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Original Article

Standardization of simplisia and methanolic extract of cemba (acacia rugata (lam.) fawc. rendle) leaves endemic plant from Massenrenpulu regency of enrekang

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ABSTRACT

Massenrenpuluis an area thatstillmaintainsthe cultureof traditionalmedicineandthe use ofspices. Cemba(Acacia rugata(Lam.) Fawc. Rendle) is an endemic plantin Enrekangand is arareplantin South Sulawesi. By community Enrekang, Cemba leaves are used as spice, but it is also used to neutralize the fat from the meat so as not to cause hypertension, so the potentialis very large plant for the treatmentof hypertension or hyperglycemia. The aims of this study are to determine how to manufacture the simplisia and standardized extracts of Cemba leaves and to know the parameter and the term of raw material quality standard of traditional medicine cemba leaves (Acacia rugata (Lam.) Fawc. Rendle). The research method used was experimental. Preparation of extracts with sonicator method used methanol solvent. The extract quality requirements consists of inspection of common standard parameter and specific standard parameter based on the requirement of Indonesian Herbal Pharmacopeia. Based on the results of study of simplisia characterization and methanolic extract of Cemba (*Acacia rugata* (Lam.) Fawc. Rendle) leaves, it met with the quality requirement as raw material for traditional medicine.

Key Words: *Acacia rugata* (Lam.) Fawc. Rendle), *cemba, Massenrenpulu, standarization.*

INTRODUCTION

In Indonesia, it is known many tribes, whether officially recognized or not. In South Sulawesi, it is known three tribes namely Makassar, Bugisand Toraja. However, the Enrekang (Masserenpulu) has a different culture and behavior with other tribes. Many of the differences between Massenrempulu and Bugisor Makassar. Actualization, when writing down the Bugis origin is the unity of all the coverage contained in the region of South Sulawesi. It is both in terms of language, accent, dialect, or cultural roots. Massenrempulu is very different from Bugis and Makassar tribes. It's agricultural and generally mountainous area is as cultural differentiator with the coastal Bugis and merchants. In language, it is also very different, there are many differences which is sometimes substantial, but there are some words which both of its pronunciation and meaning almost same. There has not been much research on the history or historical research Massenrempulu ancestors. Some say that the people of Japanin habited the region and then developed into Massenrempulu[1]. The regency of Enrekangis one of the Massenrempulu group with mountainously geographical area so that it is extremely rich of plant or typical spices. One of them which are still commonly used today is cemba leaves. Leaves cembais one of typical spices of Enrekang which often used when cooking. Cemba (*Acacia rugata* (Lam.) Fawc. Rendle) is one of endemic plant in south sulawesi especially in regency of Enrekang and is a rare plant in south Sulawesi. By the citizen of Enrekang, the leaf of Cemba (*Acacia rugata*(Lam.) Fawc. Rendle) is used as spices, beside that it is also used to neutralize the fat of meat in order it will not cause the hypertension, so that this plant has potency to the healing of hypertension and hyperglycemia. Even though this plant is popular to the citizen of Massenrepulu, the regency of Enrekang, but the research and supporting scientific data have not been reported yet. Therefore, it is important to conduct research about the standardization of cemba leaves so that its use is more acceptable and it can be improved as one of Indonesian original plant which can be used as the material for traditional medicine.

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RESEARCH METHOD

The Collection and the processing of Samples: Cemba leaves samples were accommodated in Massanrepulu area, exactly in the village of Salukanan, the sub district of Baraka, the regency of Enrekang,the province of South Sulawesi, Indonesia.Cembaleaveswhichhad been obtainedweresortedwetly fromdirt, then it was driedusingan ovenwith a temperature of45 °C, after it was dry, it was smoothed andit was ready tobeused asresearchmaterial.

Simplisia Characterization

Organoleptic test: Examination of simplisia powder of cemba leaves includes shape, color, smell and taste[2,3].

Microscopic Test: Cemba simplicia leaf powder was placed on an object glass, dropped in to a solution of kloralhidrat, then it was fixed and observed the fragment using a microscope[2,3].

Determination of Drying Loss: 1gram of simplisia was carefully weighed and put into closed porcelain that had previously been heated at a temperature of105ºCfor 30minutes. The simplisia was put evenly in the crucible porcelain by shaking the crucible evenly. Put it in the oven, open the lid crucible, heated at a temperature of100ºCto105ºC, weighed and repeated heating up till the constant weigh reached[2].

Determination of Total Ash Level: A total of 2 grams of simplisia was weighed carefully, put in a porcelain crucible that has been heated and calibrated, then heated slowly till the charcoal depleted, cooled and weighed. If in this way the charcoal cannot be eliminated, added hot water, stirred, filtered through ash-free filter paper. Filter paper along filtering rest was heated in the same crucible. The filtrate was put into the crucible, evaporated and heated until the weight remains. Total ash content was calculated to weight of the extract, and expressed in% w /w[2].

Determination of acid-insoluble Ash Level: The ash produced on the determination of total ash level was heated with 25 mL of concentrated hydrochloride acid, washed using hot water, heated till the mass constant. The ash level which was insoluble in acid was calculated to the weight of simplisia, expressed in & w/w[2].

Determination of water soluble essence level: A total of 5 grams of simplisia powder was macerated with 100 ml of chloroform P (2.5 mL chloroform in 1000 mL of distilled water) for 24 hours using a pumpkin while occasionally shaken during the first 6 hours, then allowed to stand. Quickly filtered, 20 mL of the filtrate was evaporated in a shallow dish based on average (which has ditara) over a water bath until dry, the rest is heated at a temperature of 105ºC until the weight remains. Levels calculated in percent of the material that has been dried in the air[2].

Determination of ethanol soluble essence level: A total of 5 g of simplisia is macerated with 100mL of ethanol for 24 h as indicated in the monograph; using pumpkin while occasionally shaken during the first 6 hours, then allowed standing. Quickly filtered, 20mL of the filtrate was evaporated in a shallow dish based on average 4(which has been calibrated) over a water bath until dry, residual heat at temperatures up to105ºC fixed weights. Levels calculated in percent of the material that has been dried in the air.

Phytochemical profile Test: Phytochemical profile test to the content of secondary metabolites was conducted on viscous methanolic leaf extract cemba. A total of 5 gram of powder was macerated by using ethanol, then it was boiled, filtered in hot condition, and the filtrate was dried over a water bath. Then it was added by water and kloroforomas much (5: 5), and then shaken and left strong for some time to form two layers. Water layer is used to test flavonoids, phenolics and saponins. Kloroforomlayer is used to test compounds terpenoids, steroids, andalkaloids[4,5]:

Alkaloid test: Some drops of chloroform layer were added a few drops of 2N sulfuricacid, strong shaken, then allowed to stand until separation occurs. Acid layer was taken and added 1-2drops of Mayer's reagent or Dragendorff reagent, if it was formed white precipitate with Mayer reagent or orange color with Dragendorff reagent showed positive results for alkaloids.

Flavonoid test: Some drops of water layer on drop plate were added by 10 grains of magnesium metal and some drops of concentrated hydrochloric acid, the color orange, pink to red indicates the presence of flavonoid compounds

Phenolic test: A few drops of water layer on drop plate were added by 1-2 drops of iron (III) chloride 1% solution. When it was formed green to blue, it meant that there was a phenolic compound.

Saponin test: The water layer in a test tube was shaken. If the foam was formed which lasted for 5 minutes, then I meant that the samples contained saponin.

Terpenoid and Steroid test: Chloroform layer was filtered through a pipette containing it. The filtrate was pipette for about 2-3drops and allowed to dry on the drop plate. After drying, it was added by Burchad Lieberman reagent (2 drops of acetic acid anhydride and 1 drop of concentrated sulfuric acid). The formation of the red /pink/ violet means

positive terpenoids, where as if it is green or blue means positive for the presence of steroids

Extract Manufacturing: A total of 100 grams of simplisia powder of cemba leaves was extracted with ultrasonicator method using methanol as solvent. Simplisia powder was added to the closing Erlenmeyer which was added by methanol solvent in sonicator for 15 minutes and was allowed to stand for 15 minutes, filtered and performed re-extraction process 2 times. Liquid extract obtained was concentrated using a vacuum evaporator[6,7].

Extract Characterization:

Organoleptic test: Organoleptic test consists of shape, color, smell and flavor of extract[2,5].

TLC(Thin Layer Chromatography) Profile of Extract: The chromatography profile of methanolic extract of Cemba leaves was made using TLC F254 aluminium plate as stationary phase and chloroform : methanol (1:1) as mobile phase by using gallic acid as the comparison.

Determination Bacteria Total and Fungus Total

Determination of Bacteria Total: With sterile pipette, 1 mL of extract Dengan pipet steril10^{-4} dilution was implanted in NA medium, then it was incubated at 37^0C temperature for 24 hours. It was observed and calculated the colony amount growing and multiplied by dilution factor[5].

Determination of Fungus Total: The extract with 10^{-4} dilution was pipette by using sterile pipette and implanted in PDA medium, then it was incubated at 25^0C for 3 days. It was observed and calculated the colony amount growing and multiplied by dilution factor[5].

Test of Heavy Metal Contamination: The determination Pb limit at ion in the methanolic extract was conducted by usung wet destruction with nitric acid and hydrogen peroxide. Pb levels were determined by atomic absorption spectrophotometer[5,8].

RESULT OF STUDY

The collection of samples: The samples of cemba leaves was collected in Massanrepulu in Salukanan village, subdistrict of Baraka, regency of Enrekang, province of South Sulawesi.

Samples Determination: Determination samples would be made by sending sample preparation example including the roots, stems and leaves toBogoriensis Herbarium, Research Center for Biology, Indonesian Institute of Sciences (LIPI). The samplespecimenhad beendepositedat the Laboratory of Pharmacognosy-Phytochemistry.

Simplisia Characterization:

Table 1. Simplisia Characteristic Data

Test	Result
Organoleptic Test	Flavor : Unique Taste : Acid Colour : greenish yellow
Microscopic test	Fragment : Stomata, epidermis.
Determination of drying losses	Not more than 10%
Total ash level	Not more than 8,5%
Acid-Insoluble ash level	Not more than1,45%
Water-soluble essence level	Not less than32%
Ethanol-soluble essence level	Not less than10%
Phytochemical screening (chemical compound)	Essensial oil (+); Flavonoid (+); Saponin (+) Terpenoid (+); Steroid (-); Alkaloid (+)

Extract Characteristic:

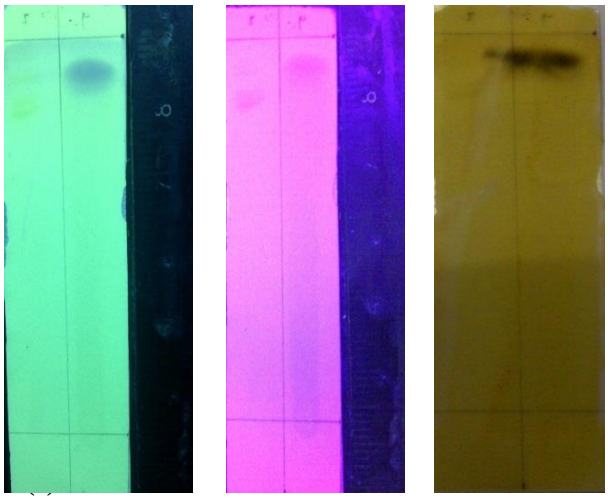
Table 2. Extract characteristic

Flavor	unique
Taste	Acid
Color	Brackish red

Table 3. Extract rendamen

Solvent	Solvent volume (mL)	Dried sample weight (g)	Extract weight (g)	Rendamen (%)
Methanol	2000	100	24,75	24,75

TLC profile of Extract:



(a) (b) (c)

Figure 1. TLC profile of phenolic compound on methanolic extract of Cemba leaves

Table 4. Metal contamination test result

Parameter	Unit	Result	Test method/technique
Timbal (Pb)	mg/kg	31,8877	SNI 01-2896-1998 Butir 5
Kadmium (Cd)	mg/kg	<0,001	SNI 01-2896-1998 Butir 5
Merkuri (Pb)	mg/kg	<0,005	SNI 01-2896-1998 Butir 5

DISCUSSION

Cemba plant is wild and very easy plant to grow in Massenrenpulu (Kab. Enrekang), by local people it is used as aspicein meat and as a typical food of Enrekang regency. Plant samples were obtained from Salukanan Village, District of Baraka, Enrekang, South Sulawesi and had been identified at the Herbarium, Research Center for Biology, Indonesian Institute of Sciences(LIPI). And it was known that its Latin name Acaciarugata(Lam.) Fawc. Rendle simplisia including FamilyFabaceae. The Processing of simplisia with artificial drying method used a special drying cabinet and oven with a temperature of not more than 50ºC, it is intended at a controlled temperature in order it will not affect the chemical compounds that are thermol abilein simplisia. Characterization test of simplisiareferred to the Indonesian Herbal Pharmacopoeia (FHI) which includes the organoleptic tests, microscopic tests, the determination of drying shrinkage, the total odor assay, the levels of acid insoluble ash, water soluble extract assay and methanol soluble and phytochemical screening (identification of chemical constituents). In organoleptic test using smell and taste organ, it was observed that the simplisia was unique in favor, greenish yellow in color and acidy in taste. At the microscopic test, a specific fragment contained the epidermis and stomata, which are known specific fragment on leaves is by the presence of stomata the drying losses test was not more than 10%. The determination of total ash level was not more than 8,5% with insoluble acid ash level not more than 1,45%, it showed that there were still inorganic content in simplisiadan still in standard range because of not more than 2%. The water soluble essence level was not more than 32% dan ethanol soluble was not more than 10%, These results indicated the essence ability of cemba leaves simplisia. The results of phytochemical screening showed that cemba leaves simplisia contained multiple compounds such as essensial oil, flavonoid, saponin, terpenoid dan alkaloid. These chemical compounds have a potency to provide biological activity of camba leaves (*Acacia rugata* (Lam.) Fawc. Rendle)simplisia. Characterization test of cemba leaf extract refers to the Indonesian Herbal Pharmacopoeia Standards and Parameters (2000). Preparation of the extract refers to the method estraksimaceration(Depkes RI, 2000) which maceration it self is extraction method without involving heating so that it will be safe for sample containing thermolabile. The extraction process used methanol as that solvent is a universal

solvent that can dissolve both polar and non polar compounds, so it is better to be used in screening study. A total of 100 gram of simplisia powder produced 24,75 gram extract with 24,75% rendamen number. The manufacturing of extract TLC profile used aluminium silica gel F254 plate as stationary phase to separate the chemical compounds according to their polarity, with methanol :chloroform (1:1) as mobile phase (eluent). The use of comparison or identical compound was gallic acid (phenolic group). The result of TLC profile showed that there was a compound which had Rf number similarity with gallic acid. Microbe contamination test aimed to observe whether camba extract contaminated by microbe or not which was able to badly affect to our health. It is also the metal It was also the metal contamination test aimed to observe the presence of metal compound in extract which intolerable in drug compound such as Pb, Cdand Hg. From the result of the test, it was found that only Pb identified in cemba leaves extract. It was caused by the contamination of air pollution where the plant was growing, but the level was still in tolerable range that was 31,8877 mg/kg. The result test of somplisia characterization and methanolic extract of cemba leaves, it can be concluded that the sample meets the requirement as traditional medicine raw material according to Indonesian Herbal Pharmacopeia and Indonesia extract monograph.

CONCLUSION

Based on the result of study, it can be concluded that the simplisia and methanolic extract of cemba leaves meets the quality requirement to be used as traditional medicine raw material.

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REFERENCES

1. Tang M. Bertindak Sendiri :SatuSistemSosialBudaya Orang Bugis, Tesis S2, Universitas Indonesia, Depok.2007
2. DepkesRepublik Indonesia. Parameter StandarUmumPembuatanEkstrakTumbuhanObat. Jakarta, 2000.
3. DepkesRepublik Indonesia, Farmakope Herbal Indonesia :Edisi I, Jakarta, 2008.
4. DepkesRepublik Indonesia. MateriaMedikaIndonesia :Jilid V. Jakarta, 1989.
5. Helmi A et al., StandardisasiEkstrakEtanolDaun Eugenia CuminiMerr. Journal Sains. 2006 : 11-2.
6. Bayon M et al. Evaluation of different sample extraction strategies for selenium determination in selenium-enriched plants (Allium sativumand Brassica juncea) and Sespeciation by HPLC-ICP-MS, Elsivier Journal Talanta 68. 2006 :1287–1293.
7. Eng At et al. Evaluation of surfactant assisted pressurized liquid extraction for the determination of glycyrrhizin and ephedrine in medicinal plants, Elsivier Journal AnalyticaChimicaActa. 2007;583: 289–295
8. Rivai H et al. KarakterisasiEkstrakDaunDewa (Gynurapseudochina (L.) DC) denganKromatografiCairKinerjaTinggi, JurnalFarmasi Indonesia 2011; 5:134 -141