was carried out to accommodate the viscosity every 15 minutes using a stormer viscometer for 60 minutes. The test was carried out 3 times replication.
3. Result

3.1 Phytochemical Screening Results

| Reagent                | Phytochemical Test | Result                          | Standard                                                                 | Conclusion                                                                 |
|------------------------|--------------------|---------------------------------|---------------------------------------------------------------------------|-----------------------------------------------------------------------------|
| Dragendorf              | Alkaloids          | Red sedimentary brick           | Deposits of light brown to yellow and brick red deposits (Marjoni, 2016) | Positive                                                                   |
| Bouchardat             | Black chocolate   | Black chocolate deposits        | Black chocolate deposits (Marjoni, Positive 2016)                         |                                                                             |
| Mg + HCl Concentrated  | Flavonoids         | The occurrence of yellow and pink color | The occurrence color of Red, Positive yellow, orange, in the amyl alcohol layer (Marjoni, 2016) |                                                                             |
| Forth                  |                    | A stable foam is formed         | If the foam does not disappear, Positive showing the presence of saponins (Marjoni, 2016) |                                                                             |
| Libermann-bourchard     | Saponin            | Formed white color with red background | It appears white color with a red Positive background formed immediately or after some time (Endang, 2016) |                                                                             |
| FeCl₃ 1% Reagent        | Tanin              | The formation of a blackish green color | The formation of a gray-green Positive color when reacted with FeCl₃ solution (Robinson, 1995) |                                                                             |
| NaOH 1N                | Quinon             | color changes to yellow         | The formation of a gray-green Positive color when reacted with FeCl₃ solution (Robinson, 1995) |                                                                             |
| Libermann-bourchard     | Triterpenoid       | give a pink color               | Purple or red color then turns to Positive blue green indicating the presence of steroid triterpenoids (Marjoni, 2016) |                                                                             |

3.2 Mucolytic Activity Test Results

| Time/Minutes | Control (+) | Control (-) | Control (+) | Control (-) |
|--------------|-------------|-------------|-------------|-------------|
| -15          | 57.3        | 33.3        | 71.3        | 39.3        |
| 0            | 32          | 32          | 64.3        | 34.3        |
| 15           | 24          | 30.3        | 59.3        | 24          |
| 30           | 15.3        | 28.6        | 43.3        | 18.6        |
| 45           | 11.3        | 26.3        | 39          | 13          |
| 60           | 6.6         | 25.6        | 33          | 10.6        |

From the table above can be illustrated as follows:
**Figure 1.** The average value of decreasing viscosity of mucus analogues for 1 hour (comparison of test groups 10%-50% with controls – and +)

**Figure 2.** The average value of decreasing viscosity of mucus analogues for 1 hour (comparison of test groups 60% - 100% with controls – and +)

**Table 3.** Average Percentage of Viscosity Decrease for One Hour

| Group       | Uji Tahap 1 Stage 1 Test (%) | Uji Tahap 2 Stage 1 Test (%) | Uji Tahap 3 Stage 1 Test (%) | Rata-rata±Sd Average     |
|-------------|-------------------------------|-------------------------------|-------------------------------|---------------------------|
| kontrol (+) | 80.1                          | 85                            | 90                            | 85.03±4.95                |
| kontrol (-) | 15.6                          | 22.7                          | 33.3                          | 23.87±8.91                |
| Uji 10%     | 71.4                          | 44.2                          | 70.5                          | 62.03±15.46               |
| Uji 20%     | 73                            | 68.75                         | 76.4                          | 72.72±3.83                |
| Uji 30%     | 71                            | 73.4                          | 92.8                          | 79.07±11.95               |
| Uji 40%     | 60                            | 77.1                          | 95.4                          | 77.5±17.7                 |
| Uji 50%     | 76.1                          | 88.4                          | 96.6                          | 87.03±10.32               |
| Uji 60%     | 84.4                          | 90.3                          | 75                            | 83.23±7.72                |
| Uji 70%     | 83.3                          | 93.9                          | 81.2                          | 86.13±6.81                |
| Uji 80%     | 86                            | 93.6                          | 81.2                          | 88.07±4.84                |
| Uji 90%     | 88.2                          | 88.8                          | 77.2                          | 84.73±6.53                |
| Uji 100%    | 94                            | 96.1                          | 31                            | 73.7±3.69                 |

Information: Statistical calculation with ANOVA Post Hoc Test LSD at p<0.05
4. Discussion
Phytochemical screening was carried out to find out the chemical compounds contained in sente stem juice. Seen from the results of phytochemical screening, sente stem juice has mucolytic activity because [5] explained that chemical compounds that have mucolytic activity are groups of saponins, tannins, flavonoids and alkaloids.

Observation of the mean values of viscosity stages 1, 2 and 3, shows a decrease in the viscosity value of each test group, these results indicate the presence of mucolytic activity of the sente stem sap we tested.

Statistical test results with Post Hoc ANOVA LSD Test at p<0.05, all group test were significantly different from the negative control group and not significantly different from the positive control group, this confirmed that the possibility that sente stem juice had mucolytic activity.

5. Conclusion
Sente stem juice (Alocasia Macrorrhizos) positively contains chemical compounds alkaloids, flavonoids, saponins, tannins, quinones and triterpenoids. All concentration of Sente stem juice (Alocasia Macrorrhizos) have mucolytic activity.

6. References
[1] Endang, H. 2016 Analisis Fitokimia. Jakarta: Buku Kedokteran EGC.
[2] Fatmawati, S. 2018 Senyawa Metabolit Sekunder dan Aspek Farmakologi Alocasia Macrorrhizos, 3(1), 141–158.
[3] Fitria, V. 2014 STUDI EFEK TERATOGENIK EKSTRAK DAUN BINAHONG (Adredera cordifolia (Ten.) Steenis) PADA TIKUS WISTAR, Tesis, Program Studi Farmasi. Bandung: Institut Teknologi Bandung.
[4] Goodman, G. 2002 Dasar Farmakologi Terapi (10, Vol.2, ed.). Penerbit Buku Kedokteran ECG.
[5] Linn, A., Kalmegh, N., & Linn, A. V. 2010 Review Article HERBAL ANTITUSSIVES AND EXPECTORANTS - A REVIEW cough and are often prescribed in combination. Before dealing with the particular type of drug used, it is production, its role in disease and desirability of The sale of pediatric and adult cough medication represents a significant proportion of the pharmaceutical dose combinations because using two or more drugs in clear the respiratory passages of foreign material and reflex. It occurs due to stimulation of mechano-or to suppress the debilitating cough suffered by such Available online at www.globalresearchonline.net, 5(2), 5–9.
[6] Marjoni, R. 2016 Dasar-dasar Fitokimia untuk Diploma III Farmasi. Jakarta Timur: CV Trans Info Media.
[7] Robinson, T. 1995 Kandungan Organik Tumbuhan Tinggi, Penerjemah: Kosasih Padmawinata. Bandung: penerbit ITB.
[8] Tjay, T. h., & rahardja, K. 2007 Obat-obat Penting. Jakarta: Elex Media Komputindo.