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Mini Review: Quality Control Study of Crude Drug of *Justicia gendarussa* Burm. f. Leaves as Male Contraceptive

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ABSTRACT: *Justicia gendarussa* Burm. f. (*Gendarussa*) leaves contain gendarusin A, which is a potential anti-fertility agent. Preclinical researches (in vitro, in vivo, and toxicity) and clinical trials have been carried out proving that *Gendarussa* leaves are safe to be used as a male contraceptive. Quality control of medicine must be carried out from the beginning when selecting crude drugs until the production process. Quality control of the crude drug is needed to maintain the quality, safety, and efficacy of its crude drug. That standardization has been done in Kediri, Mojokerto, and Ponorogo. The research output data used were specific and non-specific parameters (macroscopic and microscopic assay, determination of ash content, compound levels, etc.). The quality of crude drugs is different in each region. Crude drug from Mojokerto is better than crude drug from Kediri and Ponorogo. This is influenced by environmental difference factors on how plants grow. This review describes the differences in the quality control results of crude drug of *Gendarussa* from several areas. Hence, in the future, the quality of this plant can be guaranteed to be used as a contraceptive drug.

Keywords: crude drug; *Justicia gendarussa* Burm. f.; quality control

1. Introduction

Justicia gendarussa Burm. f. (Gendarussa) is a plant from the Acanthaceae family spread in Indonesia and several Asian countries such as Sri Lanka, India, and Malaysia [1]. This plant is often used as a folk remedy to cure rheumatic, bronchitis, eczema, fever, etc. [2]. Apigenin and vitexin glycosides in Gendarussa can be used as anti-inflammatory and anti-tumor [3]. However, *in vitro* and *in vivo* anti-fertility tests of these leaves show competitive and reversible inhibition of the hyaluronidase enzyme in spermatozoa [4]. Therefore, this plant has the potential to be developed into a non-hormonal male contraceptive drug [5,6]. Capsule of Gendarussa extract as an anti-fertility have been tested in phase III clinical trials [7].

Gendarussa leaves contain potassium, flavonoids, justicin, steroids and alkaloids [4]. In addition, these leaves also contain gendarusin A (6,8-di-C- α -L-arabinopyranosyl-4',5,7-trihydroxyflavone) as a major component, gendarusin B (6-C- α -L-arabinopyranosyl-4',5,7-trihydroxy-8-C- β -D-cylopyranosyl-flavone), gendarusin C, gendarusin D, and gendarusin E as minor components [8]. Gendarusin is a compound of the more acid-resistant C-glycosides flavonoid group [9]. Analysis to determine the compounds contained in the plant is necessary to help identify the side effects of these active compounds so as to improve the quality of the drug [10,11].

Quality control is a process involved in maintaining the quality and validity of the resulting products [12]. This process needs to be done in a production activity to produce phytopharmaceutical with better quality [13]. Efforts to ensure the quality and safety of the drug must be carried out from the beginning of the production process from the selection of crude drug until the end of production so that the product is then ready to be distributed in the community [14]. The economic value added in a crude drug that meets the standard is much greater than the unstandardized crude drug [15]. Standardization of crude drugs

is carried out to determine the quality, safety, and efficacy requirements of the Gendarussa leaves crude drug. Standardization parameters consist of specific and non-specific parameters.

Research on the quality control test of Gendarussa crude drug has been conducted in several areas, for example, Kediri, Mojokerto, and Ponorogo [16,17]. The quality of some of these crude drugs varies. This is because drugs produced from natural materials have varying compound content. These variations occur due to several factors, such as genetic, environmental, agronomy engineering, and harvest [18]. Therefore, this review will discuss the information related to the quality control of Gendarussa crude drugs as a reference in the process of developing drugs from natural materials.

2. Method

2.1. Article selection criteria and sources

The search of scientific articles was conducted on electronic databases namely PubMed, SCOPUS, ScienceDirect, Google Scholar, and Universitas Airlangga Repository. The search was limited until September 2020. The keywords used in the search were Gendarussa (*Justicia gendarussa* Burm. f.), quality control, and crude drug which were included in research articles/dissertation/thesis. From the searching results, the topic that focused on the research on quality control of Gendarussa crude drug was selected and used in this review. The inclusion criteria used as a reference in this review are scientific articles on quality control of Gendarussa with the research year no less than 2001. The exclusion criteria in this study were articles describing the quality control of Gendarussa extracts or granules.

2.2. Output research and data extraction

The research output data used were specific and non-specific parameters that included macroscopic and microscopic tests, total ash content, extract content, water content, and com-

pound levels. The data of subsequent research articles extracted in this case included the author, year of publication, research location, research design, and research results.

3. Results and discussion

Crude drug quality control is done by analyzing several specific and non-specific parameters. The specific parameters include macroscopic, microscopic, powder identification, water-soluble content, ethanol-soluble content, essential oil content, and gendarusin A levels. *Gendarussa* leaves harvested on average 9 months old will obtain high gendarusin A levels [19].

3.1. Specific parameters of quality control

Macroscopic tests are conducted by observing the morphology of the leaves covering the shape, color, and smell of the crude drug. They were single leaves with stalks of about 0.5-2 cm in size, long lancet-shaped, smooth hairless surface, and the edges of the leaves slightly edged. Then the ends of the leaves and the base of the leaves were spiky and dark green. The length of the leaves was about 5-20 cm, the width of the leaves was about 1-3.5 cm, and the leaves' bone was squinting and purple [20]. The morphology of *Gendarussa* plant is shown in Figure 1.



Figure 1. *J. gendarussa* Burm. f. plants [17]

Microscopic tests are carried out using a microscope to determine the anatomical structure of the leaves. *Gendarussa* leaves are sorted to separate the leaves from other unwanted parts of the plant. After that, the

leaves are washed to remove dust and dried at room temperature which then is called a crude drug (Figure 2). This is done to avoid crude drug damage due to enzymatic reactions and microbial growth [21]. Microscopic tests were conducted by anatomically observing the cross-section of the *Gendarussa* leaves (Figure 3).



Figure 2. Crude drugs (a) and leaf powder (b) of *J. gendarussa* Burm. f. [21]

The crude drug is mashed until it becomes powder. Microscopic identification of powder is carried out to determine the tissues characteristics of the crude drugs. Crude drug powder was yellowish-green, had typical aromatic smells, and tasted bitter [21]. The identification of *Gendarussa* powder showed the presence of stomata fragments, stair thickening xylem, sponge tissue, systolic, and glandular hair (Figure 4).

Crude drug powder is soaked by citric acid at pH 3. This acidification aims to eliminate alkaloids inside the crude drug because it causes toxic effects [4]. After that, the crude drug is washed with running water to remove the remaining acid. The crude drug that does not contain alkaloids is referred to as an alkaloid-free crude drug. The content of metabolites in the crude drugs before and after acidification is different. Crude drug before acidification contains amino benzyl alkaloid derivatives that are not detected in alkaloid-free samples [22-26].

Gendarussa contains several other compounds such as essential oils, aromatic amines, stigmasterol, tannins, saponins, amino acids, lupeol, and flavonoids [27-31]. The flavonoid compound used as a marker in this plant is gendarusin A (6,8-di-C- α -L-arabinopyranosyl-4',5,7-trihydroxyflavone, or 6,8-di-C- α -L-

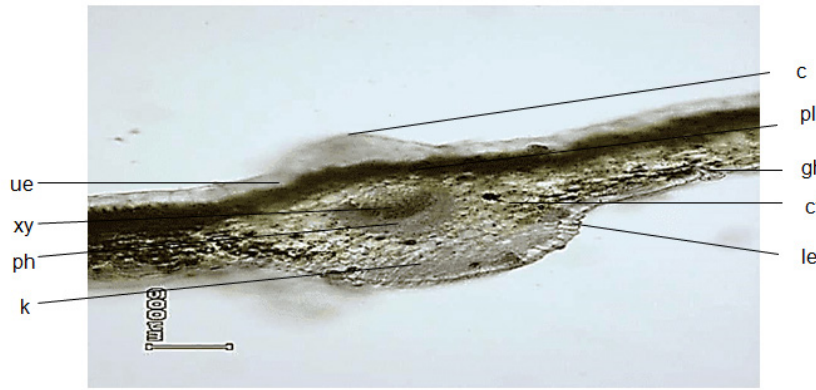


Figure 3. Transverse slices of Gendarussa leaf [17] with ue = upper epidermis, xy = xylem, ph = phloem, k = collenchyma, c = cuticle, pl = palisade, gh = glandular hair, cf = coral flower, le = lower epidermis [21]

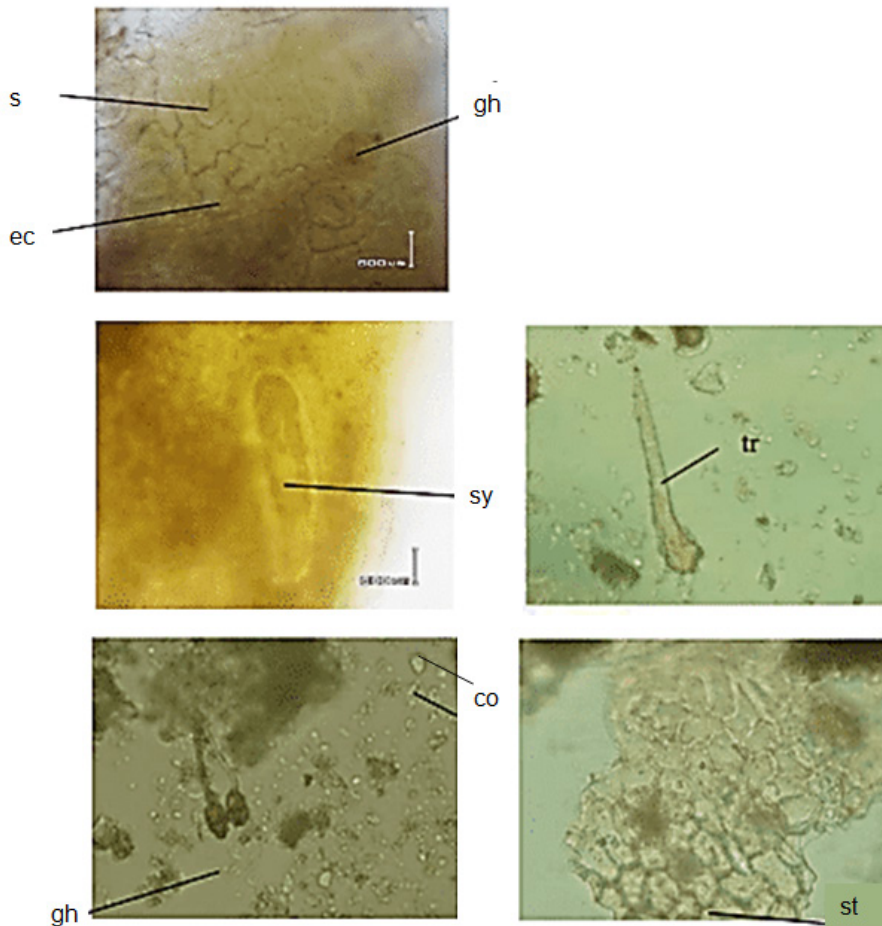


Figure 4. Gendarussa leaf powder fragments [21] with ec = epidermis cells, gh = glandular hair, s = stomata, sy = systolic, xy = xylem, co = calcium oxalate crystals, tr = trachea, st = sponge tissue [21]

arabinocylapigenin). Therefore, gendarusin A levels (as a major compound) in crude drugs need to be measured in quality control tests as marker. There are other compounds such as

6-C- α -L-arabinopyranosyl-4',5,7-trihydroxy-8-C- β -D-cylopyranosyl-flavone or 6-C- α -L-arabinocyl-8-C- β -D-cylocilapigenin (gendarusin B), gendarusin C, gendarusin D, and gendarusin

of plants, both qualitatively and quantitatively, causing differences in activities. Several studies have shown that this plant has antibacterial, antifungal, anti-HIV, anticancer, antioxidant, hepatoprotective, antiangiogenic, and anthelmintic activities [33-44] that are related to gendarusin A.

Analysis of chemical content in gendarussa leaves from several different places (Purwodadi, Pacet, and Makassar) using LC-MS instruments results in different chemical content and different metabolite profiles [24]. In addition, there are differences in metabolites concentrations of Justridusamid A, Justridusamid B, and Gendarusin A from various regions in Indonesia [26]. Thirty-six metabolite profiles have been found on Gendarussa leaves using LC-HR-MS/MS [45].

Therefore, it is important to determine and know the phytochemical content contained in herbal medicine to ensure reliability, repeatability in clinical and pharmacological studies, as well as to know the bioactivity and side effects that may occur. The appropriate method for quality control of herbal products namely by metabolite profiling [46]. It can be applied as a quality control tool for phytopharmaceutical, especially if the specifications of marker compounds have not been determined [47]. Chromatography and spectroscopy techniques can be used for the complete analysis of phytopharmaceutical products by producing metabolic fingerprinting that contributes to the determination of equations and differences of various samples [48].

There were several other parameters measured in quality control, in addition to macroscopic, microscopic, and levels of major compounds in crude drugs which will be used as phytopharmaceutical drugs. That drugs must be manufactured according to the standards required to produce good quality products. The production process must comply with GMP standards. One of the most important GMP requirements is raw material [49].

3.2. Non-specific parameters of quality control

The non-specific parameters include acid-insoluble ash, ash content, water content, contamination of heavy metal, microbes, and pesticides. Table 1 showed the quality of the Gendarussa crude drug varied by region. Based on the results of quality control from five locations (Kediri, Mojokerto-1, Mojokerto-2, Mojokerto-3, and Ponorogo), the best crude drug according to the standard test requirements was a crude drug derived from Mojokerto-3. The value of water-soluble extract of Gendarussa crude drug in Kediri and Mojokerto-1 and the value of acid-insoluble ash of Gendarussa crude drug in Mojokerto-2, Mojokerto-3, and Ponorogo have not met the requirements of *Materia Medika Indonesia*. Then, the values of ash content and water content in all five locations do not match the standards. Ash content does not meet the standard because the manufacturing process of crude drugs, especially washing, is less than perfect where there is still a lot of dirt sticking to the leaves. High water content is caused by incomplete drying of the crude drug and the humidity level of the environment during storage. Drying time is also influenced by the method of drying. Sequentially, the effective methods which are used are oven, sun & blower, blower, sun and wind. But Leaf drying as a crude drug should not be directly exposed to sunlight because it will change chlorophyll compounds in the leaf [50]. In addition, the length of storage before inspection can also affect the water content [17]. The temperature in Kediri is 27.2°C, Mojokerto is 24.8°C, and Ponorogo is 27.8°C. Moreover, the average humidity at all locations is between 70-85%.

Contamination contained in crude drugs such as heavy metal contamination, microbes, and pesticide residues can also be measured to determine the quality of a crude drug (Table 2). The rate of contamination should not exceed the standards set by WHO. Based on the research results, microbial contamination parameters and pesticide residues at all locations are suitable with WHO requirements. The total plate count of the molds and yeast is no more than 10³ colonies/

Table 1. Quality control results of crude drug of Gendarussa leaves

Author (year)	Research design	Sampling time	Sampling location	Research results	Requirements*
Kurniasari (2001)	Leaves were cleaned from impurities, dried by the wind, and made into powder	2009	Kediri	<ul style="list-style-type: none"> - Ash content 12.27±0.02% - Acid-insoluble ash 1.79±0.02% - Water-soluble extract 23.29±0.17% - Ethanol soluble extract 7.15±0.08% - Water content 11.00% - Essential oil content 0.06% - Tannin levels 1.55±0.06% - Flavonoid levels 0.16±5.77x10⁻³% - Gendarusin A levels (not measured) 	<ul style="list-style-type: none"> - Ash content less than 8% - Acid-insoluble ash more than 1% - Water-soluble extract more than 24% - Ethanol-soluble extract more than 6% - Water content less than 10%
			Mojokerto-1	<ul style="list-style-type: none"> - Ash content 12.65±0.05% - Acid-insoluble ash 1.93±0.07% - Water-soluble extract 18.30±0.13% - Ethanol soluble extract 6.90±0.07% - Water content 12.00% - Essential oil content 0.06% - Tannin levels 1.30% - Flavonoid levels 0.15±5.77x10⁻³% - Gendarusin A levels (not measured) 	
Rizqa (2010)	Leaves were cleaned from impurities, dried by wind and made into powder	February, 2009	Mojokerto-2	<ul style="list-style-type: none"> - Ash content 12.02±0.10% - Acid-insoluble ash 0.91±0.05% - Water-soluble extract 40.92±0.17% - Ethanol soluble extract 4.92±0.07% - Water content 10.22±0.39% - Essential oil content 0.04% - Tannin levels (not measured) - Flavonoid levels (not measured) - Gendarusin A levels 0.15±0.01% 	
			Mojokerto-3	<ul style="list-style-type: none"> - Ash content 13.99±0.86% - Acid-insoluble ash 0.67±0.06% - Water-soluble extract 48.28±0.26% - Ethanol soluble extract 7.40±0.45% - Water content 12.89±0.20% - Essential oil content 0.04% - Tannin levels (not measured) - Flavonoid levels (not measured) - Gendarusin A levels 0.21% 	
			Ponorogo	<ul style="list-style-type: none"> - Ash content 13.53±1.08% - Acid-insoluble ash 0.71±0.04% - Water-soluble extract 42.76±1.29% - Ethanol soluble extract 5.61±0.39% - Water content 10.11±0.19% - Essential oil content 0.04% - Tannin levels (not measured) - Flavonoid levels (not measured) - Gendarusin A levels 0.27±0.01% 	

* based on *Materia Medika Indonesia*

mL. The examination results of several bacteria such as *Salmonella* sp., *E. coli*, *P. aeruginosa*, and *S. aureus* and pesticide residues against the carbamate, organophosphate, and organochlorine groups showed negative results. However, heavy

metal contamination (Hg and Cd) in Ponorogo does not meet the standards. The maximum heavy metal content for lead is 10 ppm, mercury is 1 ppm, cadmium is 0.02 ppm, and arsenic is 10 ppm [51]. These nonspecific parameters can

Table 2. Contamination of heavy metal, microbes, and pesticides contained in crude drug of *Justicia gendarussa* Burm.f.

Author (year)	Sampling time	Sampling location	Research results			Requirements*
			Contamination of heavy metal	Contamination of microbes	Contamination of pesticides	
Rizqa (2010)	February, 2009	Mojokerto-2	- Lead (Pb) 0.382 ppm - Mercury (Hg) 0.000 ppm - Cadmium (Cd) 0.107 ppm - Arsenic (As) 0.000 ppm	- Total Plate Count (TPC) 36700 col/ml - TPC of molds 700 col/ml - TPC of yeast 0 col/ml - <i>Salmonella sp.</i> (negative) - <i>E. coli</i> (negative) - <i>P. aeruginosa</i> (negative) - <i>S. aureus</i> (negative)	- Carbamate (negative) - Organophosphate (negative) - Organochlorine (negative)	- Lead (Pb) less than 10 ppm - Mercury (Hg) less than 0.5 ppm - Cadmium (Cd) less than 0.3 ppm - Arsenic (As) less than 5 ppm - TPC of molds or yeast less than 10 ³ /g - <i>E. coli</i> less than 10/g - <i>P. aeruginosa</i> negative - <i>S. aureus</i> negative
		Mojokerto-3	- Lead (Pb) 0.427 ppm - Mercury (Hg) 0.000 ppm - Cadmium (Cd) 0.098 ppm - Arsenic (As) 0.000 ppm	- Total Plate Count (TPC) 26700 col/ml - TPC of molds 780 col/ml - TPC of yeast 0 col/ml - <i>Salmonella sp.</i> (negative) - <i>E. coli</i> (negative) - <i>P. aeruginosa</i> (negative) - <i>S. aureus</i> (negative)	- Carbamate (negative) - Organophosphate (negative) - Organochlorine (negative)	
		Ponorogo	- Lead (Pb) 0.000 ppm - Mercury (Hg) 1.829 ppm - Cadmium (Cd) 0.438 ppm - Arsenic (As) 0.000 ppm	- Total Plate Count (TPC) 27700 col/ml - TPC of molds 800 col/ml - TPC of yeast 0 col/ml - <i>Salmonella sp.</i> (negative) - <i>E. coli</i> (negative) - <i>P. aeruginosa</i> (negative) - <i>S. aureus</i> (negative)	- Carbamate (negative) - Organophosphate (negative) - Organochlorine (negative)	
Riwanti (2016)	May, 2015	Mojokerto-4	- Lead (Pb) 0.097 ppm - Mercury (Hg) 0.000 ppm - Cadmium (Cd) 0.012 ppm - Arsenic (As) <0.001 ppm	- Total Plate Count (TPC) 7200 col/ml - TPC of molds 30 col/ml - TPC of yeast 4,200 col/ml - <i>Salmonella sp.</i> (negative) - <i>E. coli</i> (negative) - <i>P. aeruginosa</i> (negative) - <i>S. aureus</i> (negative)	- Carbamate (negative) - Organophosphate (negative) - Organochlorine (negative)	

* based on WHO

be applied to simple standardization of herbal medicines if QA marker (chemical profiles) have not been determined [52].

5. Conclusion

The quality of *Justicia gendarussa* Burm. f. leaves crude drug is different in each location. This is due to differences in environmental conditions in which plants grow, but still need other research such as the effect of plant's age for the quality of Gendarussa crude drugs. The limitation of this review is the limited number of articles that discuss about QC of Gendarussa.

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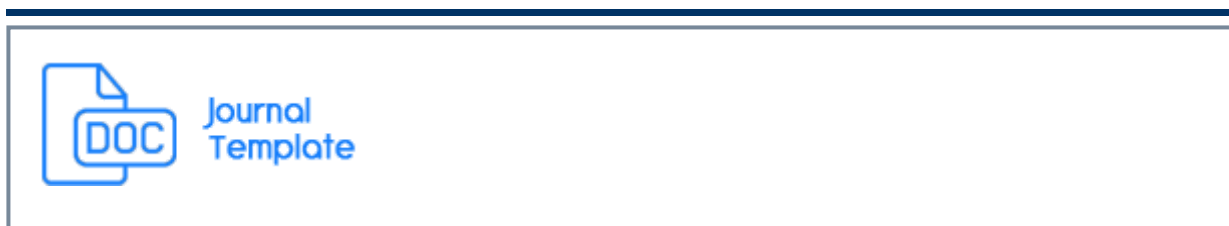
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