International Journal of Applied Pharmaceutics

ISSN - 0975 - 7058 Vol 12, Special Issue 1, 2020

Research Article

SPECIFIC AND NON-SPECIFIC PARAMETERS BASED ON SIMPLICIA AND ETHYL ACETATE FRACTIONS OF STAR FRUIT LEAVES (*AVERRHOA CARAMBOLA* L.)

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Received: 20 September 2019, Revised and Accepted: 20 December 2019

ABSTRACT

Objective: Star fruit (*Averrhoa carambola* L.) is a plant with numerous benefits, including anti-skin inflammation properties. The aim of the present study was to evaluate simplicia and ethyl acetate fractions of star fruit leaves collected from three regions in Indonesia, including Depok, Subang, and Sukabumi, and to characterize specific and non-specific parameters.

Methods: Extraction was performed through maceration. Next, liquid partitioning was performed to obtain ethyl acetate fractions. Chromatograms for simplicia and ethyl acetate fractions for samples from three regions were obtained using chloroform: methanol:water (8:2:0.5) with apigenin as the standard.

Results: The yield of the ethyl acetate fraction from the star fruit leaves was 4.2–6.2%. Water content, total ash content, and acid-soluble ash contents were 4.79%, 1.55%, and 0.064%, respectively, and total flavonoid levels were 14.63–22.14 mg QE/g of a fraction. The water-soluble and ethanol-soluble extracts were 14.32% and 9.69%, respectively, and total flavonoid contents were 0.12–0.18 mg QE/g simplicia. In addition, drying losses, ash content, and acid-soluble ash content were 9.70%, 7.14%, and 0.31%, respectively. The results of solvent residues and heavy metal contaminant (Hg, Pb, Cd, and As) tests indicated that the ethyl acetate fraction did not contain solvent residue or heavy metal contamination.

Conclusion: The specific and non-specific parameters based on simplicia and ethyl acetate fractions from star fruit leaves meet the requirement.

Keywords: Apigenin, *Averrhoa carambola*, Specific parameters, Non-specific parameters.

 DOI: http://dx.doi.org/10.22159/ijap.2020.v12s1.FF022

INTRODUCTION

Standardization is necessary to obtain uniform raw materials which would guarantee consistent pharmacological effects of medicinal plants [1,11]. In addition, the efficacy of a medicinal plant depends on the chemical constituents, which is under the influence of numerous factors, such as the place of growth, climate, and rainfall, in addition to harvesting practices [4,7,10]. A key challenge, therefore, is how to determine the consistency of the quality of crude drugs and extracts of a plant cultivated across regions with different altitudes, soil conditions, and weather [9].

Quality requirements for extracts and botanicals consist of specific predefined parameters and various general standards (nonspecific) [2,3]. Specific parameters include the identity of the extract, the organoleptic extract, and the concentrations of substances to be dissolved in certain solvents, while the non-specific parameters include drying shrinkage, moisture content, total ash content, residual solvents, pesticide residues, and contamination with heavy metals, in addition to microbial contamination [12].

Averrhoa carambola L., which is commonly known in Indonesian society as star fruit, is a plant used extensively in traditional medicine [5,8]. Star fruit leaves have numerous health benefits, for example, they have antiulcer, anthelmintics, and antitumor properties, in addition to being used to manage hypotension [15]. In addition, studies have shown that the ethyl acetate fraction of star fruit leaves contains high levels of apigenin most and exhibits the highest lipoxygenase enzyme inhibitory activity [13,14].

Considering the potential pharmacological applications of extracts from star fruit leaves, the manufacture of ethyl acetate fractions, in addition to their standardization, with the aim of maintaining consistency with regard to quality, and guaranteeing safety and efficacy, need to be explored. In addition, the present study aimed to establish key specific and non-specific parameters for crude drugs and obtain

Table 1: Organoleptic examination of *Simplicia* powder from star fruit leaves (*Averrhoa carambola* L.)

Type of examination	Result		
Organoleptic	**Depok**	**Subang**	**Sukabumi**
Form	Dry powder	Dry powder	Dry powder
Odor	Distinctive aroma	Distinctive aroma	Distinctive aroma
Color	Blackish-green	Brownish-green	Green
Taste	Bitter	Bitter	Bitter

Table 2: Macroscopic examination of *Simplicia* from star fruit leaves (*Averrhoa carambola* L.)

S. No.	Sample	Results
1.	Depok	Dried round leaves, short stems, pointed edges, blunt or rounded bases, flat-leaf edges, pinnate leaf bones, and blackish-green
2.	Subang	Dried round leaves, short stems, pointed edges, blunt or rounded bases, flat-leaf edges, pinnate leaf bones, and brownish-green
3.	Sukabumi	Dried round leaves, short stems, pointed edges, blunt or rounded bases, flat-leaf edges, pinnate leaf bones, and brownish color

ethyl acetate fractions from star fruit leaves, which could facilitate their standardization and application in the management of diverse conditions.

Table 3: Phytochemical screening of *Simplicia* and ethyl acetate fraction of star fruit leaves (*Averrhoa carambola* L.)

S. No.	Phytochemical parameters	Result		
		Depok	Subang	Sukabumi
1.	Flavonoid	(+)	(+)	(+)
2.	Alkaloid			
	Dragendorff	(−)	(−)	(−)
	Mayer	(−)	(−)	(−)
	Bouchardat	(−)	(−)	(−)
3.	Tanin	(+)	(+)	(+)
4.	Saponin	(+)	(+)	(+)
5.	Terpenoid	(+)	(+)	(+)
6.	Glikosida	(+)	(+)	(+)
7.	Anthraquinone	(−)	(−)	(−)

MATERIALS AND METHODS

Materials

A. carambola leaves were obtained from Depok, Subang, and Sukabumi regions in Indonesia.

Extraction and fractionation

A. carambola leaves were obtained from Depok, Subang, and Sukabumi. *Simplicia* powder was extracted from the leaves using 70% ethanol using the maceration method, with a powder: solvent ratio of 1:10. The maceration process began with soaking *Simplicia* for 6 h while stirring occasionally and then the mixture was allowed to stand for 18 h. The mixture was evaporated on a rotary vacuum evaporator to reduce the solvent until a sticky extract was obtained. The extract was fractionated to purify the compounds using n-hexane, ethyl acetate, and water. The extract was ground into powder, suspended in n-hexane, and homogenized using a sonicator for 10 min. The suspension was filtered using filter paper and then the filtrate was evaporated on a rotary evaporator until a viscous extract was obtained. The viscous extract was then dried using a vacuum oven at 40°C–50°C until a constant weight.

Table 4: Specific and non-specific parameters from star fruit (*Averrhoa carambola* L.) *Simplicia*

Examination	Origin			Range
	Depok	Subang	Sukabumi	
1. Specific parameters				
Water-soluble extract	14.32%	22.68%	24.03%	14.32%–4.03%
Ethanol-soluble extract	9.69%	25.74%	30.25 %	9.62%–30.25%
Total flavonoid	0.12 mg QE/g simplicia	0.18 mg QE/g simplicia	0.18 mg QE/g simplicia	0.12–0.18 mg QE/g simplicia
2. Non-specific parameters				
Drying losses (%)	9.70	8.89	8.75	8.75–9.75
Total ash content (%)	7.14	5.92	6.63	5.92–7.14
Acid-soluble ash content (%)	0.31	0.24	0.29	0.24–0.31

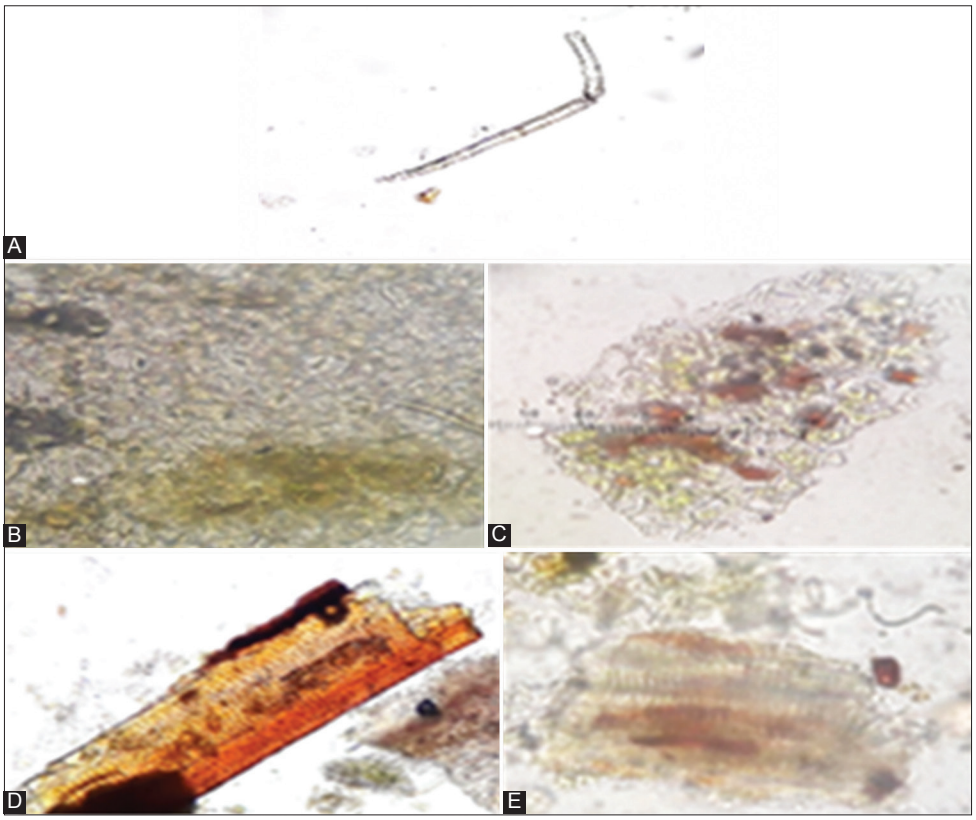

Fig. 1: Microscopic examination of simplicia of star fruit leaves at 10×. One- to three-celled hairs (A), parasitic type stomata (B), oil gland cell (C), vascular tissue with oil (D), vascular tissue with spiral (E).

Afterward, the residue was dissolved in ethyl acetate and homogenized using sonicator for 10 min. The solution was then partitioned by adding distilled water, shaken in a separator funnel, and allowed to stand for 30–60 min until two layers were formed (ethyl acetate layer on top and distilled layer water at the bottom). The layers formed were then separated. The ethyl acetate and water layers were evaporated on a rotary evaporator until a viscous extract was obtained.

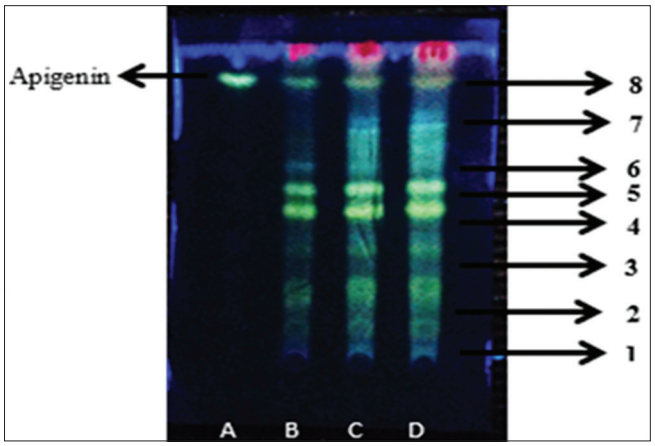

Fig. 2: Chromatogram of simplicia from star fruit leaves (*Averrhoa carambola* L.). Standard (A); Depok (B); Subang (C); Sukabumi (D)

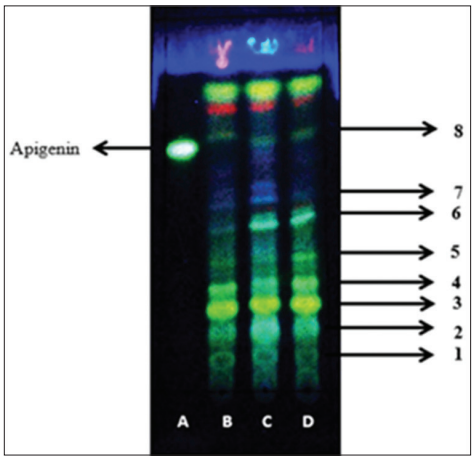

Fig. 3: Chromatogram of ethyl acetate fractions from star fruit leaves. (A) Standard, (B) ethyl acetate fraction of Depok, (C) ethyl acetate fraction of Subang, (D) ethyl acetate fraction of Sukabumi)

Table 5: Organoleptic examination of ethyl acetate fraction from star fruit leaves from three regions

Type of examination	Result		
	Depok	Subang	Sukabumi
Organoleptic			
Form	Thick extract	Thick extract	Thick extract
Odor	Distinctive aroma	Distinctive aroma	Distinctive aroma
Color	Blackish-brown	Blackish-brown	Brownish-green
Taste	Bitter	Bitter	Bitter

RESULTS AND DISCUSSION

Organoleptic parameters are specified using the senses, which are used to describe the properties of crude drugs based on shapes, smell, color, and taste. According to the results, the samples from the three regions exhibited similar organoleptic properties, with a distinct smell and a slightly tart flavor. However, the plants from the three regions could be distinguished based on a different color. The different color from leaves may be influenced by environmental factors, in addition to the time harvest and the drying process [6]. Organoleptic test results are listed in Table 1.

The first stage in the present study was the macroscopic observation of star fruit levels. Traits observed included leaf shape and color of the star fruit plants. The results of the macroscopic examinations of star fruit leaves from the three regions are listed in Table 2. The star fruit leaves from the three locations had star fruit leaves with similar shapes, which were circular. In addition, leaf petioles were short, with pointed tips, bases were obtuse or rounded, and the leaf edges were flat. The only factor that could be used to distinguish the leaves from the three regions was color.

Microscopic examinations were performed to determine the fragments that could be used to characterize *Simplicia*. Microscopic plant tissue from specific parts displays unique features so that they could be used to distinguish similar parts from different plants, based on sections referred to as identification fragments, as illustrated in Fig. 1. Microscopic examination of simplicia from star fruit leaves at a 10 × revealed identifiable fragments in the form of one- to three-celled cover hairs, parasitic stomata, and oil gland cells. We used chloroform: methanol:water mobile phase with a ratio of 8:2:0.5 to reveal chromatograms of the simplicia and ethyl acetate fractions. The eluted plate was then sprayed with 5% $AlCl_3$ and observed under 366 nm ultraviolet light. The chromatogram results of the simplicia and ethyl acetate fractions are presented in Figs. 2 and 3, respectively. In the simplicia results, there are identical spots between the standards and the samples; therefore, the simplicia from star fruit leaves contained apigenin flavonoids.

Phytochemical screening was performed for the simplicia from the three regions. According to the results, the simplicia contained flavonoids, tannins, saponins, terpenoids, and glycosides (Table 3). The specific and non-specific parameters of simplicia from star fruit leaves are listed in Table 4, organoleptic examination of ethyl acetate fraction from star fruit leaves from three regions are listed in Table 5, while the specific and non-specific parameters of the ethyl acetate fractions are listed in Table 6.

CONCLUSION

The simplicia of the *A. carambola* leaves produced a dry powder, ranging in color from green to brown and with a distinctive smell.

Table 6: Specific and non-specific parameters of ethyl acetate fractions from star fruit leaves (*Averrhoa carambola* L.)

Examination	Origin			Range
	Depok	Subang	Sukabumi	
1. Specific parameters				
Total flavonoid levels	14.63 mg QE/g simplicia	16.40 mg QE/g simplicia	22.18 mg QE/g simplicia	14.63–22.18 mg QE/g simplicia
2. Non-specific parameters				
Water content %(v/b)	4.79	4.59	4.59	4.59–4.79
Total ash content (%)	1.23	0.88	1.55	0.88–1.55
Acid-soluble ash content	0.064	0.049	0.049	0.049–0.064

They had a somewhat tart taste. The leaves were circular in shape and the leaf petioles had short pointed tips, and the leaves had obtuse or rounded bases, flat-leaf edges, and green-brown color. Microscopic examination of fragments revealed identifiers on the hair bulbs, with one- to three-celled hairs, parasitic type stomata, and oil gland cells, file thickening carrier with nets and with oil present, with thickening of the ladder carrier file. In addition, water-soluble compound contents were not lower than 14.23% and ethanol-soluble compound contents were not lower than 9.62%.

Phytochemical screening tests revealed that the presence of flavonoids, tannins, saponins, terpenoids, and glycosides, and total flavonoid contents was not lower than 0.12 mg QE/g of bulbs. Non-specific parameters included drying shrinkage not greater than 9.70%, total ash content not greater than 7.14%, and acid-insoluble ash content not greater than 0.31%. The organoleptic properties of the ethyl acetate fraction from star fruit leaves included a thick extract with a blackish-brown color, a distinctive smell, and a bitter taste.

CONFLICTS OF INTEREST

We declare that we have no conflicts of interest.

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