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Standardization of a Crude Drug *Moringa oleifera* Leaf from Africa, India and Local (Indonesian) which Cultivated in Bojonegoro Indonesia

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ABSTRACT

Purpose: The research was to determine specific parameters (macroscopy and microscopy) and non-specific parameters (loss on drying, total ash content, acid insoluble ash content, water-soluble extractive matter, ethanol-soluble extractive matter), heavy metal contaminant (Pb, Hg, As, Cu) and microbial contaminant (Total Plate Count and Total Yeast Mold). Research also determined a total flavonoid of M. oleifera leaf.
Methodology: The method carried out according to the procedure stated in the Materia Medika Indonesia 5th edition. Result test compared to the standard stated in Indonesian Herbal Pharmacopoeia 2nd edition. Methods to detection Contaminants were referred to WHO guidelines for assessing the quality of herbal medicines. The determination of total flavonoid was performed by spectroscopic method.
Results: Crude drug of M. oleifera leaf meet the specific parameters (macroscopy and microscopy). Non-specific parameters of Africa, India, Local M. oleifera leaf shows that loss on drying (8.06 ± 0.03; 8.89 ± 0.31; 7.56 ± 0.17) %, total ash content (8.64 ± 0.43; 10.64 ± 0.90; 15.31 ± 0.87) %, acid insoluble ash content (0.56 ± 0.08; 0.35 ± 0.01; 0.36 ± 0.06)%, water-soluble extractive matter (21.38 ± 1.39; 30.12 ± 2.06; 12.68 ± 1.12) %, ethanol-soluble extractive matter (39.37 ± 1.51; 27.74 ± 2.44; 27.09 ± 1.43)%, contaminant test including heavy metal contaminant shows that Pb, Cd, As and Hg were not to be detected. Microbial contaminant (Total Plate Count and Total Yeast Mold) under limits of WHO standart. Total flavonoid content of Africa, India, Local M. oleifera leaf were 8.12 ± 0.52 mg/ 100 mg QE; 10.69 ± 0.15 mg/ 100 mg QE dan 13.08 ± 0.08 mg/ 100 mg QE respectively.
Conclusion: Moringa Leaves meet specific parameters (macroscopy and microscopy test). Non-specific parameter tests of Africa, India, Local Moringa Leaf shows that loss on drying, acid insoluble ash content, water-soluble extractive matter, ethanol-soluble extractive matter, contaminant test including heavy metal contaminant shows that (Pb, Cd, As and Hg) and microbial contaminant (Total Plate Count and Total Yeast Mold) meet the standard required, only total ash content did not meet the standard. Total Flavonoid content shows that the extract of M. oleifera from local (Indonesia) variety is higher than others.
Key words: M. oleifera leaf, Standardization, Crude Drug
Applications/Originality/Value: Quality of Crude drug of M. oleifera leaf from Africa, India and Local (Indonesian) which Cultivated in Bojonegoro Indonesia. There was no study compare a different M.oleifera variety which cultivated at the same places. The findings could be helpful in the identification and authentication of M. oleifera in future for further research and utilization

INTRODUCTION

Morinaga oleifera (Moringaceae) contains flavonoid compound such as apigenin, quercetin, ramnetin and camferin (Misra et al, 2012; Leone et al, 2015) and alkaloid moringinin (Mehra et al, 2017). Moringa leaf with various potential active pharmacology compound used as antioxidant, anti-ulcer, hyper cholesterol, anti-hypertension, anti-inflammatory, anti-tumour, anti-microbial and anti-diabetes (Asiedu-Gyekye et al, 2014; Ferreira et al, 2014). M.oleifera leaf also high nutritive content besides of the medicinal functions. The micronutrients content is 7 times the vitamin C of oranges, 4 times the vitamin A of carrots, 4 times the calcium of milk, 3 times the potassium of bananas, and protein in the two yogurts (Aminah et al, 2015). Widely use of moringa leaf in the community encourages the important thing of standardization. Standardization of herbal drugs means confirmation of its identity, Quality and purity (Bijauliya et al, 2017). Standardization aims to ensure the quality of herbs used as raw material for herbal medicine product and compared with standard quality of crude drug in Indonesian Herbal Pharmacopoeia 2nd edition (DEPKES RI, 2017).

Standardisation parameter including specific parameters and non-specific parameters. Specific parameters of standardization including plant identity, organoleptic, macroscopy and microscopy, marker / active compound content (total flavonoid content, phenolic content, volatile oil, etc). Non-specific parameters including loss

on drying, total ash content, acid insoluble ash content, water-soluble extractive matter, ethanol-soluble extractive matter, residual solvents, pesticide residues, heavy metal contaminant and microbial contaminant (DEPKES, 2000).

Quality control of herbal plant refers to WHO standard including pesticides residues, heavy metals contaminant and microbial contaminants. Contaminant was an undesired introduction of impurities of a chemical or microbiological nature, or foreign matter, into or onto a starting material, intermediate product or finished herbal product during production, sampling, packaging or repackaging, storage or transport (WHO, 2011).

WHO declared that heavy metal contaminant including Lead (Pb) and Cadmium (Cd). BPOM RI (National agency of drug and food Republic of Indonesia) declared that heavy metal contaminant Lead (Pb), Cadmium (Cd), Arsenic (As) and Mercury (Hg). Microbial contaminant in WHO regulatory, including total aerobic bacteria, total mould and yeast, enterobacteria, gram-negative bacteria, *Eschericia coli* and *Salmonella*. Furthermore, microbial contaminant based on BPOM RI (Indonesian Food and Drug Agency) including total plate count, total mould-yeast, aflatoxin and bacteria *Eschericia coli*, *Salmonella spp*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* must be absent. Many factors, such as geographical location, climate, temperature, agricultural process, harvesting process, post-harvesting process (drying method, storage) influence the quality of herbal material and chemical content of them. The chemical content of the medicinal plant was determined by the species of medicinal plants to their varieties (Dewoto, 2007 ; de Freitas Araujo& Bauab, 2012).

Plant material

Crude drug of *M. oleifera* leaf was collected on March 2019 from Bogo village, Kapas, Bojonegoro District, Indonesia. The plant leaves collected from 3 different varieties: Africa, India, Local (Indonesia) which cultivated in Bogo. *M. oleifera* leaf was identified and authenticated by Forest Farmer Group (Kelompok Tani Hutan) Bojonegoro.

METHODS

Extraction

M. oleifera leaf was dried at room temperature and powdered with blender, then sifted with sieve 60 mesh. The sample was extracted by maceration method with ethanol 96% at room temperature for 3x24 h. The liquid extract was evaporated with a rotary evaporator.

Specific Parameter of Standardization

Specific parameters of standardization including the identity of the plant, organoleptic, macroscopy and microscopy, marker / active compound content (total flavonoid content, phenolic content, volatile oil). Macroscopy and microscopy of the *M. oleifera* leaves was studied according to the method in Materia Medica Indonesia (DEPKES RI, 1989) and Indonesia Herbal Pharmacopoeia 2nd edition (DEPKES RI, 2017). Identification a powder of *M.Oleifera* leaf using microscopes binocular Olympus CX-23.

Non-Specific Parameter of Standardization

Non-specific parameters including loss on drying, total ash content, acid insoluble ash content, water-soluble extract content, ethanol-soluble content according to the method in Materia Medika Indonesia (DEPKES RI, 1989) and Herbal Pharmacopoeia 2nd edition (DEPKES RI, 2017). Methods for detection Heavy metal contaminant and microbial contaminant referred to WHO guidelines for assessing the quality of herbal medicines with references to contaminants and residues.

Determination of Total Flavonoid

Total flavonoid was analyzed using aluminium chloride colourimetric method according to Sulastri *et al* (2018). Quercetin was used to make the calibration curve. 10 mg of quercetin was dissolved in ethanol 96% and diluted to 8, 10, 12, 14, 16 and 18 µg/mL. 1 ml of each concentration of standard solutions, as well as 1 ml of each sample solution, were mixed with 3 mL ethanol 96%, 0.2 mL of aluminium chloride 10%, 0.2 mL potassium acetate 1 M and 5.6 mL of distilled water. The mixture was incubated at room temperature for 10 min with intermittent shaking. The absorbance was measured at 376 nm using Shimadzu B UV-Vis spectrophotometer. Total flavonoid was calculated as mean ± SD (n = 3) and expressed as weight of quercetin equivalent (QE)/ 100 mg extract.

RESULTS

Specific Parameter of Standardization

Macroscopy

M. oleifera can be cultivated at low and high altitude. Macroscopy of crude drug *M.oleifera* leaves was green to brownish green, *cimcumcriptio* was *ovatus* or *obovatus*. Leaf length of 10 mm to 30 mm and width 4 mm to 10 mm. *Apex Folii* was *obtusus, Basis Folii* was *rotundatus* and *Margo Folii* was *integer*. Macroscopy of *M.oleifera* leaf shown in Figure 1 and Table 1.

(a) Local Variety (b) African Variety (c) India Variety

Figure 1. Macroscopy Crude Drug *M.oleifera* Leaf

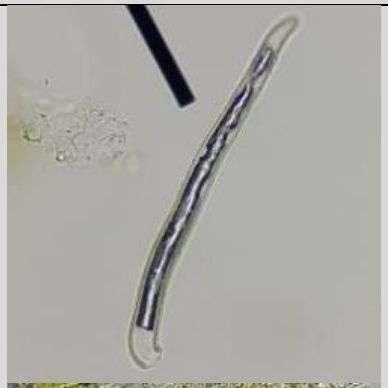
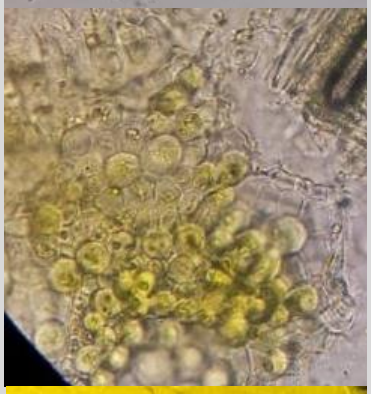
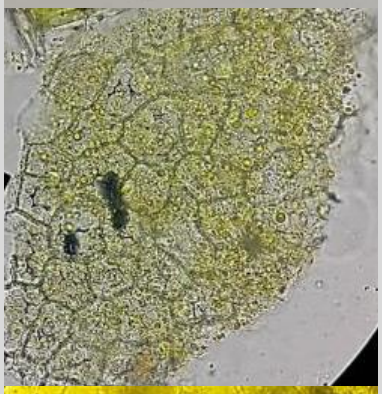
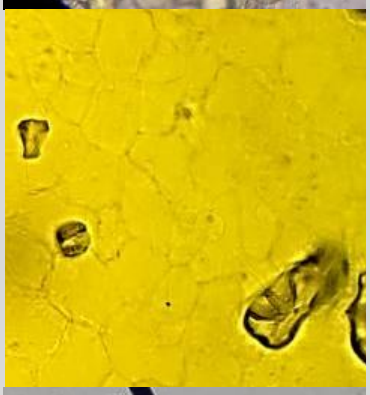
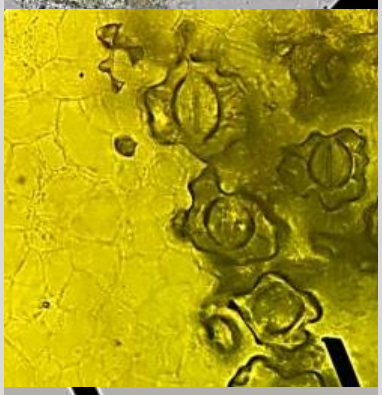
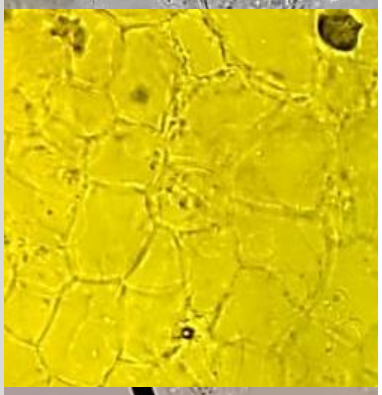
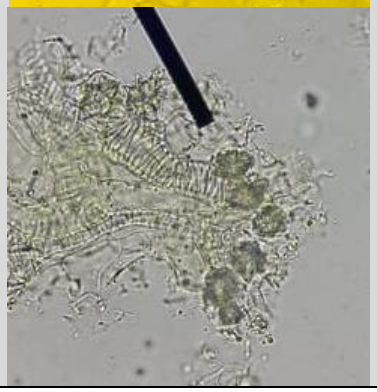
Table 1. Macroscopy of *M. oleifera* Leaf

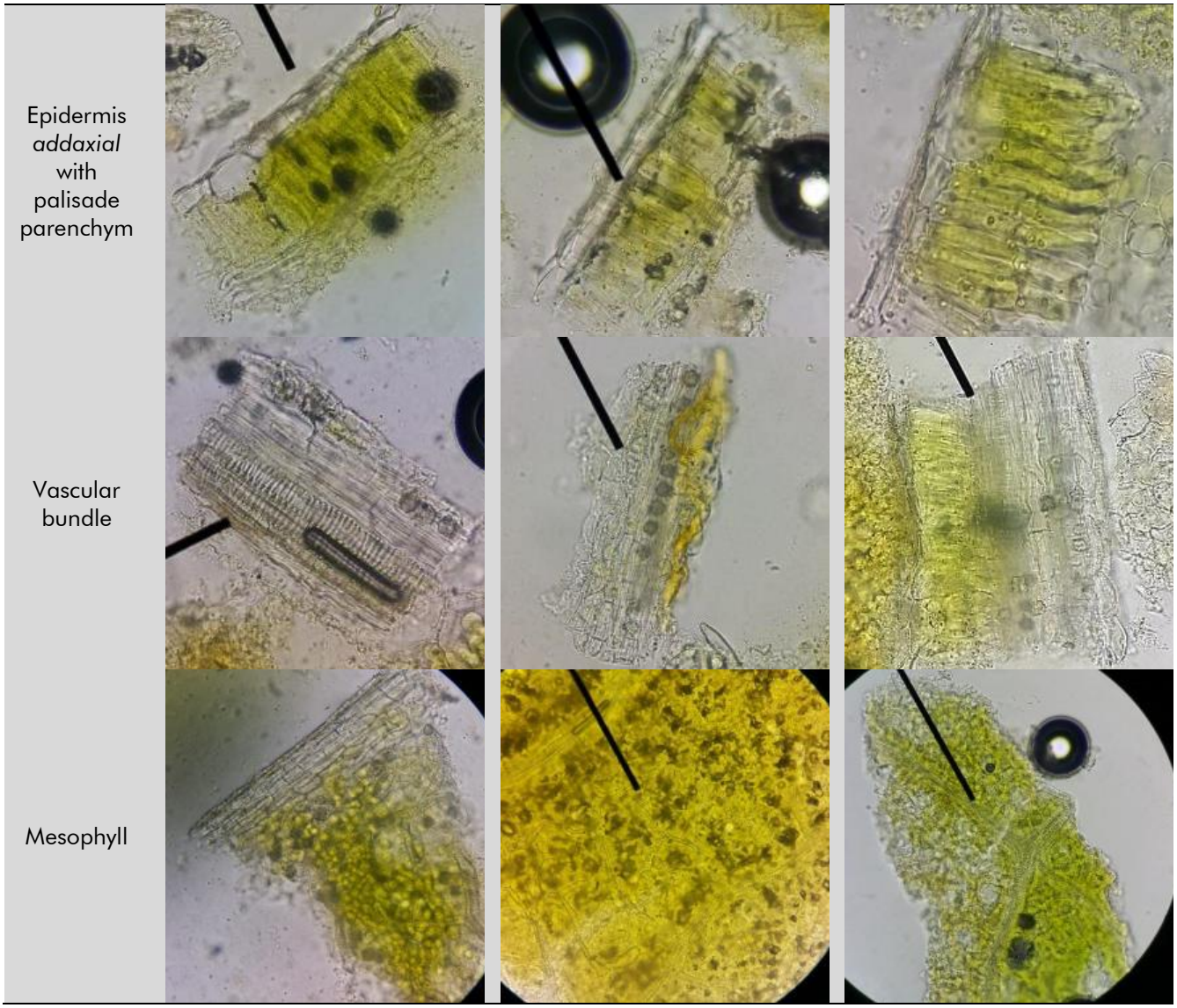
Parameters	Indonesia Herbal Pharmacopoeia 2nd edition (DEPKES RI, 2017)	Materia Medica Indonesia 5th edition (DEPKES RI, 1989)	Result		
			Africa	India	Local (Indonesian)
circumscri-ptio	*orbicularis* or *ovatus- oblongus*	*Ovatus* or *obovatus*	*ovatus-oblongus*	*Ovatus* or *obovatus*	*Ovatus* or *obovatus*
Apex folii	*Obtusus* or *rotundatus*	*obtusus*	*obtusus*	*rotundatus*	*rotundatus*
Basis folii	*acutus*	*rotundatus*	*rotundatus*	*rotundatus*	*rotundatus*
Margo folii	*integer*	*integer*	*integer*	*integer*	*integer*
Nervatio	*penninervis*	*penninervis*	*penninervis*	*penninervis*	*penninervis*
Folium Compositum	*pinnatus*	*imparipin-natus*	*imparipinnatus*	*imparipinnatus*	*imparipinnatus*
Colour	Green, yellowish green, brownish green	Green, brownish green	Dark green	green	green
Lenght	-	10 mm – 30 mm	15 mm – 25 mm	15 cm – 25 mm	15 mm – 2 mm
Width	-	4 mm – 10 mm	5 mm – 10 mm	10 mm – 13 mm	5 mm – 10 mm
Smell	odorless	specific	specific	specific	specific
Taste	-	-	Slightly bitter	Slightly bitter	Slightly bitter

Microscopy

Microscopy of M.oleifera observed with an enlargement 400x of a binocular microscope. Identified or specific fragment of powder crude drug M.oleifera leaf including trichomata, epidermis addaxial, xylem with spiral thickening and Calcium oxalate crystal. Table 2 shows microscopy of M. oleifera.

Table 2 Microscopy of M. oleifera Leaf

Parameters	Result		
	Africa	India	Local (Indonesian)
Trichomata			
Pallisade parenchym			
Epidermis *abbaxial* with anomositic stomata			
Calcium oxalate Cyrstal			



Macroscopy and microscopy of crude drug *M. oleifera* leaf shows that Africa, India and local (Indonesian) varieties meet the criteria on Materia Medica Indonesia 5th edition (DEPKES RI, 1989) and Indonesia Herbal Pharmacopoeia 2nd edition (DEPKES RI, 2017).

Non-Specific Parameter of Standardization

Non-specific parameters including loss on drying, total ash content, acid insoluble ash content, water-soluble extractive matter, ethanol-soluble extractive matter, heavy metal contaminant and microbial contaminant was analyzed. Table 3 shows the non-specific parameter of crude drug *M.oleifera* leaf.

Table 3. Non Specific Parameter of Crude Drug *M.oleifera* Leaves.

Parameters	Indonesia Herbal Pharmacopoeia 2nd edition (DEPKES RI, 2017)	Materia Medica Indonesia 5th edition (DEPKES RI, 1989)	WHO guidelines for assesing quality of herbal medicines (2007)	BPOM RI (Indonesian Food and Drug Agency) (2014)	Result		
					Africa	India	Local (Indonesian)
Loss on drying (%)	< 10	-			8.06 ± 0.03	8.89 ± 0.31	7.56 ± 0.17
Total Ash	< 7.5	< 11			8.64 ±	10.64 ±	15.31 ±

Content (%)					0.43*	0.90*	0.87*
Acid Insoluble Ash Content (%)	< 0.9	< 1			0.56 ± 0.08	0.35 ± 0.01	0.36 ± 0.06
Water-soluble extractive matter (%)	> 4.9	> 5			21.38 ± 1.39	30.12 ± 2.06	12.68 ± 1.12
Ethanol-soluble extractive matter (%)	> 5.0	> 5			39.37 ± 1.51	27.74 ± 2.44	27.09 ± 1.43
Heavy Metal Contaminant (ppm)							
Pb			≤ 10	≤ 10	Not detected**	Not detected**	Not detected**
Cd			≤ 0.3	≤ 0.3	Not detected**	Not detected**	Not detected**
As				≤ 5	Not detected**	Not detected**	Not detected**
Hg				≤ 0.5	Not detected**	Not detected**	Not detected**
Total Plate Count (colony/ g)				≤ 10^6	6.1 x 10^3	1.8 x 10^2	6.3 x 10^4
Total Yeast Mold (colony/ g)				≤ 10^4			
Yeast					< 10	< 10	< 10
Mold					4.0 x 10^1	< 10	3.8 x 10^2

*Parameters did not meet the standard required

***Detection limit of* Cd, Pb: 0.1 ppm, *Detection limit of* As, Hg: 0.001 ppm

Loss on drying was a measurement of the residual of a crude drug after drying which is counted as a percentage. Loss on drying aim to provide a maximum limit (range) a quantity of compound has been lost after the drying process (DEPKES RI, 2017). The result shows that loss on drying for *M.oleifera* leaf African, India, Local (Indonesia) was 8.06 ± 0.03, 8.89 ± 0.31, 7.56 ± 0.17 respectively. All the sample meet the required standard of Indonesia Herbal Pharmacopoeia 2nd edition. Strongly recommends effective drying to reduce moisture content to less than 10%. However, since the products easily attracts and reabsorbs moisture during milling and storage, contamination with moulds and other potential pathogens is a serious concern (Adu-Gyamfi & Mahami, 2014).

Total ash content aims to describe the internal and external material content of crude drug *M.oleifera* leaf. The material contaminant has been found since the harvesting process, time to harvesting, drying process until the methods using for extraction process. The sample of crude drug *M.oleifera* has been heated at high temperature (600^0 C at the furnace) until al material becomes ash. All the organic compound will be reducted and evaporated, only an inorganic material has been found. Total ash and acid-insoluble ash contents are important indices to illustrate the quality as well as purity of herbal medicine. Total ash includes ``physiological ash", which is derived from the plant tissue itself, and ``non-physiological ash", which is often from environmental contaminations such as sand and soil. Total ash content alone is not sufficient to reflect the quality of herbal medicines, since the plant materials often contain considerable levels of physiological ash, calcium oxalate in particular. Thus, the acid-insoluble ash content is another index to illustrate the quality of herbal (Rao & Xiang, 2009). The result shows that Total ash content for *M.oleifera* leaf African, India, Local (Indonesia) was 8.64 ± 0.43, 10.64 ± 0.90, 15.31 ± 0.87 respectively. all the sample did not meet the required standard of Indonesia Herbal Pharmacopoeia 2nd edition, and only Local (Indonesia) variety did not meet the required standard of Materia Medika Indonesia 5th edition (DEPKES RI, 1989). High mineral content and inorganic compounds such as calcium, oxalate, potassium, sodium, chloride and alkali can be found in the herbal plant material (WHO, 2011). The result shows that acid insoluble ash content for *M.oleifera* leaf African, India, Local (Indonesia) was 0.56 ± 0.08, 0.35 ±

0.01, 0.36 ± 0.06 respectively. All the sample meet the required standard of Indonesia Herbal Pharmacopoeia 2nd edition and Materia Medika Indonesia 5th edition (DEPKES RI, 1989).

Water-soluble extractive matter and Ethanol-soluble extractive matter were described a quantity of active compound has been extracted after maceration 1x24 h with the selective solvent (water or ethanol). The result shows that Water-soluble extractive matter for *M.oleifera* leaf African, India, Local (Indonesia) was 21.38 ± 1.39, 30.12 ± 2.06, 12.68 ± 1.12 and for Ethanol-soluble extractive matter was 39.37 ± 1.51, 27.74 ± 2.44 and 27.09 ± 1.43 respectively. All the sample meet the required standard of Indonesia Herbal Pharmacopoeia 2nd edition and Materia Medika Indonesia 5th edition (DEPKES RI, 1989).

Contamination of heavy metal must be limited because can induce a serious health problem in the human body. Crude drug as a raw material for herbal medicine must be meet the quality standard limitation of contaminant. 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. Arsenic (As) can induce lung cancer and skin disorders, and mercury (Hg) cause neurological disorder and kidney damage (Shaban, Abdou & Y.Hasal, 2016). The result shows that contamination of heavy metal (Pb, Cd, As, Hg) for *M.oleifera* leaf African, India, Local (Indonesia) was not detected at the sample (*Detection limit of* Cd, Pb: 0.1 ppm, *Detection limit of* As, Hg: 0.001 ppm). Plants accumulate minerals essential for their growth from the environment and may accumulate some metals which have no known direct benefit to the plants. Herbal can be contaminated by these heavy metals via root uptake or by direct deposition of contaminants from the atmosphere onto plant surfaces. The study further suggested that *M. olifera* in polluted sites may accumulate trace metals in any of its parts. When the plant is to be considered for medicinal purpose and the need to regulate where the plants should be cultivated because of its ability to uptake trace metals (Agboola *et al*, 2016).

The herbal product is not permitted contains a microbial as a contaminant. Contamination of microbial (bacteria or yeast) can cause a problem- related health such as diarrhoea, febris, and other condition that unwanted. Preparation on the herbal product must be a concern with hygiene and sanitary as in CPOTB (The good procedure to prepare herbal medicine) based on Indonesian regulatory or GMP (Good Manufacturing Product). The total number of microbial and fungi are limited, but microbe such as *E.coli*, *Salmonella spp*, and *P. aeruginosa* must be absent. The microbial contaminant can be present due to the preparation process did not refer to CPOTB or GMP. The results show that microbial contaminant (Total Plate Count (colony/ g) and Total Yeast Mold (colony/ g) for *M.oleifera* leaf African, India, Local (Indonesia) was under the limit quality standard of BPOM RI (2014) (National agency of drug and food Republic of Indonesia). *M.oleifera* leaf powder when boiled (a rolling boil for 5 min) contains risk of contamination from B. cereus, C. perfringens type A, Cronobacter, enterohemorrhagic E. coli, L. monocytogenes, Salmonella spp., and S. aureus is low to moderate, with only a moderate to serious risk posed by C. perfringens type C. However, when the is not boiled before consumption, the food safety risk is increased for all of the evaluated pathogens. Risk, can be mitigated when the powder is stored under the appropriate conditions to ensure there is no ingress of moisture and then processed in a hygienic manner to reduce contamination and/or cross-contamination by following hazard analysis critical control point or similar procedures (even in a home setting) including a heat treatment, i.e., boiling, to further reduce microbial hazards (Walia, Kapoor, & Farber, 2019).

Total Flavonoid Content

Flavonoid was an active compound in *M.oleifera* leaf, it was polar and soluble in water or ethanol (Sulastri *et al* (2018). Based on Indonesia Herbal Pharmacopoeia 2nd edition (DEPKES RI, 2017) flavonoid content of extract *M.oleifera* leaf not less than 6.3% as a quercetin equivalent. Determination of total flavonoid use quercetin as standards where the calibration curve equations obtained were $y = 0.025x + 0.111$ ($R^2 = 0.987$). The result of total flavonoid shows in Table 4. Total Flavonoid content showed that the extract of *M. oleifera* from Local (Indonesia) variety is higher than others.

Table 4 Total Flavonoid Content of M. oleifera leaf

Parameters	Indonesia Herbal Pharmacopoeia edition 2 (DEPKES RI, 2017)	Result		
		Africa	India	Local (Indonesia)
Total Flavonoid Content	6.3 % QE	8.12 ± 0.52 mg/ 100 mg QE	10.69 ± 0.15 mg/ 100 mg QE	13.08 ± 0.08 mg/ 100 mg QE

CONCLUSION

Moringa Leaves meet specific parameters (macroscopy and microscopy test). Non-specific parameter tests of Africa, India, Local Moringa Leaf shows that loss on drying, acid insoluble ash content, water-soluble extractive matter, ethanol-soluble extractive matter, contaminant test including heavy metal contaminant shows that (Pb, Cd, As and Hg) and microbial contaminant (Total Plate Count and Total Yeast Mold) meet the standard required, only total ash content did not meet the standard. Total Flavonoid content show that the extract of *M. oleifera* from local (Indonesia) variety is higher than others.

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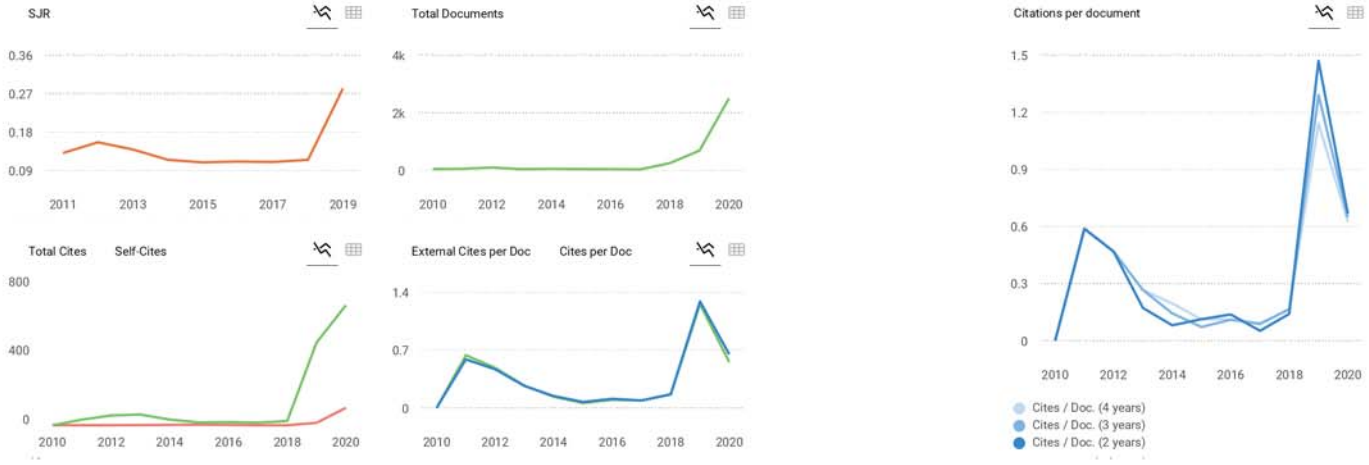

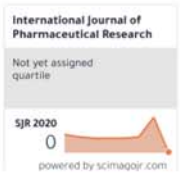

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