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Standardization of Sambiloto (*Andrographis paniculata* Ness) Extract Obtained by Hydrotropic Microwave Assisted Extraction

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Abstract: This recent days, the utilization of herbal remedies has increased tremendously. Even though herbal remedies are reported have several benefits, its utilization require several step before it can be used for medicinal purposes. One of them is called standardization process. Standardization of hydrotropic extract of sambiloto (*Andrographis paniculata* Ness) obtained by microwave assisted extraction process was conducted by testing the specific and nonspecific parameters of the plant extract. The hydrotrope used in the microwave assisted extraction was sodium benzoate. The microwave extraction process was conducted at hydrotrope concentration of 2 M, extraction duration of 15 min, and power level of 39.9 W. The specific parameters are including organoleptic analysis, solubility, and chemical analysis. The nonspecific parameters are including lost on drying, heavy metal contamination, microbial contamination, and ash content. The determination process identify that the herbs was species of *Andrographis paniculata* Ness. The research showed that the water soluble extractive value was found to be 73.6 %, while the alcohol soluble extractive value was found to be 73.2%. The extract was analysed by using TLC to get the chromatogram profile. The non specifik parameter analysis revealed that the loss on drying was found to be 8.67%, the heavy metal and microbial contamination were higher than the WHO standard.
Keywords : sambiloto, hydrotropic, sodium benzoate, standardization, extract, microwave assisted extraction, *Andrographis paniculata* Ness.

Introduction

All culture throughout history has been using herbs as medicine for their healthcare. Over the past three decades, the use of herbal medicinal products and supplements has increased tremendously. It is now not less than 80% of people worldwide relying on them for some part of primary healthcare[1]. World Health Organization encourages, recommends and promotes herbal medicine in natural health care programmes. It is mainly due to herbal remedies are easily available at low cost, safe and people have faith in them[2].

Even though herbal remedies are reported have several benefits, its utilization require several step before it can be used for medicinal purposes. The National Agency of Drug and Food Control of Indonesia require both pharmacology and toxicity test, and also standardization on the development of traditional medicine into standardized herbal medicine.

Standardization is a quality assurance program for production and manufacturing of herbal drugs. It is an important step for the establishment of a consistent biological activity, a consistent chemical profile[3]. Standardization of herbal medicines can also be describe as the process of prescribing a set of inherent characteristics, constant parameters, definitive qualitative and quantitative values that carry an assurance of quality, efficacy, safety and reproducibility[4]. Standardization also involves the study from birth of plant to its clinical application[5].

The WHO guidelines stated that there are six major methods for evaluation and standardization of herbal drugs[5]. The methods and the evaluation parameters are including: (i) authentication (parts of plant collect, regional status, family, biological source, and chemical constituent); (ii) organoleptic evaluation (odor, taste, size, shape and special feature); (iii) microscopy evaluation (leaf content, trichome, stomata, and quantitative microscopy); (iv) chemical evaluation (chemical assay, chemical test, and physicochemical screening); (v) Physical evaluation (moisture content, viscosity, melting point, solubility, optical rotation, refractive index, ash value, extractive value, volatile oil content and foreign matter); (vi) biological evaluation (microbial contamination, pesticides contamination, and pharmacological activity of drugs)[5].

Furthermore, the quality of plant extract was influenced by several parameters including extraction methods, extracting liquid, solid solvent ratio[4,6]. In the case of sambiloto extraction, extraction methods employed were maceration[7], soxhletation[8], microwave assisted extraction[9], refluxation[9], and supercritical fluid extraction[10]. Extraction liquid commonly applied in the sambiloto extraction were water, methanol, ethanol[8-10].

Standardization of extract obtained from those methods already reported. Singh et al. (2012) reported the standardization of *Andrographis paniculata* Ness extract obtained by hydro-alcoholic extraction[11]. Meanwhile, the application of microwave assisted extraction of *Andrographis paniculata* Ness with hydrotrope solution (sodium benzoate) as the medium was firstly introduced[12]. Combination of hydrotrope and microwave assisted extraction was proved to be a safe and effective medium for the extraction process of Sambiloto[13]. The finding of the optimum condition for the hydrotropic-microwave assisted extraction of sambiloto was then followed by the standardization process.

The National Agency of Drug and Food Control of Indonesia recommended common standardization parameters for medicinal herbal extract. The parameters consist of non specific and specific parameter. Non specific parameter are including lost on drying, ash content, water content, heavy metal contamination, and microbial contamination. The specific parameters are including extract identity, organoleptic analysis, solubility in certain solvent, and chemical analysis.

Experimental

Raw Material and Chemicals

The aerial parts of Andrographis paniculata were collected from local plantation in Gunungpati, Semarang, Central Java, Indonesia. The hydrotrope used as the medium of the microwave assisted extraction was sodium benzoate (Sigma-Aldrich, 99%).

Apparatus

The extraction process was conducted in a microwave extractor. The extractor was a modified domestic microwave, equipped with extraction flask and a spiral condensor.

Extractive preparation

Aerial parts of Andrographis paniculata were collected, dried and powdered. Twenty grams of dried powder was subjected in 200 ml of hydrotrope solution (2M). The mixture placed in 500 ml round bottom flask and extracted in a modified microwave extractor for 15 minutes at system powers of 39,9W. The mixture then was allowed to stand for 1 hour and then filtered. The residu was washed with water and double volume of demin water was added. The extract was then centrifuges for 15 minutes at 4000G, and dried. The extract used for evaluation of both specific and nonspecific parameters.

Determination of specific parameters

Extract identity

Extract identity investigated by conducted determination process.

Organoleptic analysis

Organoleptic analysis was conducted by visually observation for its color and consistency), and determined using sensory organs for its smell and taste.

Water soluble extractive value

Five grams of extract (W_1) were taken in a glass stoppered flask. It was then macerated for 24 hours in 100 ml of chloroform. The mixture was stirred for the first 6 hours, and let for the remaining 18 hours. The extract was filtered and 20 ml of the filtrate was pipette out in a pre-weighed shallow and flat pan (W_2) and evaporated to dryness on a water bath. It was kept in a hot air oven for 5 hr at l05°C, cooled in desiccators for 30 minutes and weighed (W_3).

The water soluble extractive value can be calculated by Equation 1.

$$\text{Percentage of water soluble extractive value} = \frac{W_3 - W_2}{W_1} x100\% \quad (1)$$

Alcohol soluble extractive value

Five grams of extract (W_1) were taken in a glass stoppered flask. It was then macerated for 24 hours in 100 ml of ethanol (96%). The mixture was stirred for the first 6 hours, and let for the remaining 18 hours. The extract was filtered in an accurate way to prevent ethanolic evaporation. Twenty ml of the filtrate was pipette out in a pre-weighed shallow and flat pan (W_2) and evaporated to dryness on a water bath. It was kept in a hot air oven for 5 hr at l05°C, cooled in desiccators for 30 minutes and weighed (W_3).

The alcohol soluble extractive value can be calculated by Equation 2.

$$\textit{Percentage of alcohol soluble extractive value} = \frac{W_3 - W_2}{W_1} x100\% \quad (2)$$

Chemical analysis

The extract was analysed by using TLC to get the chromatogram profile

Determination of nonspecific parameters

Lost On Drying

One gram of the extract was weighed (W_1) in a careful way. The extracts put in a shallow capped weighing bottle, which has been heated under a temperature of 105°C for 30 minutes, and make it even (W_2). The shallow capped was then put in the drying room under a temperature of 105°C to reach a constant weight. Before the drying phase, add 1 gram of drying silica, which has been carefully weighed, dried, and stored in an exicator under room temperature. Mix the silica in an even way into the hot extract, then dry the mixture again under a temperature of 105°C to reach a constant weight.

Ash content

Two grams of the extract (W_1) was accurately taken in a previously ignited and tarred Silica dish (W_2). The material was spread evenly and ignited in a muffle furnace. The temperature is gradually increased to 600°C until the colour of the sample turn white which indicated the absence of the carbon. The crucible was cooled in desiccators and allowed to stand for 30 minutes and weighed (W_3).

$$\text{Percentage of ash content} = \frac{W_3 - W_2}{W_1} x100\% \quad (3)$$

Heavy metal contamination

The cadmium and lead content of the extract were determined by using Atomic Adsorption Spectrofotometry

Microbial contamination

One gram of extract was suspended in a 10 ml of PDF in a sterile glass jar. The samples were dilute and mixed with PCA medium. It was then incubated under a temperature of 35-37°C for 24 hours. The colony was then observed and counted for its colony number.

Result and Discussion

Specific parameter

Extract identity

The extract identity was conducted through determination process which was in the Laboratory of Ecology and Biosystematic, Diponegoro University. The determination process revealed that the plant checked was Sambiloto (*Andrographis paniculata* Nees) with plant classification as follows:

Classification

Kingdom : Plantae
Division : Spermatophyta
Class : Dicotyledoneae
Ordo : Lamiales
Family : Acanthaceae
Genus : Andrographis
Species : Andrographis paniculata Ness. (Sambiloto)

Determination

1b, 2b, 3b, 4b, 12b,13b,14b,17b,18b, 19b,20b, 21b, 22b, 23b, 24b, 25b, 26b, 27b, 28b, 29b, 30b, 31b, 403b, 404b, 405b,414a, 451b. 466b, 467b, 468b, 469b, 470e, 541b, 542c, 549b, 550b, 551b, 560b, 561b, 562e, 570b, 576b, 577b, 578a, 579b, 580b, 581a, 582c, 583b, 584b, 601a, 602a, 603b, 604c, 605b, 606b, 610b, 612a,.....Family 187: Acanthaceae.....1b, 36b, 39b, 40b, 42a, 43a, 44a.....Genus 37. Andrographis...Species: *Andrographis paniculata* Ness. (Sambiloto)

Organoleptic analysis

Organoleptic analysis utilized sensory organs in order to describe the consistency, color, smell, and taste of the *Androgprahis paniculata* extract obtained by hydrotropic-microwave assisted extraction. The consistency of the extract was solid of a dry powder. The color of the extract was green as shown on figure 1, which gave an aromatic smell and bitter taste.

Figure 1. *Andrographis paniculata* Ness extract obtained from hydrotropic microwave assisted extraction

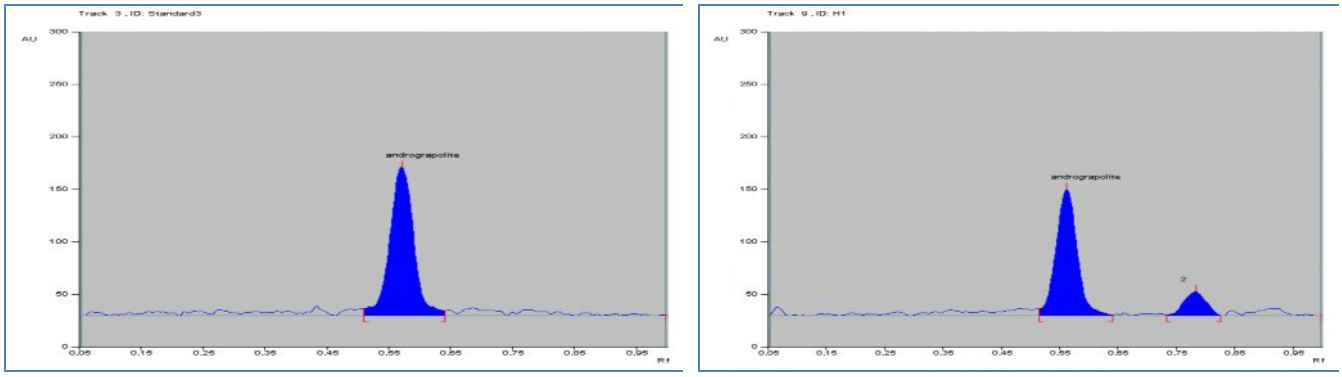
Singh et al (2012) standardized hydro-alcoholic extract of *Andrographis paniculata*. They found that the colour of their extract was brown. Hydrotropic microwave assisted extraction has proven to give a better colour of the extract. Microwave assisted extraction has also reported yielded a better color and stabile extract[14].

Water and alcohol soluble extractive

The presence of the sugar, acids and inorganic compounds can be indicated from its water-soluble extractive value. While the presence of polar constituents like phenols, alkaloids, steroids, glycosides, flavonoids and secondary metabolites in the plant sample can be indicated from its alcohol-soluble extractive value The water and alcohol soluble extractive value in the *Andrographis paniculata* extract obtained from hydrotropic microwave assisted extraction were found to be 73,6 % w/w and 73,2% w/w, respectively. The water and alcohol soluble extractive value of the *Andrographis paniculata* extract obtained from hydrotropic microwave assisted extraction were higher than the limit of the water and alcohol soluble extractive value stated by WHO. The WHO monographs stated that the water and alcohol soluble extractive of *Andrographis paniculata* Ness not less than 18 and 13%, respectively[15].

Chemical analysis

The chemical analysis was conducted by using Thin Layer Chromatography. The chromatogram profile of andrographolide standard was shown on Figure 2.a. The chromatogram profile of andrographolide standard has shown at retention factor of 0.56-0.58. While the chromatogram profile of sambiloto hydrotropic-microwave assisted extraction extract was shown on Figure 2.b.



(a) **(b)**

Figure 2. Chromatogram profile of: (a) andrographolide standard, (b) sambiloto hydrotropic-microwave assisted extraction extract

The andrographolide content of *Andrographis paniculata* Ness hydrotropic-microwave assisted extraction extract was 1.32%. It was higher than the andrographolide content of sambiloto extract obtained by cold maceration with a 1 : 1 mixture of dichloromethane and methanol[16]. It was found that the andrographolide content of the extract form maceration process of *Andrographispaniculata* Ness harvested after it was flowering, was up to 1.2%.

Non specific parameter

Lost on drying

Loss on drying test is designed to measure the amount of water and volatile matters in a sample when the sample is dried under specified conditions. Loss on drying can also indicate the hygroscopicity of extract[17]. The research showed that the sambiloto hydrotropic microwave assisted extraction extract was hygroscopic, where the loss on drying of *Andrographis paniculata* Ness hydrotropic microwave assisted extraction extract at 105°C was found to be 8.67%. It was higher than the loss on drying of hydro-alcoholic extract of *Andrographis paniculata Nees extract (4.54%)[11]. WHO monographs stated that the loss on drying of Andrographis* paniculata Ness is not more than 10%[15].

The high value of loss on drying indicate the high water content and volatile matters of the hydrotropic microwave assisted extraction extract. It could be due to the utilization of hydrotrope as the solubilization agent. Hydrotropes are compound that solubilises hydrophobic substances in aqueous solution. Hydrotropes are able to increase solute solubility in water due to the formation of organized assemblies of hydrotrope molecules at critical concentration[18].

Total Ash

Total ash value of plant material indicated the amount of minerals and earthy materials attached to the plant material. Analytical results showed total ash value of *Andrographis paniculata* extract was 23,4% w/w.

Heavy metal contamination

Cd concentration of the *Androgprahis paniculata* extract obtained by hydrotropic-microwave assisted extraction was 0.0728 ppm. It was lower than WHO prescribsion. WHO prescribed limit for Cd contents in medicinal plant is 0.3 mg/kg and the maximum acceptable concentration for food stuff is around 1 ppm (Ziarati, 2012). Cd intoxication can lead to kidney, bone and pulmonary damages. Moreover, the concentration of Pb of the *Androgprahis paniculata* extract obtained by hydrotropic-microwave assisted extraction was 1.868 ppm. The WHO maximum limit of Lead prescribed in herbal medicines and products is 10 ppm[19].

Microbial contamination

WHO stated that herbal drugs must meet the modern hygienic standard. The presence of microbial contaminant in non sterile products can reduce, deteriorate or even inactivate the active constituent of the products.

The research showed that the total bacteria of the *Andrographis paniculata* Ness hydrotropic microwave assisted extraction extract was up to 2,2 10^7 CFU/gr. The contamination level was failing the WHO criteria. WHO stated the maximum contamination bt aerobic bacteria was 10^5 CFU/gr[20]. The high contamination could be due to the result of a series of environmental factors influences such as temperature, humidity and pre-harvesting and post-harvesting condition, handling practices, and the storage conditions of crude and processed medicinal-plant materials. Microbial contamination on herbal product can occur during the collection of raw materials and processing them into finished products due to poor quality control and hygiene practices during manufacture.

Conclusion

The specific and non specific parameters of the *Androgprahis paniculata* extract obtained by hydrotropic-microwave assisted extraction was conducted. The research showed that the water soluble extractive value was found to be 73.6 %, while the alcohol soluble extractive value was found to be 73.2%. The lost on drying was found to be 8.67%. The heavy metal contamination was lower than the limit set by WHO, while the microbial contamination was higher.

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