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REVIEW ARTICLE

SPECIFIC AND NON-SPECIFIC PARAMETERS OF ETHANOLIC EXTRACT OF JOMBANG LEAVES (*TARAXACUM OFFICINALE* F.H. WIGG.)

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ABSTRACT

Taraxacum officinale F.H. Wigg., commonly known as Jombang in Indonesia, is one of the 30 medicinal plants processed into Scientific Jamu. Jombang has been used empirically to both prevent and treat liver illness. In order to maintain consistency in quality, safety, and efficacy, Jombang extract must be standardized; as a result, extracts used as raw materials for medicines have constant specific and non-specific parameters and are expected to meet quality requirements to be accepted in healthcare settings. This research intended to analyze the general standard parameters of the 50%-ethanolic extract of Jombang leaves from Tawangmangu, Indonesia. The specific parameters are organoleptic properties, phytochemical composition, water-soluble and ethanol-soluble extract content; while the non-specific parameters consist of water content, ash content, acid-insoluble ash content, heavy metal, and microbial contamination. Both parameters are determined using the method established by the Indonesian Ministry of Health for General Standard Parameters of Extracts. The results indicated that the 50%-ethanolic extract of Jombang leaves had thick extract, dark brown color, distinctive smell, and slightly bitter taste. Alkaloid, saponins, flavonoids, and steroids were found in the extract's phytochemical screening results. It contained 67.62% of water-soluble extract and 18.83% of ethanol-soluble extract. Mold and yeast contamination were negative (<1.0 x 10^3 colonies/ g), Pb and Cd levels were undetectable (<0,009 ppm and <0,00011 ppm, respectively), while the water content value was 13.26%, the ash content value was 9.87%, and the acid insoluble ash content value was 0.48%.

KEYWORDS

Taraxacum officinale; leaves; ethanolic extract; specific parameters; non-specific parameters

1. INTRODUCTION

Jombang (*Taraxacum officinale* F.H.Wigg) is one of the plants used in scientific herbal formulations for liver dysfunction (Balai Besar Litbang Tanaman Obat dan Obat Tradisional, 2019). Jombang, commonly called dandelion, is an annual growing plant from the Asteraceae family. This plant grows as a common weed in gardens, agricultural crops, prairies, and deserts across Europe, Asia, and North America (Di Napoli and Zucchetti, 2021). Jombang has been used for decades as a medicinal herb. Young roots and shoots are the main parts used for medicinal purposes (Rasool and Sharma, 2014). Due to the phytochemical substances included in the plant's blossoms, leaves, stems, and roots, jombang offers a wide range of therapeutic uses. The primary phytochemicals are carotenoids, flavonoids (such as chrysoeriol, quercetin, and luteolin-7-glucoside), phenolic acids (such as caffeic acid, chlorogenic acid, and chicoric acid), polysaccharides (such as inulin), sesquiterpene lactones (such as taraxinic acid, taraxacoside, 11β, 13-didydrolactucin, ixerin sterols (e.g. taraxasterol, =sitosterol, stigmasterol); triterpenes (e.g., -amyrin) (Singh, Malhotra and Subban, 2008; Mir, Sawhney and Jassal, 2013). Jombang has been widely used for the treatment of liver and spleen diseases (Schütz, Carle and Schieber 2006) and chronic liver diseases (Martinez et al., 2015; Devaraj, 2016).

Jamu Saintification aims to increase the availability, safety, and efficacy of herbs and to promote the use of herbal medicine for both self-medication and treatment in primary health care facilities (Badan Penelitian dan Pengembangan Kesehatan, 2010). The quality of raw materials is one of the main prerequisites for determining the effectiveness and safety of herbal medicines. However, clinicians who wish to join Jamu Saintification are often faced with a significant problem, the availability of quality and standardized herbal raw materials. Therefore, various efforts have been made to support the availability of quality herbal raw materials from various regions in Indonesia, including cultivation and standardization processes (Jayani and Putri, 2020).

In this study, tests were carried out on specific and non-specific parameters as part of the standardization of Jombang leaf extract (*Taraxacum officinale* F.H.Wigg). Standardization of jombang leaf extract is needed to maintain uniformity in quality, safety and efficacy; Thus extracts used as raw materials for medicines will have constant specific and non-specific parameters and are expected to meet quality requirements to be accepted in health services.

2. LITERATURE REVIEW

In the last two decades, traditional health services have become increasingly popular and in demand by the community (Purwadianto et al., 2017). About 49.53% of Indonesia's population takes herbal medicine to both maintain health and treat illness, according to Basic Health Research (Riset Kesehatan Dasar/RISKESDAS), which was conducted in

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2010. Ninety-six percent or so of the Indonesian people who used herbal medication claimed to have experienced its advantages. According to the 2010 RISKESDAS findings, 55.3% of those who used herbal medicine ingested it in liquid form (infusum or decoction), with the remaining 44.7% taking it as powder, diced, pills, capsules, or tablets (Badan Penelitian dan Pengembangan Kesehatan, 2010).

Basically, socially and culturally, jamu has been accepted by the Indonesian people as a way of traditional medicine. This is proven by the use of herbal medicine by our ancestors for generations (Purwadianto et al., 2017). However, herbal medicine has not been well received by the medical profession as an alternative therapy. This is understandable because in general herbal medicine does not yet have solid scientific evidence regarding its effectiveness and safety. Besides, the medical profession (doctors and dentists) is obliged to carry out clinical decisions (choice of therapy) based on evidence (evidence-based medicine) (Siswanto, 2012).

In an effort to elevate the culture of using traditional health, the Government of Indonesia through the Ministry of Health, has developed Jamu into Standardized Herbal Medicine and Phytopharmacy to provide scientific evidence of its effectiveness and safety. This effort includes a strategy called Jamu Scientification, which is scientific proof of herbal medicine through health service-based research stipulated in Minister of Health Regulation No. 003/I/MENKES/2010 (Purwadianto et al., 2017). Until now, there are eleven scientific herbal formulas made from a mixture of various selected ingredients that can be found in conventional herbal medicine (Balai Besar Litbang Tanaman Obat dan Obat Tradisional, 2019).

Standardization of medicinal plants is a process to ensure the identity, quality and purity of medicinal plant raw materials (Jayani and Putri, 2020). The standardization of medicinal plants is an important stage in the development of herbal medicines (Muthia *et al.*, 2021). Standardization of medicinal plants needs to be done because the raw materials obtained from nature are very diverse in terms of quality and quantity. In this context, raw materials for herbal medicines depend on many factors such as environment, soil, climate, seed quality, harvesting, and raw preparation which include drying methods and storage conditions (WHO, 2009; Indonesian Ministry of Health, 2017).

3. Research Method

3.1 Chemicals

In this study, chemicals such as anhydrous acetic acid, formic acid, ethanol, aquadest, Mayer, Wagner, and Dragendorff reagents, as well as magnesium powder, sodium hydroxide, iron (III) chloride, anhydrous acetic acid, formic acid, acetic acid, peptone, plate count agar (PCA), distilled water agar (DWA), and potato dextrose (PDA) were used

3.2 Plant Materials

The leaves of Jombang were collected from Tawangmangu, Central Java. The sample's determination was conducted at B2P2TOOT refer to letter No. KM/04/02/2/761/2022.

3.3 Extract Preparation

The Jombang leaves was properly cleaned using tap water, sorted when it still wet, coolly dried, and ground into powder. Two hundred grams of powdered simplicial were weighed, and 50% ethanol (as much as 2 L) was added and macerated for 24 hours, with repeated shakes for the initial 6 hours and left for 18 hours. The macerate was then separated using filtration. The residue was macerated four times more in the same process. The filtrate was collected and concentrated using a rotary evaporator at 50°C resulting on a pourable viscous extract (Indonesian Ministry of Health, 2017).

3.4 Identification of Phytochemistry Content

3.4.1 Alkaloid Test

A gram of the material was crushed using a mortar together with a little of sand and chloroform. Then, 5 mL of 0.05 N ammonia solutions in chloroform were added. The mixture then was agitated for a few minutes before being filtered into the test tube. Two layers form in the filtrate after adding H2SO4 2N and often shaking it. The water phase/ upper layer is separated and examined using reagents from Mayer, Wagner, and Dragendorff. The presence of alkaloid group chemicals is indicated by the formation of sediment (Al-daihan and Bhat, 2012; Solihah et al., 2018).

3.4.2 Flavonoid Test

The test tube was filled with 0.5 g of sample in total, 5 mL of ethanol, and heated for 5 minutes. The extract was next filtered, and a few drops of strong HCl were then added to the filtrate. Then 0.2 mg of magnesium powder was added. Flavonoid chemicals are present if it appears red (Al-daihan and Bhat, 2012; Solihah et al., 2018).

3.4.3 Saponin Test

About 10 mL of hot water were added to a sample of 500 mg.. Then chilled and shaken vigorously. Saponin group chemicals were found if there was a stable foam 1 cm or higher in height. Additionally, adding 1 drop of HCl 2 N will not cause the foam to disintegrate. (Indonesian Ministry of Health, 1977; Solihah et al., 2018)

3.4.4 Tannin Test

In 50 mL of distilled water, 500 mg of the sample was added in total. After 15 minutes of boiling, the mixture was cooled. One percent $FeCl_3$ was drip-injected into five milliliters of filtrate. It indicates the presence of chemicals of the tannin class if the color changed to a greenish black (Al-daihan and Bhat, 2012; Solihah et al., 2018).

3.4.5 Steroid Test

In a mortar, 2 g of materials were crushed. A little chloroform, sand, and 5 mL of 0.05 N ammonia solutions were then added. The composite then was agitated for a while before being filtered into the test tube. After shaking the filtrate often and adding H2SO4 2N, two layers began to develop. The lower layer of solution was separated, transferred to the drop plate, and given time to dry. A well-mixed addition of anhydrous acetic acid followed drying. Add three drops of strong sulfuric acid after that, and then check the color. If the color change to blue or green, this denotes the appearance of steroid chemicals (Al-daihan and Bhat, 2012; Solihah et al., 2018).

3.5 Determination of Specific Parameter

3.5.1 Organoleptic Properties

Organoleptic examination is carried out by describing the form, color, aroma and flavor of the extract (Indonesian Ministry of Health, 2000). The declaration “odorless”, “almost odorless”, “faint characteristic odor”, or others, were concluded by observation once the extract was exposed for 15 minutes to the air.

3.5.2 Water-Soluble Compounds

A total of 1.0 g of the extract was macerated using 25 mL of water-chloroform (39:1) for 24 hours using a plugged flask while shaking repeatedly for the initial 6 hours and was aged for 18 hours then filtered. The filtrate was dried through evaporation in a tarred evaporator cup. The residue was heated at 105°C until constant weight was reached. Furthermore, the percentage of compounds dissolved in water is calculated against the initial extract weight (Indonesian Ministry of Health, 2000).

3.5.3 Ethanol-Soluble Compounds

A total of 1.0 g of the extract was macerated in 25 mL of ethanol (39:1) for 24 hours in a plugged flask, stirring frequently during the first 6 hours. After 18 hours, the mixture was allowed to sit for another 18 hours before being filtered. In a tarred evaporator cup, the filtrate was dried by evaporation. The residue was heated at 105°C until constant weight was reached. Furthermore, the percentage of compounds dissolved in water is calculated against the initial extract weight (Indonesian Ministry of Health, 2000).

3.6 Determination of Non-Specific Parameter

3.6.1 Water Content

Distillation is used to determine the water content. About 5 g of the extract were added to 200 mL of xylol that had been diluted with water, and the mixture was then heated at 110°C for one hour. The water volume is measured and determined once the layers have completely separated. Weighing and calculating the water content in percent by weight (Indonesian Ministry of Health, 2000).

3.6.2 Total Ash Content

A silicate crucible containing about 2 g of the extract was heated at 650 °C using a furnace after being heated on a hot plate until no charcoal was present. After cooling the silicate crucible to room temperature in a desiccator, it was weighed, and the findings were computed, represented as%w/w, and weighed. (Ministry of Health of Indonesia, 2000).

3.6.3 Acid Insoluble Ash Content

The ash, which had a total ash content, was boiled for 5 minutes in 25 mL of diluted sulfuric acid, then the insoluble acid was collected and filtered

through ash-free filter paper. After being washed in hot water and placed in a silicate crucible, the ash was then heated in a furnace at room temperature for 650°C until all of the charcoal was gone. The material's weight in percentage w/w and weight is used to compute the amount of acid-insoluble ash. (Indonesian Ministry of Health, 2000).

3.6.4 Heavy Metal Contamination

The content of heavy metals in the extract was analyzed using atomic absorption spectrophotometry. The determination of heavy metals examined include the concentration of lead and cadmium (Indonesian Ministry of Health, 2000).

3.6.5 Contamination of Microorganisms

Total plate count: Peptone dilution solutions containing 9 mL were utilized in 5 tubes (PDF). The sample preparation homogenization results were plated using a 1 mL dilution into a tube containing the first PDF diluent until a 10^{-2} dilution was obtained, shaken to achieve homogeneity, and then further diluted until a 10^{-6} dilution was reached. Each dilution was duplicated in one milliliter on a petri dish, which was then covered with 15 to 20 milliliters of medium plate count agar (PCA). This procedure was repeated several times until the suspension was dispersed evenly throughout the petri dish. For the purpose of evaluating the sterility of the media and diluent, a blank control test was conducted. The petri dish was incubated upside down at 35–37 °C for 24–48 hours after the medium had set. Expanding colonies' numbers were counted and noted (Indonesian Ministry of Health, 2000).

Mold and yeast count: About 9 mL of distilled water agars (DWA) at a concentration of 0.05% were placed in three tubes. The sample preparation was homogenized by adding 1 mL of a 10^{-1} dilution to the first DWA tube, stirring until a 10^{-2} dilution was obtained, and then repeating the process up to 10^{-4}. The PDA's surface was coated with a total of 0.5 mL of each dilution, which was then evenly distributed and made into a duplo by shaking and spinning it. To assess the sterility of the media and diluent, a blank test was performed by placing the media on one petri dish and another petri dish filled with medium and diluent, then leaving to harden. All petri dishes were cultivated at 20 - 25°C for 5 - 7 days. After 5 days of incubation, the number of fungal colonies increased, reaching a peak at 7 days. A plate with 40-60 mold/yeast colonies was discovered. (Indonesian Ministry of Health, 2000).

5. FINDINGS AND DISCUSSION

Standardization is a mechanism that ensures that every marketed drug, particularly herbal medicines, has an active component at the appropriate quantity or dose and produces a therapeutic effect. This is an important stage in guaranteeing the uniformity of biological activity, chemical profile, or simply the quality assurance of medicinal ingredients used in the creation of herbal medicine products. Furthermore, standardization of extracts has the potential to boost the commercial worth of herbal medicine makers (Novyra Putri et al., 2021).

The existence of various chemical components in simplicia and ethanolic leaves extract was tested using standard procedures and methodologies. The findings show that flavonoids, saponins, and steroids are present (Table 1).

Table 1: Phytochemistry content of Jombang leaves

Phytochemistry Content	Screening Result
Alkaloid	+
Flavonoid	+
Saponin	+
Tanin	-
Steroid	+

By establishing specific and nonspecific extract characteristics, a 50%-ethanolic extract of Jombang leaves was standardized. Table 2 displays the results of specific and nonspecific characteristics of a 50%-ethanolic extract of Jombang (*Taraxacum officinale* F.H. Wigg.) leaves. This standardization's determination findings require a reference to demonstrate that the extract fulfills the standards. The Indonesian Ministry of Health and other sources have yet to issue an official standardization reference for a 50%-ethanolic extract of Jombang leaves.

Specific criteria are linked to the original plant ingredients and chemicals responsible for pharmacological action. On the other hand, non-specific parameters are connected to environmental factors such contamination with pollutants or the use of additives, compounds produced by the interaction of constituent compounds with contaminants, or chemicals that can alter and affect the stability and safety of drugs. (Indonesian Ministry of Health, 2000; Sari, Elya and Basah, 2020).

Table 2: Determination of specific and non-specific of ethanolic extract of Jombang Leaves

	Parameter	Result	Requirement
Specific parameter	Organoleptic	Thick extract, dark brown color, distinctive smell and slightly bitter taste	-
	Water soluble content	67.62%	-
	Ethanol soluble content	18.83%	-
Non-specific parameter	Water content	13.26%	<10%
	Total ash content	9.87%	-
	Acid insoluble ash content	0.48%	-
	Heavy metal contamination: Pb Cd	 Negative Negative	 <10 ppm <10 ppm
	Microbial contamination: Total plate count Mold and yeast	 1.6×10^3 colonies/ g Negative	 $<1.0 \times 10^4$ colonies/ g 1.0×10^3 colonies/ g

Organoleptic characteristics, water-soluble and ethanol-soluble extract content are the particular criteria. The organoleptic test of extract seeks to characterize the form, odor, color, and flavor of the extract (Indonesian Ministry of Health, 2000). It was found that the 50%-ethanolic extract of Jombang leaves had a thick extract, dark brown color, unique scent, and somewhat bitter taste. It had a water-soluble extract content of 67.62% and an ethanol-soluble extract content of 18.83%. The determination of soluble chemicals in water and ethanol aims to provide an approximation of the polar (water soluble) and semi-polar to nonpolar (soluble ethanol) active molecules. (Solihah et al., 2018).

Total ash concentration, acid insoluble ash content, Pb and Cd levels, and microbiological contamination are considered as nonspecific extract parameters. The water content of the extracts was measured in order to determine a minimal limit or range of water content left in the extract after drying (Indonesian Ministry of Health, 2000). The water content of a 50%-ethanolic extract of Jombang leaves was determined to be 13.26%. As a result, the water content in the extract exceeded the maximum level authorized by the Indonesian Ministry of Health (limit value 10%). The extract required to be dried again before use and stored in a low humidity environment because it was hygroscopic in order to prevent microbial infection. (Solihah et al., 2018).

Ash content measurement strives to provide a summary of internal and external mineral content produced during the process up until extraction. The material contamination has been discovered since the harvesting procedure, time to harvesting, drying process, and extraction methods employed (Solihah et al., 2018). The extract yielded a total ash content of 9.87% and an acid insoluble ash content of 0.48%. Total ash and acid-insoluble ash levels are crucial indicators of herbal medicine quality and purity. Total ash is made up of "physiological ash," which comes from plant tissue, and "non-physiological ash," which comes from environmental pollutants such sand and dirt. Total ash content alone is insufficient to evaluate the quality of herbal medicines because plant materials frequently contain considerable amounts of physiological ash, calcium oxalate in particular. As a result, the acid-insoluble ash concentration is another indicator of herbal quality (Dharmadasa et al., 2014).

Contamination of heavy metal must be limited because it can induce a serious health problem in the human body. Crude drug as a raw material for herbal medicine must meet the quality standard limitation of

contaminants. Toxicity due to heavy metal such as Lead (Pb) can induce neurological disorders and kidney damage. Cadmium (Cd) can induce kidney damage, lung cancer and bones abnormality (Jayani and Putri, 2020). The determination of Pb and Cd content seeks to ensure that the heavy metal content in the extract does not exceed the limit set by the Indonesian Ministry of Health for broad standardized criteria of medicinal plant extract due to its toxicity (Indonesian Ministry of Health, 2000). The extract's Pb and Cd concentration was negative (10 ppm). Toxic metal contamination can be purposeful or unintentional. Heavy metal contamination in herbal treatments, such as mercury, lead, copper, cadmium, and arsenic, can be linked to a variety of factors, including environmental pollution (Novyra Putri et al., 2021).

The intention of microbial contamination testing is to make sure that the extract does not contain pathogenic or non-pathogenic microorganisms in excess of the established limits because doing so compromises the extract's stability and poses a health risk (toxic) (Indonesian Ministry of Health, 2000). Total plate numbers and yeast numbers are used in microbial contamination testing. A total plate number of 1.6 x 10^3 colonies/ g was obtained from a 50% ethanolic preparation of Jombang leaves. The result was still within the permissible range because it was less than the maximum limit of 1.0 x 10^4 colonies/g stipulated in the Indonesian FDA's book of Monographic Extract of Medicinal Plants. The yeast number determined was negative and did not surpass the standards imposed by the Indonesian FDA of 1.0 x 10^3 colonies/g. The active chemical flavonoid included in Jombang leaves extract can also suppress the bacteria`s growth or availability of microorganisms in the extract, resulting in low growth of bacteria and mold/yeast. This microbiological development is dependent on various elements, including the environment; this has a significant impact on the overall quality of herbal products. Herbal remedies typically contain bacteria and fungus derived from the soil. Harvesting procedures, wet sorting, dry sorting, and inadequate storage can all lead to increased contamination, such as the growth of *E. coli* or *Salmonella spp*. While many bacteria and fungus are derived from natural microflora, only aerobic spore-forming bacteria frequently prevail.

6. Conclusion

The result indicated that the parameters might be used to assess the stability and safety of a 50%-ethanolic extract of Jombang leaves before its application to treat a variety of illnesses.

Limitation and Further Research

Many factors, such as geographical location, climate, temperature, agricultural process, harvesting process, and post-harvesting process (drying method, storage) influence the quality of herbal material and its chemical content.

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