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Research Article

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Physicochemical and phytochemical evaluation of the aqueous extract of safflower (*Cartamus tinctorius* Linn.)

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ABSTRACT

Aqueous extract of C. tinctorius flowers is under consideration for development into Phytopharmaca for immunostimulant supplement. This study aimed to find the nonspecific and specific standard as a reference parameter extraction repeatability. Physicochemical determinations, including proximate analysis were done by sensory examination and gravimetric, thin layer chromatography with F245 silica gel using various solvent systems, metallic content analysis by AAS and pesticide residue by HPLC. The extract was dry but hygroscopic and soluble in water, with a loss drying at 6.0-6.7 %w/w, total ash at 4,92 – 5,37%w/w, acid insoluble ash at 0,506 – 2,89%w/w, water soluble extract at 10.36 - 11.90 %w/w and ethanol soluble extract at 10.00 - 10.54 %w/w. The extracts yielded reproducible chromatograms on normal silica plates developed with various solvent systems. Metallic level including cadmium at 0.0015-0.0058 µg/g, lead at 2.912 - 6.01 µg/g but arsenic no detected. Aldrin, dieldrin and endrin as pesticide residue no detected. Phenolic total at 3.8-4.8 %b/b expressed as tannic acid. The results confirm that aqueous extract of C. tinctorius flowers possess characteristics that would allow it's possible development into Phytopharmaca and that meets standardization herb requirement.

Keywords: physicochemical, phytochemical, *C. tinctorius* and standardization

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INTRODUCTION

Measles is especially endemic in developing countries caused by a virus[1]. Enhancement of the immune system including the use of immunostimulant can cure the disease[2]. One of the plants that proven to cure measles, especially in Bugis-Makassarethnic is KasumbaTurate (*Cartamus tinctorius* Linn.) [3].

Safflower, also named *C.tinctorius*Linn.is a genus belonging to the Asteraceaefamily[4]. At traditional Chinese medicine widely used to improve blood circulation[5, 6], the medicinal parts are flowers, seeds and the oil, extending the coagulation time in mice and exhibiting a significant antithrombotic effect[7]. *C tinctorius* is used not only for its traditional medicinal purposes in but is also effective for treating breast cancer [8].

For competitive herb market we have especially concentrated to make most useful and beneficial formulations such as Phytopharmaca. This study aimed to find the specific standard as a reference parameter extraction repeatability.

EXPERIMENTAL SECTION

Chemicals and Reagents

All chemicals and solvents used in the study were of analytical grade. Tannic acid, Folin-Ciocalteu reagent, sodium carbonate, sodium chloride, sulfuric acid, hydrochloride acid, nitric acid, standard for As, Cd, and Pbwere obtained

from Merck-Indonesia. Aldrin, dieldrin and endrinwere obtained from Sigma-Aldrich. All solutions including freshly prepared doubled distilled water.

Plant collection and identification
The flowers were collected from Watangsoppeng, South Sulawesi, Indonesia in June 2014 and were identified in Herbarium Bogoriense (Bogor, West Java, Indonesia).

Extraction
Extract was made by infundation method using doubled distilled water solvent. The extract filtered through Whatman filter paper and evaporated under the vacuum at 40^{O}C (Buchi) and then dried to a powder using a freeze-dryer at -80^{O}C (Scanvac).

Lost on drying (LOD)
This was carried out using 1.0 g of extract. Drying was effected in a gravity-convention oven (Memmert) maintained at $105\text{-}110^{0}$C. The results are expressed as a range.

Physicochemical tests
The following tests, briefly described, were carried out on the extracts as per Indonesian Herbal Pharmacopoeia:

Water and ethanol soluble extract
This was determined at room temperature and expressed in terms of "parts', representing the number of milliliter (mL) of water in which 1 g of the material is soluble. The following descriptive terms are used: very soluble (< 1 part); freely soluble (1 to 10 parts); soluble (30 to 100 parts); sparingly soluble (30 to 100 parts); slightly soluble (100 to 1000 parts); Very slightly soluble (1,000 to 10,000 parts) and practically insoluble (> 10,000 parts).

Total ash (TA) and Acid insoluble ash (AIA)
TA was determined using 1.0 g of extract and a furnace (Furnace Star) which was heated gradually to the ignition temperature of $650 – 700^{0}$C. The process was repeated until at least two consecutive constant weights were obtained. The ash was dissolved in HCl 1 N and filtered with Whatman. The residue was heated until repeated at least two consecutive constant weights (which less that 0.01%) as AIA.

Phytochemical Screening
Test for Tannins
This test was taken in water, warmed and filtered. Five mL of filtrate was allowed to react with 1 mL of $FeCl_3$ 5%. Dark green or deep blue color indicated tannin is present.

Test for Alkaloid
Dragendorff test. The test extract was added Dragendorff's reagent. Formation of orange-brown precipitate indicated the presence of alkaloids.

Mayer test. To a 1 mL of test extract was added a few drops of Mayers reagent. Formation of cream colored precipitate it shows the presence of alkaloids.

Wagner test. The test extract was added Wagner reagent. Alkaloids give reddish brown precipitate.

Test for Saponins
Foam test. One mL solution of extract was diluted with double distilled water and shaken in a graduated cylinder for 15 min. Development of stable foam suggests the presence of saponins.

Test for amino acid and protein
Millon test. Two mL of Millon's reagent was added to 2 mL of test extract. White precipitate appears, which turns red upon gentle heating.

Ninhydrin test. Test extract was boiled with Ninhydrin 5%. Appearance of violet color demonstrated the presence of amino acid.
Biuret test. In to the test extract was added 4% NaOH solution and few drop of $CuSO_4$. Violet color appears indicate the presence of protein.

Test for polyphenol compounds

Phenolic test

Ferric chloride test. To the test solution and add few drop of neutral 5% ferric chloride. A dark green color indicates the presence of phenolic compounds.

Test for Flavonoid compounds

Shinoda test. The extracts were dissolved in alcohol. One piece of magnesium fallowed by concentrated hydrochloric acid was added drop wise and heated. Appearance of magenta color demonstrated the presence of flavonoids.

Total Phenols Estimation

The total phenolic content was determined by Folin-Ciocalteu reagent following the method of Stratil, with slight modification [9]. To 100 µL of extract, 2.5 mL of Folin-Ciocalteau's reagent (1/10 dilution) and 1.0 mL of Na_2CO_3 (7.5%, w/v) were added and incubated at 45º C for 15 min. The absorbance was measured at 765 nm using UV–visible spectrophotometer (Agilent). Total phenol content was expressed as tannic acid through the calibration curve with tannic acid (five point calibration).

Atomic absorption spectroscopy (AAS)

AAS is a technique used to determine the concentration of a particular metallic in anextract. All standards of analytes, proper dilutions were done by 1.0 mol/L nitric acid from the stock solutions of As, Cd, and Pb (1000 mg/L). 250 µL of test extract and standard solutions were pipetted into the vial in the measurements. Standard reference material bought was used for evaluating methods used in the determination of As, Cd, and Pb in extract.

Pesticide residue

Gas Chromatography is used to analyze the pesticide residue in extract. Firstly, liquid–liquid extraction (LLE) method to remove the residues and filtered. Stock solutions of individual standards (10 mg/mL) were prepared in toluene, considering standard purity, and stored in dark flasks at -20 ºC. The calibration standard solutions were prepared in toluene containing the three pesticides in concentrations ranging from 0.250 to 4.00 mg/mL while standard solutions prepared in acetonitrile were used for spiking test extract.

Thin Layer Chromatography (TLC)

The extract was dissolved in ethanol and applied with the help of micropipette tip on pre-coated silica gel F254 TLC plates (Merck). Different combinations of solvent system were tried for best separation of constituents [10].

RESULTS AND DISCUSSION

The sensory organoleptic examination that would have a direct bearing on handling and in-process quality control are shown in Tables 1.

Table 1. Organoleptic characteristics of the dry extracts of aqueous extract of *C. tinctorius* flowers

No.	Parameter	Result
1	Product name	Cartamus flower aqueous extract
2	Latin name	*Cartamus tinctorius*Linn.
3	Part plant used	Flower
4	Appearance	Brown granular powder
5	Odor	A characteristic aroma
6	Taste	A characteristic taste

Table 2. Physicochemical parameter of the dry extracts of aqueous extract of *C. tinctorius* flowers

No.	Parameter	Result
1	Solubility	Freely soluble in water
2	pH solution	pH of 5 % solution is 6
3	Lost on drying	6.0-6.7 %w/w
4	Total ash	4,92 – 5,37%w/w
5	Acid insoluble ash	0,506 – 2,89%w/w
6	Ethanol soluble extract	10.00 – 10.54 %w/w

Physicochemical constants can serve as a source of information usually used in judging the purity and quality of drug. The lost on drying of aqueous extract show moisture content was 6.0-6.7% which seems to be lower which necessary to support the growth of microbes such as bacteria, fungus and yeast to bring degradation of extract. In physicochemical parameter, determined in in four forms: the total ash, acid insoluble ash, ethanol soluble extract and

solubility. Aqueous extract freely soluble in water, total ash was 4,92 – 5,37%w/w, while acid insoluble ash was 0,506 – 2,89%w/w and pH solution of extract was 6 (Table 2).

Metallic residue was determined with commercial standard reference using AAS, metallic residue are arsenic (As), cadmium (Cd) and lead (Pb) (Table 3). Pesticide residue was determined using GC. Aldrin, dieldrin and edrin as pesticide residue Table 3). Long-term exposure of metallic or pesticide may cause long-term harm, such as cancer, damage to the reproductive system, the liver, the brain, and other parts of the body[11, 12].

Table 3. Metallic and pesticide residue in dry extracts of aqueous extract of *C. tinctorius* flowers

No.	Metallic	Specification	Result
1	Arsenic (As)	0.1 µg/g	No detected
2	Cadmium(Cd)	0.03 µg/g	0.0015-0.0058 µg/g
3	Lead (Pb)	7 µg/g	2.912-6.01 µg/g
4	Aldrin	LD 2.32 mcg/kg	No detected
5	Dieldrin	LD 14.47 mcg/kg	No detected
6	Edrin	LD 8.31 mcg/kg	No detected

Phytochemical screening of aqueous extract give information about chemical compounds of extract. The extract positive for amino acid, protein, phenolic and flavonoid compound but negative for tannin and saponin (Table 4). Presence of alkaloid in the plants extract may be participating in plant metabolism process [13, 14]. While no presence of tannin and saponin may be show no toxic and safety to consume[15, 16].

Table 4. Phytochemical characteristic of aqueous extract of *C. tinctorius* flowers

No.	Phytochemicals	Test	Result
1	Tannins	$FeCl_3$	-
2	Alkaloids	Dragendroff'stest	+
		Mayer,stest	+
		Wagner,stest	+
3	Saponin	Foam test	-
4	Protein and amino acid	Millon's test	+
		Ninhydrin test	+
		Biuret test	+
5	Phenolic compounds	Phenol test	+
6	Flavonoid compounds	Shinoda test	+
7	Total phenolic	Folin-Ciocalteu	3.8-4.8 %b/b

Phenolic and flavonoid compound in aqueous extract refer to the active compound of *C. tinctorius*. Total phenolic compound on extract was 3.8-4.8 %b/b (Table 4). A total of eight flavonoidswere isolated from *C. tinctorius*including a novel quercetin-7-O-(6″-O-acetyl)-β-D-glucopyranoside and seven known flavonoids, luteolin, quercetin, luteolin 7-O-β-D-glucopyranoside, luteolin-7-0-(6″-O-acetyl)-β-D-glucopyranoside, quercetin 7-O-β-D-glucopyranoside, acacetin 7-O-β-D-glucuronideand apigenin-6-C-β-D-glucopyrano syl-8-C-β-D-glucopyranoside[17]. This phytochemical compounds are the kind candidates in the medicinal value of the plant.

CONCLUSION

The results confirm that aqueous extract of *C. tinctorius* flowers possess characteristics that would allow it's possible development into Phytopharmaca and that meets standardization herb requirement.

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