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Analysis of Non Specific Parameters of Simplicia Bloodleaf Plant (*Iresine herbstii* Hook) During Storage

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ABSTRACT

Bloodleaf plant, scientifically known as *Iresine herbstii* Hook, is an ornamental shrub with possible medicinal properties. Storage of simplicia is necessary to guarantee the accessibility of raw materials from *I. herbstii*. Prolonged storage of simplicia can impact its quality, including many non-specific factors. Indeterminate parameters suggest a relationship between the characteristics of Hook's *I. herbstii* simplicia during extended storage. This study seeks to ascertain the impact and correlation between the duration of storage of simplicia and the non-specific characteristics of *I. herbstii* simplicia. Moisture content, extractive value, and ash content were examined in samples with shelf lives of 0 month (IH0), 4 months (IH4), 10 months (IH10), 20 months (IH20), and 32 months (IH32). The acquired data were analyzed using DMRT (Duncan's Multiple Range Test) and MFA (Multiple Factor Analysis). The analysis results indicated that the storage duration impacted all parameters examined except acid-insoluble ash concentration. Prolonged storage of *I. herbstii* simplicia decreased the concentration of water-soluble and alcohol-soluble extractive value. At the same time, the water content and total ash content increased. The MFA analysis revealed a strong link between non-specific parameters and the duration of storage of *I. herbstii* simplicia, except for acid-insoluble ash concentration.

KEYWORDS:
Iresine herbstii Hook
Storage duration
Quality of simplicia
MFA

1. INTRODUCTION

The perennial plant *Iresine herbstii* Hook is a plant from the Amaranthaceae. Widespread in tropical and subtropical areas, including Indonesia (Jaafar and Jaafar, 2021). Indonesian people have used natural ingredients from plants as a form of treatment. One plant that has the potential to be used as traditional medicine is bloodleaf plant, but so far this plant is mostly known as an ornamental plant due to its vibrant red leaves. *I. herbstii* has alternative names such as chicken gizzard, beefsteak plant, and herbst's bloodleaf (Jaafar & Jaafar, 2021). *I. herbstii* originates from tropical South America and is believed to have been initially gathered in Brazil, it which be found in the tropical forest across many regions of India and tropical Asia (Flores-Olvera, Zumaya & Borsch, 2016). Bloodleaf plant possesses wound healing properties, exhibits low antioxidant activity, affects the central nervous system, demonstrates affinity for various cerebral receptors, and shows potential as an antiviral agent (Andleeb et al., 2020). Apart from being an ornamental plant, this is also a medicinal plant containing alkaloids, flavonoids, and anthocyanins (Iswoyo et al., 2023; Safrina and Joko Priyambodo, 2018; Asikin et al., 2014). People use the compounds in *I. herbstii* as repellant for waste remediation, as antibacterial agents, antiviral agents, and natural dyes (Efendi et al., 1970; Andleeb et al., 2020; Spórna-Kucab et al., 2020; Agustina et al., 2022; Kiran et al., 2022; Sai-Ut et al., 2023).

The increasing number of people adopting the Back to Nature lifestyle has influenced the increase in demand for raw materials for herbal medicines, but there are still problems in maintaining the sustainability of raw materials. Simplicia possesses the benefit of being capable of being preserved in a simple form for an extended period, which can impact the quality of simplicia due to the proliferation of microorganisms, leading to alterations in color, structure, scent, taste, and biological activity (Safrina & Supriadi, 2020). Hence, proper storage plays a crucial role in the processing of medicinal plants to safeguard the integrity of their chemical constituents (Nurapni et al., 2023). Sustaining an industry or business depends on the continuous supply of raw materials. Stock storage is crucial to maintain continuity when field production is inconsistent (Hossain et al., 2023). Despite sufficient supplies, various problems can disrupt raw material stability (Saptadi et al., 2023). Likewise, with the stock of *I. herbstii* as raw material for medicinal plants, requiring controlled environment conditions, strict inspection, regular maintenance, until appropriate storage systems to maintain quality (Ching et al., 2023; Tejesh and S. J., 2023; Li et al., 2022).

Non-specific parameters are important parameters in the quality of simplicia. These parameters include water content, ash value, and extractive value (Kementerian Kesehatan Republik Indonesia, 2000). Water content indicates the minimum amount of water contained in the extract when it is excessive during storage because of environmental conditions causing the growth of microbes and damage to the stability of the simplicia (Maciel & Steppe, 2017). The total ash content is used to describe the mineral content found on the inside and outside of the simplicia so that a high ash content can indicate that the simplicia contains many minerals during storage (Sari, Elya & Katrin, 2019). The extractive value shows the quality of the simplicia by knowing the content of the compounds extracted in the solvent used (Safrina, Herera & Kusumadewi, 2021). The opportunity to develop *I.herbstii* as a raw material for traditional medicine is still very large, so specific and accurate data is needed, one of which is the sustainability of raw material availability. Research on the effect of shelf life on extracted value, water content, and ash value can be used to determine the extent to which simplicia *I. herbstii* still has good quality for use after storage. This study aims to determine the effect of shelf life on non-specific *I. herbstii* simplicia parameters and the correlation between the two.

2. MATERIALS AND METHODS

2.1. Sample Preparation

The *I. herbstii* plants used as samples were grown at an elevation of around 1800 meters above sea level at the medicinal plant garden of Tlogodlingo Village in Tawangmangu District. Planting, maintaining, harvesting, and seeding are all part of *I. herbstii* farming. The samples of *I. herbstii* plants used in the study were obtained from the Tlogodlingo medicinal plant garden, approximately 1800 meters above sea level. The garden covers an area of 1200 m^2, and the plants are spaced 30 cm x 30 cm apart. The application rate of fertilizer is 20 tonnes per hectare of manure. Flowers were collected as specimens when they were three months old after planting. During the harvesting phase, *I. herbstii* undergoes a sorting procedure to remove any impurities from the material. The samples were subsequently washed in a continuous flow of water until they were completely free from contaminants and then left to dry. The *I. herbstii* leaves undergo processing using a leaf chopper machine, yielding fragments measuring 8-10 cm long. The samples were then dehydrated in an oven maintained at around 43 ^oC for approximately 4-5 days until their water content decreased to 10% or below. The simplicia is contained within HDPE plastic packaging, with a precise thickness of 0.13 mm. Furthermore, each bottle is equipped with ten silica gel sachets. The packaging step entails employing the Hoover technique. The processed herbal materials are stored in the postharvest laboratory warehouse.

2.2.Storage of Research Samples

The *I. herbstii* simplicia plants are kept in the simplicia warehouse at a temperature ranging from 20 °C to 25 °C and a humidity level between 65% and 75%. Observations were conducted to assess the storage period of the simplicia, considering the water content and non-specific simplicia. The observed samples were of simplicia that were preserved for various durations: 0 months (IH1), 4 months (IH2), 9 months (IH3), 10 months (IH4), 20 months (IH5), and 32 months (IH6). Every storage period consists of three repetitions. The storage duration for *I. herbstii* simplicia is determined based on the stock inventory data in the Simplicia warehouse. The parameters observed include moisture content (MC), water soluble extractive (WSE), alcohol soluble extractive (ASE), total ash (TA), and acid insoluble ash (AIA).

2.3. Non-Specific Parameters

2.3.1. Moisture content (MC)

Determination of water content was carried out using the gravimetric method using a Moisture Analyzer MFX-50. The temperature used to determine water content is 105 ^oC.

2.3.2. Determination extractive value

2.3.2.1. Water soluble extractive (WSE)

Preparation of the test equipment includes preparing a porcelain cup, heating it to a temperature of 105 ^oC, placing it in a desiccator, and then cooling it. Next, weigh until the weight is constant and the results are recorded. Next is the preparation of the water-chloroform test material. Preparation of the water-chloroform test material is to take 2.5 mL of chloroform (CH_3Cl) PA, add distilled water to 1000 mL in a 1000 mL beaker, and transfer it to a media dispenser bottle. The steps for determining the water-soluble essence content are carefully weighing 5 g of the medicinal plant simplicia powder, putting it in a laboratory bottle with a lid, and adding 100 mL of water chloroform. Shake using a shaker at 80 rpm for 6 hours and leave for 18 hours. Filter the entire filtrate, take 20 mL, put it in a porcelain cup, and evaporate the filtrate until dry. Heat the remainder at 105 ^oC, place in a desiccator, then weigh until constant weight.

2.3.2.2. Alcohol soluble extractive (ASE)

Preparation of the test equipment includes preparing a porcelain cup, heating it to a temperature of 105 ^oC then placing it in a desiccator. Next, weigh until the weight is constant and the results are recorded. Then, 96% ethanol was put into the container connected to the Finnpipette dispenser. The steps for determining the ethanol-soluble essence content are carefully weighing 5 g of the simplicia powder and putting it in a laboratory bottle with a lid. Moreover, adding 100 mL of 96% ethanol. Shake using a shaker at 80 rpm for 6 hours and leave for 18 hours. Filter the entire filtrate, take 20 mL, put it in a porcelain cup, and evaporate until dry. Heat at 105 ^oC until constant weight.

2.3.3. Determination ash value

2.3.3.1. Total Ash (TA)

Preparation of the test equipment includes preparing a silicate crucible, heating it to a temperature of 800 ^oC, then placing it in a desiccator. Next, weigh until the weight is constant and the results are recorded. The procedure for determining the TA content is carried out carefully weighing 2 g of the simplicia powder, placing it in a silicate crucible, gently igniting it until the charcoal runs out, placing it in a desiccator, and weighing it until the weight is constant. If the charcoal cannot be removed, add hot water and filter using ash-free filter paper. Then, filter the remainder in filter paper in the same crucible. Place in a desiccator and weigh until constant weight.

2.3.3.2. Acid insoluble ash (AIA)

Preparation of the test equipment includes preparing a silicate crucible, heating it to a temperature of 800 ^{o}C and placing it in a desiccator. Next, weigh until the weight is constant and the results are recorded. 226 mL HCl solution into a 1 L beaker, add distilled water to 1 L, and put it in a dilute HCl container. The procedure for determining the acid-insoluble ash content is to boil the ash obtained from determining the TA content with 25 mL of dilute HCl, collect the part that is not soluble in acid, filter it using ash-free filter paper, let it stand, put it in a desiccator, and weigh it until constant.

2.4. Data Analysis

Data analysis was carried out with DMRT and R–statistics software. Multiple factor analysis (MFA) summarizing and visualizing a complex data table in which individuals are described by several sets of variables (quantitative and/or qualitative) structured into groups. The effect of storage time for *I. herbstii* simplicia was used as the main factor.

3. RESULTS AND DISCUSSION

During the storage process of *I. herbstii* there was a decrease in WSE and ASE but there was an increase in the MC of TA, and AIA (**Table 1**). DMRT analysis showed that the length of storage of *I. herbstii* simplicia influenced MC, WSE, ASE, TA, but had no effect on AIA.

Table 1. Non-specific parameters of *I. herbstii* simplicia during storage simplicia during storage results

Samples	MC		WSE		ASE		TA		AIA	
IH0	6.97 ± 0.18	a	3.43 ± 0.01	d	0.88 ± 0.02	d	12.30 ± 0.05	a	1.56 ± 0.56	a
IH4	8.5 ± 0.39	b	3.16 ± 0.05	c	0.84 ± 0.01	c	13.33 ± 0.38	b	1.82 ± 0.88	a
IH10	9.33 ± 0.51	c	2.91 ± 0.06	b	0.66 ± 0.03	b	14.66 ± 0.07	c	2.42 ± 0.40	a
IH20	10.29 ± 0.56	d	2.83 ± 0.08	a	0.59 ± 0.01	a	14.73 ± 0.17	c	2.46 ± 0.26	a
IH32	12.38 ± 0.29	e	2.79 ± 0.02	a	0.59 ± 0.02	a	14.82 ± 0.02	c	2.49 ± 0.13	a

Remarks: The value is average value ± deviation standard; n=3. The same alphabet on the same column shows no significant difference at 5%.

Changes in water content in simplicia *I. herbstii* are influenced by temperature and humidity during storage. Storage of *I. herbstii* simplicia for 32 months increased by 4.9%. WSE was reduced by 0.65% and ASE was reduced by 0.31%. Based on the research results, the value of the WSE in each treatment was higher when compared to the ASE, indicating that the constituent components are more polar in nature. The decrease in WSE in simplicia is caused by increased microbial activity due to an increase in water vapor during storage so that it can damage the compounds contained during storage (Safrina, Herera & Kusumadewi, 2021). WSE is higher than ASE because the content of most secondary metabolite compounds is polar in nature found in *I. herbstii* compared to semi-polar secondary metabolite compounds, so that these compounds will easily dissolve in water compared to alcohol (Febrianti et al., 2019). There was no significant decrease in WSE and ASE after storage of simplicia for 20 months because the content of soluble compounds is relatively stable against bacterial damage. AIA describes soil and sand contamination in the simplicia production process (Fatimawali, Kepel & Bodhi, 2020). Storage duration did not have a significant effect on AIA because there was no increase in sand or soil content in the vacuum-packed simplisia in plastic containers.

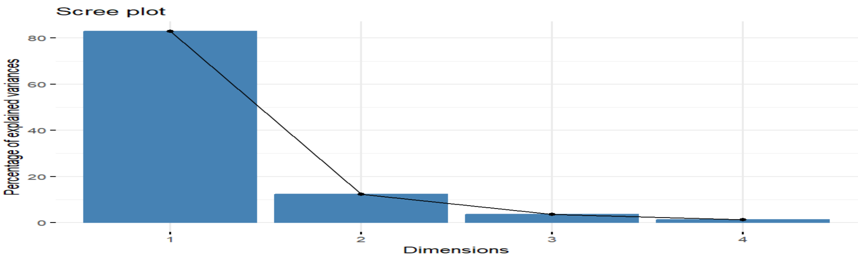

Figure 1. Distribution of explained variance across dimensions of non-specific variables parameters of simplicia *Iresine herbstii* during storage.

A scree plot uses multiple-factor analysis to visually depict the relationship between dimensions and the proportion of explained variations (**Fig. 1**). The X-axis represents the quantity of dimensions considered in the investigation. Simultaneously, the Y-axis represents the proportion of the overall variability accounted for by each dimension in the four-dimensional graph. The first dimension represents 82.96% of the total, and the second dimension contributes 12.2%. Collectively, these two dimensions account for over 95% of the entire sum. The percentage of explained variance experiences a significant decrease from the first to the second dimension, and this loss continues, albeit at a slower pace, from the second dimension onwards. This trend indicates that most of the variation in the dataset can be accounted for by the initial dimension, with declining benefits from including other dimensions. The sharp decline following the initial dimension indicates that the first principal component holds considerably greater significance than the others in elucidating the variation within the sample of simplicia *I. herbstii* Hook during storage. The scree plot indicates the reduction of the data dimensionality from five principal components to the first two dimensions due to representing 95% variability of the majority of the information from all variables (Abdi, Williams & Valentin, 2013; Mewengkang, Mananohas & Komalig, 2022).

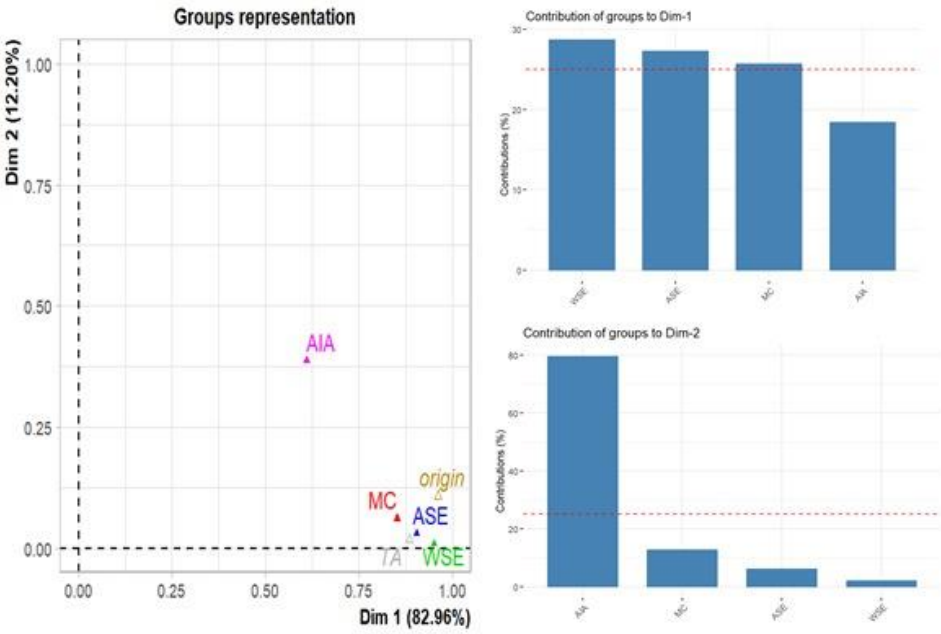

Figure 2. Results of multiple factor analysis-groups of variables non-specific parameters of simplicia *Iresine herbstii* Hook during storage (2a); Percentage contribution of the non-

specific variable group Simplicia *I. herbstii* during storage in dimension 1 (2b); Percentage contribution of the non-specific variable group Simplicia *I. herbstii* during storage in dimension 2 (2c).

The results of MFA-group variables show a correlation between variables and dimensions (**Fig. 2a**) where the active variable group is shown in a plot located in dimension 1 (contribution of 82.96%) that is used in this study for the non-specific parameters since the high contribution value illustrates the strong correlation between simplified storage time and changes in the values of WSE, ASE, and MC (Abdi, Williams & Valentin, 2013). In this study, the more extended storage of simplicia (*I. herbstii*) can reduce the value of WSE, reduce the value of ASE, and increase the MC of Simplicia. The coordinates of these three groups of variables are almost the same, which means they make almost the same contribution to dimension 1 (**Fig. 2b**). The complementary variable group is shown in purple on the variable plot and is in dimension 2 (contribution 12.2%). The variable AIA is the variable that is most strongly correlated with dimension 2. In this study, AIA provided the most dominant contribution in dimension 2 (**Fig. 2c)**

The results of previous research explain that simplicia's MC is an essential component in its storage process. The stored medicinal plant simplicia must have a low MC because high MC will make it easier for fungi and mold to grow, which can damage the simplicial (Pusmarani et al., 2019). Other active parameters are WSE, and ASE. The WSE can describe the number of compounds contained in the simplicia that are polar or have the same polarity as water. The higher the value of the WSE of a simplicia, the expectation is that the chemical compounds dissolved in water will also be higher. The ASE describes the number of compounds that dissolve in alcohol. In terms of quality, the aim of determining the ASE is almost the same as determining the WSE (Husni, Ismed & Awaliana A, 2021).

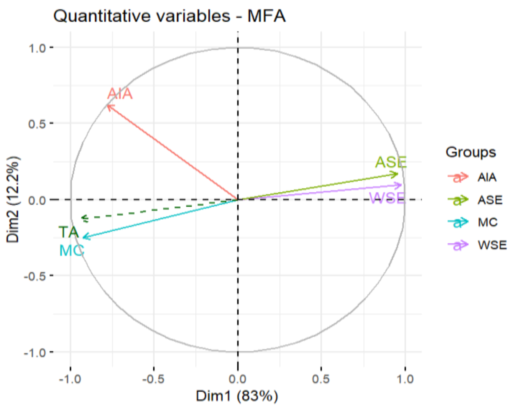

Figure 3. Results of multiple factor quantitative analysis of non-specific variables parameters of simplicia *Iresine herbstii* during storage

The results of multiple factor analysis-quantitative variables (**Fig. 3**) show that three groups of variables group/cluster each other. The WSE and ASE are two variables grouped into 1. The MC of simplicia and TA of simplicia are grouped into 1, with the plots being close together. This shows that the WSE and ASE levels are correlated with the storage time of the simplicial *I. herbstii* (Lê, Josse & Husson, 2008). The longer *I. herbstii* simplicia is stored, the WSE and ASE levels will decrease. Meanwhile, *I. herbstii* simplicia, which is stored for a long time, will increase the MC and TA of the simplicia.

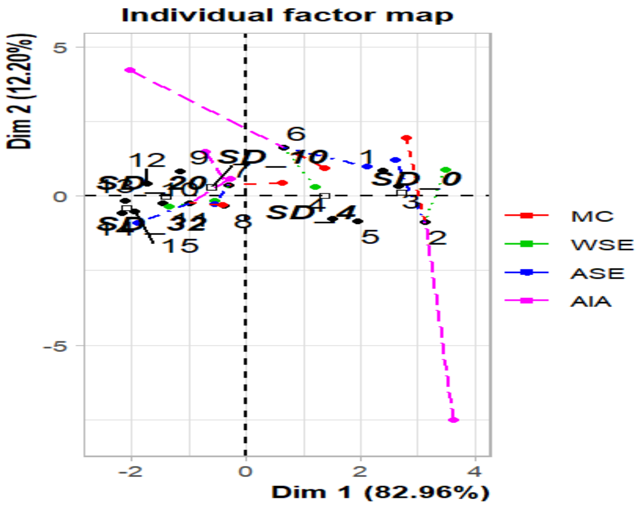

Figure 4. Individual factor map of non-specific variables parameters of simplicia *Iresine herbstii* during storage.

The graph **(Fig. 4)** illustrates the distribution of different data points over two main dimensions. The x-axis represents Dim 1, which explains a large percentage of the variance, exactly 82.96%, making it the primary axis of variation among the data points. Dim 2, which is represented on the y-axis, explains a lesser amount of the variation, exactly 12.28%. The data points are numerically labeled from 0 to 32 and are distributed randomly on the graph. Every point in the collection represents a distinct observation or case. The dashed vertical line at x=0 symbolizes the mean or median of Dim 1. It separates the data into two groups based on their scores in relation to this center value. The dashed magenta line could perhaps indicate a trend line or a distinct threshold inside Dim 2, emphasizing a certain element or subdivision within the data. The AIA exhibits a clear trajectory, progressing from the lower left to the top right quadrant, indicating a robust correlation with both dimensions. The concentration of MC around the center indicates a moderate impact on both dimensions. The WSE and ASE are distributed unevenly, suggesting different levels of impact on the dimensions. The various factors contribute to the primary dimensions, likely drawn from a broader set of underlying data. The arrangement and distribution of the data points can facilitate the detection of patterns, correlations, or groups among the variables during storing *I. herbstii* simplicia.

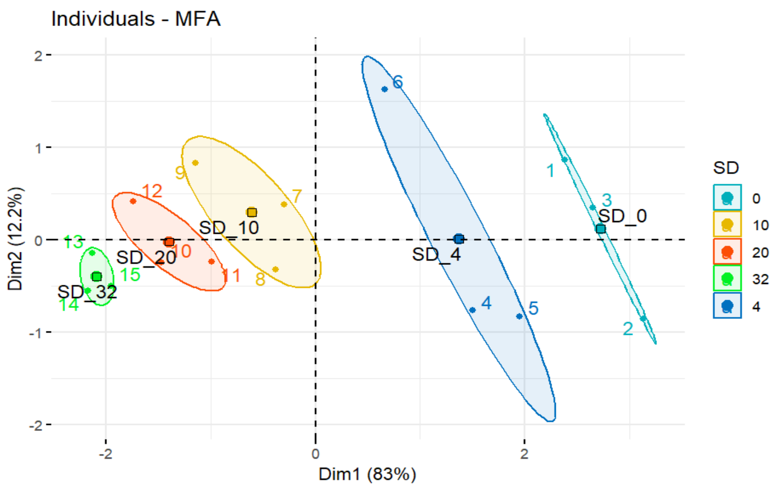

Figure 5. Results of multiple factorial analysis-individual variable non-specific parameters of simplicia *Iresine herbstii* during storage

Individual multiple-factor analysis is an analysis that can group individuals with similar profiles who are close to one another (**Fig. 5**). Individuals analyzed in MFA had storage times ranging from 0 to 32 months. The results of the individual multiple factor map analysis show that storage duration 0 (SD_0) and storage duration 4 (SD_4) are in the first-dimension plot; this shows that fresh simplicia (0 months storage duration) and four months storage duration are the best storage time of *I. herbstii* simplicia (positive coordinates). This is in sharp contrast to the storage duration 32 (SD_32) plot, which is closest to dimension two, which is a negative coordinate (Abdi, Williams & Valentin, 2013). Storing *I. herbstii* simplicia for 32 months is the worst storage method because it can reduce the quality of the product. This research shows that the longer the simplicia is stored, the higher the MC. This means that the longer the simplicia is stored, the worse its quality. Other parameters that indicate simplicia's quality is worsening after long storage are the decrease in WSE and the decrease in ASE (Sari, Elya & Basah, 2020).

4. CONCLUSIONS

The DMRT study revealed that the duration of storage of *I. herbstii* simplicia had a significant impact on MC, WSE, ASE, and TA, but did not affect AIA. The MFA analysis demonstrated a significant correlation between general parameters and the length of storage of *I. herbstii* simplicia, with the exception of the concentration of AIA. There is a positive correlation between the storage period of 0 month and 4 months on non-specific factors, indicating that the optimal storage period is up to 4 months.

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