GSC Biological and Pharmaceutical Sciences

eISSN: 2581-3250 CODEN (USA): GBPSC2

Cross Ref DOI: 10.30574/gscbps

Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

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Specific and non-specific parameters determination and chemical content tests of the combination of ethanol extracts of *Acalypha indica* Linn and *Peperomia pellucida* (L) H.B.K.

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GSC Biological and Pharmaceutical Sciences, 2024, 26(01), 147–154

Publication history: Received on 27 November 2023; revised on 07 January 2024; accepted on 09 January 2024

Article DOI: https://doi.org/10.30574/gscbps.2024.26.1.0003

Abstract

Indonesian people have long used herbal plants as medicine. Herbal plants used by Indonesian people include cat root herb (*Acalypha indica* Linn) and suruhan herb (*Peperomia pellucida* (L) H.B.K). Previous research showed that combining cat root herb extract and surah herb was safe for rat kidneys. This research aims to test specific and non-specific standard parameters in the combination of the two extracts so that they are safe and have good quality that meets the requirements and to know the chemical content of the extracts combination. The specific parameters used in this research were dissolved compound levels (water and ethanol soluble compound levels) and non-specific parameters, namely drying loss, water content, and ash content. The water-soluble compound content obtained was 7.44%, and the ethanol-soluble compound content was 4.9%. The drying loss was 3.29%, extract water content was 4.41%, total ash content was 7.39%, and acid-insoluble ash content was 4.37%. Based on the results of this research, it can be concluded that several specific and non-specific parameters meet the established quality standards. The identification of the extracts combination shows the presence of alkaloids, flavonoids, tannins, saponins, glycosides and cyanogenic glycosides, but does not contain anthraquinone glycosides, and the total alkaloid content is 0.31%.

Keywords: Cat root herb; Suruhan herb; Specific parameters; Non-specific parameters; Chemical content

1. Introduction

Indonesian people have long used herbal plants as medicine. The herbal plants consumed by the community are in the form of herbal medicine. Jamu itself comes from the ancient Javanese language, jampi or usada, which means healing using medicinal ingredients, prayers, and magic spells (Djojoseputro, 2012). According to Minister of Health Regulation no. 003/Menkes/Per/I/2010, herbal medicine is traditional medicine consisting of herbal ingredients, ingredients in the form of plant ingredients, animal ingredients, mineral ingredients, galenic preparations, or mixtures of these ingredients, which have been used for generations to treat dams. can be implemented by the norms applicable in society (Menteri Kesehatan Republik Indonesia. 2010).

Traditional medicine in Indonesia is grouped into three categories: herbal medicine, standardized herbal medicine, and phytopharmaca. These three groups of traditional medicines must have safety criteria and comply with established quality requirements. The difference between the three is in the efficacy claims given, where efficacy claims for herbal

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medicine must be based on empirical data. Standardized herbal medicines must meet the required efficacy claims through scientific evidence, pre-clinical testing, and standardizing the raw materials used in the finished product. Furthermore, phytopharmaceutical products have the same criteria for health claims; the difference is in the use claims, where standard herbal medicines meet general and medium claims, while phytopharmaceutical products must meet medium to high use claims (BPOM, 2004).

Herbal medicines used by Indonesian people include cat root (*Acalypha indica* Linn) and suruhan (*Peperomia pellucida* (L). H. B. K). In previous research, it was found that the combination of cat root and suruhan herb extracts affected the kidney function of white rats, namely by using high doses, namely 0.4 g of cat root herb and 10.8 g of cat root herb/200 g BW, there was no significant difference in all parameters. on the kidneys of male and female mice compared to the control group (Ambarwati et al., 2020). Furthermore, in making this combination of extracts, standardization of the extract is required, namely measuring specific and non-specific parameters. Standardization itself is a series of processes that guarantee that the final product (drug, extract, or extract product) has certain parameters that are constant and determined in advance (Direktorat Pengawasan Obat Tradisional. 2000). The specific parameters used in this research were the levels of water-soluble compounds and ethanol and the non-specific parameters were drying loss, water content and ash content. Hopefully, this research can become a further reference in making cat root herbal extracts and herbal medicines in the development of herbal medicines in Indonesia.

2. Material and methods

2.1. Plant material

The test materials used were cat root herb extract (*Acalypha indica* Linn) and suruhan herb extract (*Peperomia pellucida* (L) H.B.K).

2.2. Chemical materials

The solvent and chemicals used are 95% ethanol, aquadest, chloroform, and sulfuric acid.

2.3. Equipment

The equipment used includes glassware, analytical scales (Metler Toledo), an oven (Memmert), a water bath (Imperial IV), and a furnace.

2.4. Methods of Specific Parameters Determination

2.4.1. Levels of Water-Soluble Compounds

Carefully weigh approximately 5 g of powder (4/18) dried in air. Put it in a stoppered flask, add 100 mL of chloroform-saturated water, shake repeatedly for the first 6 hours, and leave for 18 hours. Strain and evaporate 20.0 mL of the filtrate until dry in a shallow, flat-bottomed cup heated to 105° and set aside; heat the remainder at 105° until the weight remains constant. Calculate the content in % water soluble essence (Kementerian Kesehatan Republik Indonesia, 2022). The extraction method used in this study is based on the techniques described in the work of Bassene in 2012 and Vercauteren (2013-2014).

2.4.2. Levels of compounds that dissolve in ethanol

Carefully weigh approximately 5 g of powder (4/18) dried in air. Place in a stoppered flask, add 100 mL of ethanol P, shake repeatedly for the first 6 hours, and leave for 18 hours. Filter quickly to avoid evaporation of the ethanol; evaporate 20.0 mL of the filtrate until dry in a shallow, flat-bottomed cup heated to 105° and set aside; heat the remainder at a temperature of 105° until the weight remains constant. Calculate the content in % of ethanol soluble essence (Kementerian Kesehatan Republik Indonesia, 2022).

2.5. Methods of Non-Specific Parameters Determination

2.5.1. Drying shrinkage

Drying loss is the reduction in weight of a material after it has been dried in a predetermined manner. Unless stated otherwise in each monograph, simplicia must be in powder form with an acceptable degree of number 8, a drying temperature of 105° and drying loss determined as follows: Accurately weigh 1 to 2 g of simplicia in a shallow weighing bottle with a lid that has previously been heated to the specified temperature and tare. Flatten the material in the

weighing bottle by shaking it until it forms a layer approximately 5 to 10 mm thick, put it in the drying chamber, open the lid, and dry at the specified temperature until the weight remains constant. Before each drying, let the closed bottle cool in a desiccator to room temperature (Kementerian Kesehatan Republik Indonesia. 2022).

2.5.2. Water content

Determination of water content can be done using the azeotropic method and gravimetric method. The method used in this research is the gravimetric method.

Carefully weigh approximately 10 g of the test substance and put it in a container that has been weighed. Dry at 105° for 5 hours, and weigh. Continue drying and weighing at 1-hour intervals until the difference between two consecutive weighing's is no more than 0.25% (Kementerian Kesehatan Republik Indonesia, 2022).

2.5.3. Ash content

Determination of Total Ash Content

Carefully weigh 2 to 3 g of the ground test material and put it in a silicate crucible that has been ignited and tared; ignite slowly until the charcoal runs out, calm, and weigh. If the charcoal cannot be removed by this method, add hot water, stir, and filter through ash-free filter paper. Place the filter paper and the rest of the filter in the same crucible. Put the filtrate into a crucible, evaporate, and ignite until the weight remains at a temperature of 800 ± 25º. The total ash content is calculated from the weight of the test material, expressed in % w/w (Kementerian Kesehatan Republik Indonesia, 2022).

Determination of acid-insoluble ash content

Boil the ash obtained in the Total Ash Content Determination with 25 mL of dilute hydrochloric acid LP for 5 minutes. Collect the part that does not dissolve in acid, filter through ash-free filter paper, wash with hot water, ignite in a crucible until the weight remains at a temperature of 800 ± 25º. The ash content that is insoluble in acid is calculated from the weight of the test material, expressed in % w/w (Kementerian Kesehatan Republik Indonesia, 2022).

2.6. Methods of Chemical Content Tests

2.6.1. Identify the chemical content of the extract combination

Identify Alkaloids

500 mg of extract was added with 1 mL of 2 N hydrochloric acid and 9 ML of water, heated over a water bath for 2 minutes, cooled, and filtered. The filtrate is divided into four parts on a watch glass and Mayer LP reagent is added to each, a positive result is indicated by the formation of a white or yellow lumpy precipitate; Bouchardat LP reagent forms a brown to black precipitate; Dragendorf LP reagent forms a brick red precipitate; and with the Solutio Iodii reagent a brown precipitate is formed (Ditjen POM, 1995).

Identification of Glycosides

3 grams of the extract was added to 30 mL of a mixture of 7 parts by volume of ethanol (95%) P and 3 parts by volume of water in a reverse water cooler for 10 minutes, cooled, and then filtered. 20 mL of filtrate, 25 mL of water, and 25 mL of 0.4 M lead (II) acetate were added, shaken, and allowed to stand for 5 minutes, then filtered. The filtrate was filtered three times each with 20 mL of a mixture of 3 volume parts of chloroform P and 2 volume parts of isopropanol P. Anhydrous sodium sulfate P was added to the juice collection, filtered and evaporated at a temperature of not more than 50°C. The remainder was dissolved in 2 mL of methanol. Furthermore, the solution in methanol is referred to as the experimental solution (Ditjen POM, 1995).

A 0.1 mL of test solution was placed in a test tube and evaporated over a water bath. Add 2 mL of water and 5 drops of Molisch Lp to the remainder. Then carefully add 2 mL of sulfuric acid P, a purple ring forms at the liquid boundary indicating the presence of sugar bonds (Molish reaction).

Identification of Anthraquinone Glycoside

A total of 200 mg of extract was added with 5 mL of 2 N H_2SO_4, heated briefly, and cooled. Then, 10 mL of benzene P was added, shaken, and allowed to stand. The benzene layer was separated and filtered. The yellow filtrate indicates

the presence of anthraquinone. The benzene layer was shaken with 1 mL to 2 mL of 2N sodium hydroxide and allowed to stand. The water layer is intensely red, and the benzene layer is colorless (Ditjen POM 1995).

Identify Saponins

A total of 0.5 grams of extract in a test tube was added to 10 mL of hot water, cooled, and then shaken vigorously for 10 seconds. Positive results are indicated by the formation of stable foam for no less than 10 minutes as high as 1 cm to 10 cm. When adding 1 drop of 2N hydrochloric acid, the foam does not disappear (Ditjen POM 1995).

Identiy Flavonoids

Approximately 2 grams of the extract was evaporated in a water bath, and the remainder was dissolved in 1-2 mL of ethanol, added 0.5 grams of P zinc powder and 2 mL of 2N hydrochloric acid, then left for 1 minute. Then, 10 drops of concentrated hydrochloric acid P are added. If, within 2-5 minutes, an intensive red color occurs, this indicates the presence of flavonoids (glycoside-3-flavonol).

A total of 2 grams of the extract was evaporated over a water bath; the remainder was dissolved in 1 mL of 95% P ethanol. Then add 0.1 gram of magnesium P powder and 10 drops of concentrated hydrochloric acid P. If a red-orange to purple-red color occurs, it indicates the presence of flavones, chalcones, and aurones.

A total of 2 grams of the extract was evaporated over a water bath; then, the remainder was wetted with acetone P. Add a little fine powder of boric acid P and oxalic acid P to it, heat carefully over a water bath, and avoid overheating. The remainder obtained was mixed with 10 mL of P ether. Then, the color change was observed with 366 nm UV light, and the presence of flavonoids was indicated by intensive yellow fluorescence (Ditjen POM 1995).

2.6.2. Identify Tannins

Approximately 0.2 grams of the extract is diluted with 20 mL of hot distilled water and then shaken homogeneously; after cooling, it is centrifuged, and the above liquid is decanted. Then, 5 drops of 10% sodium chloride solution were added and filtered. The filtrate is divided into 2 parts, the first is added with a 1% gelatin solution in 10% NaCl to create a precipitate, the second is added with a 3% iron (III) chloride solution to create a blackish or brownish solution (Evans, 2002).

2.6.3. Determination of Total Alkaloid Content

Carefully weigh 10 grams of the combination of extracts, add 30 mL of distilled water and 10 mL of 1 N HCl then heat over a water bath for 30 minutes at a temperature of 40 - 60°C and cool then filter. Add concentrated ammonia to the pH of the chloroform solution as much as 20 mL using the first 125 mL separating funnel and shake carefully. The chloroform layer was taken and heated at 50°C. Filtering is carried out until the alkaloid test results in the water layer are negative. Then, the weighing is carried out in a container that has been tared (Harborne, 1987; Robinson, 1995).

2.6.4. Thin Layer Chromatography (TLC) Profile

The extract is dissolved in hot distilled water, then filtered. Next, the filtrate was filtered using ethyl acetate. The ethyl acetate layer was taken and used as a test solution (Robinson, 1995). The test solution was spotted on a silica gel plate, then eluted with various types of mobile phase combinations suitable for the flavonoid group, namely n-butanol-glacial acetic acid-water (40:10:50) which took the upper phase, chloroform-methanol (5:1), chloroform-methanol (9:1), chloroform-methanol (95:5) and chloroform-methanol-water (80:12:2). Then the plate was sprayed with 5% AlCl3 in methanol (Bladth & Zgainski, 1984).

3. Results

3.1. Determination of Specific Parameters

In this research, the specific parameters used were the levels of water-soluble compounds and the levels of ethanol-soluble compounds. Based on the test results, it was found that the combination of cat root simplicia and suruhan simplicia was 7.44% ± 0.641 and 4.9% ± 0.011 (In Table 1)

Table 1 Extract specific parameter test results

Test parameters	Dissolved compound content (%)
Water soluble compound content	7.44 ± 0,641
Ethanol soluble compound content	4.90 ± 0.011

3.2. Determination of Non-Specific Parameters

The non-specific parameters used in this research consisted of drying loss, water content, total ash content, and acid-insoluble ash content.

Determination of water content aims to determine the water residue left after the drying process. The water content obtained in this study was 4.41% ± 0.131

The drying shrinkage level obtained was 3.29% ± 0.118. The summary of the results is listed in Table 2.

Table 2 Test results for non-specific extract parameters

Test parameters	Concentration (%)
Drying shrinkage	3.29% ± 0.118
Water content	4.41% ±0.131
Total ash content	7.39% ± 0.023
Acid insoluble ash content	4.37% ± 0.09

3.3. Chemical Content Tests

In testing the chemical content of the extract, it was found that the phytochemical content in the extract was alkaloids, glycosides, saponins, flavonoids, and tannins (Table 3)

Table 3 Results of the chemical content identification test of the extract

Chemical Content of Test Results	Hasil Uji
Alkaloid	+
Glycoside	+
Anthraquinone glycosides	-
Saponin	+
Flavonoids	+
Tannin	+

The total alkaloid content contained in the extract is 0.31% ± 0.015.

Based on the thin layer chromatography profile test after eluting using chloroform – methanol eluent, the results were obtained as in Figure 1.

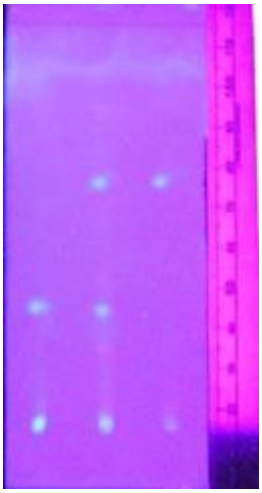

Figure 1 Chromatogram pattern using chloroform-methanol (95:5) eluent; A: A, indica (hRf = 31), B: combination of A. indica & P. pellucida (hRf = 31, 62), C: P. pellucida (hRf = 62), under 366 nm UV light with AlCl3 color reagent light blue

4. Discussion

4.1. Specific Parameters

Determination of the levels of water and ethanol soluble compounds aims to determine the content of active compounds dissolved in water and ethanol based on their polarity (Direktorat Pengawasan Obat Tradisional. 2000). This parameter can be used to determine optimal extraction to obtain bioactive compounds in the extract (Mlcek et & Plaskova, 2023). This test shows that there are more compounds dissolved in water than compounds dissolved in water. Water is an efficient solvent for extracting various polar compounds and increases the efficiency of the extraction process through the diffusion process (Mlcek & Plaskova, 2023). Apart from that, ethanol can also be used for the extraction of polar compounds with a lower polarity than water, namely 0.654, which is soluble in water and can also extract secondary metabolite compounds (Abubakar & Haque, 2020).

4.2. Determination of Non-Specific Parameters

The non-specific parameters used in this research consisted of drying loss, water content, total ash content, and acid-insoluble ash content. Determination of water content aims to determine the water residue left after the drying process. The water content obtained in this study meets the requirements, namely <10% (Saifuddin et al., 2011).

Determination of drying losses aims to determine the limits and maximum range of the number of compounds lost and evaporated in the drying process (Komala & Haryoto, 2020). The drying trace levels obtained can be used to determine the appropriate drying process by considering heat-resistant compounds, volatile compounds, flavonoid content, and color components present in the plant (Mukherjee, 2019). The size of the drying shrinkage level is one of the standard parameters in maintaining quality to avoid mold growth.

Determination of total ash content and ash content aims to determine the mineral content contained in the extraction process (Direktorat Pengawasan Obat Tradisional. 2000). In principle, determining the ash content is done by heating at high temperatures using a furnace where water and organic substances will be evaporated, organic substances that are heated in the presence of oxygen will be converted into carbon dioxide (CO_2) and oxides of nitrogen (N_2). Most minerals are converted into oxides, sulfates, phosphates, chlorides, and silicates (Mukherjee, 2019).

4.3. Chemical Content Test

Identification of alkaloids with the reagents Dragendorf, Bouchardat, Mayer, and Solutio Iodii showed positive results. These four reagents have the same principle, namely precipitation. The Dragendorf reagent produces a brick red precipitate due to the formation of water-insoluble addition compounds. In Bouchardat's reagent, a black precipitate is formed because of the formation of complex compounds with alkaloids. In the Mayer reagent a white precipitate is formed due to the reaction between the heavy metal Hg2+. In contrast, in the Solutio Iodii reagent a brown precipitate is formed due to the absence of starch/starch in the test filtrate (Evans, 2002).

Positive results for glycosides are indicated by the formation of purple rings due to the reaction between 1-naphthol and carbohydrates contained in the extract (Stephen & Merrifield, 2005). Testing for anthraquinone glycosides was carried out using the Borntrager reaction. The principle of the reaction is that an alkalization reaction occurs with the phenolic ions contained in the extract, resulting in a red color if the test is positive (Diaz-Muñoz et al., 2018). If the extracted compound contains very stable anthraquinone glycosides or derivative compounds from reduced anthranol, the test will produce a negative test, or no red color will be formed (Evans, 2002).

Saponin, which shows positive results in the extract, is a chemical reaction from the formation of a colloidal solution and also the formation of foam (Harborne, 1987). The reaction principle in the flavonoid test is the reduction of the Zn and Mg metals used. Mg has a stronger reducing effect compared to Zn, while Zn has a greater electrode potential value compared to Mg. In this case, the Mg reduction reaction is faster and more sensitive compared to Zn reduction (Harborne, 1987).

Tannin identification was carried out by adding sodium chloride gelatin solution, which gave positive results, namely a brown precipitate. This happens because gelatin is a protein so that it will precipitate tannin. Here, a reaction occurs between tannin and gelatin to form a stable copolymer (precipitate), which is insoluble in water. The addition of sodium chloride solution causes the solution to become saturated so that the gelatin becomes easier to precipitate in other words, apart from precipitation by protein, a salting-out process also occurs by sodium chloride (Harborne, 1987).

To determine alkaloid content, an acid solution is used so that the solution has a pH < 7. At pH < 7, the alkaloid will be in a protonated state, which makes it more soluble in water but insoluble in organic solvents. Dissolving in acid also functions to remove non-alkaloid substances. Then, the alkaloids are given a chloroform solution to produce free alkaloids (Wink, 2016). One of the levels that influences the alkaloid content in a plant is the soil. The pH of the soil will affect the active content or secondary metabolites in plants. Nutrient supply also influences alkaloid levels. Lack of nitrogen nutrition will cause the accumulation of nitrogen-free secondary metabolites such as phenol. Conversely, this will also promote the synthesis of nitrogen-containing secondary metabolites such as alkaloids and cyanogenic glycosides (Yuan, et al., 2020).

Thin-layer chromatography has many advantages compared to other methods in separating specific compounds using the least amount of solvent. The polarity of the solvent or type of solvent mixture can be changed easily and quickly (Touchstone & Dobbins, 1983). The spots obtained from the combination of extracts consisted of two, namely hRf 31 from cat root extract and hRf 62 from suruhan extract. The spots obtained fluoresce blue, indicating the presence of substituted 5-OH and 3-OH flavonols (Mabry et al., 1970).

5. Conclusion

The results of determining the extract combination parameters include water-soluble compound content of 7.22%, ethanol-soluble compound content of 4.90%, drying loss of 3.29%, water content 4.41%, total ash content 7.39%, ash content acid insoluble 4.37%, chemical content identification shows the presence of alkaloids, flavonoids, tannins, saponins, glycosides and cyanogenic glycosides, but does not contain anthraquinone glycosides, and the total alkaloid content is 0.31%.

Compliance with ethical standards

Acknowledgment

The authors would like to thank the Lembaga Penelitian dan Pengabdian kepada Masyarakat Universitas Negeri Jakarta Program Penelitian Vokasi (Penelitian Kerjasama-Dalam Negeri) with agreement letter number 1/SPK/UN39.14/PPK.01.APTV/VI/2023.

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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