EXTRACTION TIME EFFECT ON ACTIVE COMPOUNDS LEVELS IN CAT WHISKERS (*ORTHOSIPHON ARISTATUS* (BLUME) MIQ.)

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ABSTRACT

Objective: Determine the best time to boil cat whiskers by observing the impact of boiling time on the quantities of rosmarinic acid in cat whiskers.

Methods: For the extraction process, water is boiled for 10, 20, and 30 min at 90 degrees Celsius. High-Performance Liquid Chromatography (HPLC) was used to measure the quantities of rosmarinic acid and validate the analytical procedures in terms of accuracy, precision, linearity, and specificity. The one-way ANOVA test and Duncan's test were used to analyse the data; a p-value of 0.05 was used to indicate a statistically significant difference.

Results: The lowest quantities of rosmarinic acid were found in the study's results during a shorter boiling duration of 10 min, or 2.07% w/w. The highest concentrations of rosmarinic acid were found after a prolonged boiling period of 20 min, at 2.32 % w/w. Meanwhile, rosmarinic acid levels dropped to 2.15 % w/w after a 30 min overboiling period. Rosmarinic acid levels from the three boiling durations differed significantly, according to statistical analysis (p=0.000; p<0.05).

Conclusion: It was determined that 20 min was the ideal boiling duration for extracting rosmarinic acid from purple cat whiskers.

Keywords: Cat whiskers, Purple variety, Rosmarinic acid, Heating time, Validation, HPLC

INTRODUCTION

Cat whiskers are grouped based on the morphology of the flower into three varieties, namely purple, white-purple and white varieties [1, 2]. The purple variety of cat whiskers contains the most secondary metabolites compared to the white variety [3]. Rosmarinic acid, eupatorin and sinensetin are the three secondary metabolites in cat whiskers [4]. The purple cat's whiskers have the highest levels of rosmarinic acid and sinensetin, while the white-purple cat's whiskers have the highest eupatorin levels [3, 5].

Cat whiskers are empirically well-known diuretic and antidiabetic [6, 7]. Indonesian people use cat's whiskers traditionally by consuming boiled leaves of cat's whiskers which are believed to overcome several health problems, such as difficulty urinating if consumed as much as one glass three times a day [8, 9]. Boiling by the community is one of the extraction techniques to extract active compounds from plants, in this case, extracting rosmarinic acid from the cat's whiskers plant.

Rosmarinic acid belongs to a class of phenolic compounds derived from caffeic acid [10, 11]. It is known that in the extraction of phenolic compounds, the influence of extraction time plays an important role. If the time is too fast, the phenolic compounds have not been completely attracted because the dissolution process with solvents has not been maximized [12-15]. Meanwhile, suppose the extraction time is too long. In that case, oxidation of phenolic compounds will occur due to too high oxygen exposure [16], so the levels of extracted phenolic compounds will decrease. This oxidation process is one of the mechanisms for the decomposition of a compound (degradation) caused by the presence of oxygen.

In addition, the boiling time (extraction) can affect the amount of rosmarinic acid in the extract produced. The extract produced will increase if the boiling time is longer; therefore, the amount of rosmarinic acid will be affected, too. However, the longer the boiling time, the longer the plant will experience contact with hot temperatures, which can cause compound degradation and reduced compound stability, so the levels of extracted secondary metabolites will decrease [17-19]. The reason for choosing this boiling method is because of the polarity of rosmarinic acid, which is polar and can

dissolve in water because it has one carboxylic group and four hydroxyl group. Another reason for choosing the boiling method is based on the community's empirical use of the cat's whiskers plant through the boiling process, in which the community consumes the decoction of the cat's whiskers leaves, which are believed to be able to overcome various health problems [8, 20].

In addition to the effect of boiling time, there are several factors that can affect levels of cat's whiskers that have been reported in previous studies, including the effect of fermentation time [21–24] and the effect of different solvents used [20, 25, 26]. The levels of secondary metabolites tested in this study were rosmarinic acid levels. An instrument with high analytical sensitivity is needed to determine the levels of rosmarinic acid in cat whiskers. The sample used is a natural product that is not isolated until it is pure first, so it has a diverse secondary metabolite content. Therefore, the instrument used is High-Performance Liquid Chromatography (HPLC). The advantages of the HPLC method are high analytical sensitivity, fast, good resolution and the column can be reused [27, 28].

The cat's whiskers plant has pharmacological activity. As well as the traditional use of cat's whiskers in the community through the boiling process, the boiling does not pay attention to the exact boiling time, while the stability and levels of active compounds in the plant can be affected, thus affecting the pharmacological activity produced. There are two main compounds in cat's whiskers, rosmarinic acid and sinensetin. But, the levels of rosmarinic acid are higher than sinensetin. So this study focuses on rosmarinic acid [2] Because of the rosmarinic acid's polarity and solubility, it is necessary to conduct this research to determine the effect of boiling time on rosmarinic acid levels in the purple variety of cat's whiskers in order to ensure the optimal time for boiling the purple variety of cat's whiskers so that the levels of rosmarinic acid can be guaranteed.

MATERIALS AND METHODS

Plant sample preparation

The purple variety of cat's whiskers plants was collected from the

Medicinal Plant Garden, Faculty of Pharmacy, Jenderal Achmad Yani University in Cimahi. Cimahi City is located at an altitude of 685 meters above sea level (m sea level), and the rainfall is around 2000-5000 mm/y. This location is the optimal location for the growth of cat whiskers because the optimal location for cat whiskers to grow is between 500-1,200 meters above sea level and rainfall of around 3,000 mm/year [29].

Raw material preparation

Samples of the cat's whiskers were collected from Medicinal Plant Garden Pharmacy UNJANI in December 2021. The sampled cat's whiskers were the top 3 parts of the leaf shoots collected as much as 1 kg. This part were chosen because the leaves have high levels of active compounds. Samples were determined in Padjadjaran University Plant Taxonomy Laboratory (UNPAD) Bandung, located on Jl. Raya Bandung Sumedang, Jatinangor, West Java. Samples were washed with running water, then sorted wet and weighed. Samples were put into the oven to dry at 40 °C. Dry sorting and chopping samples were carried out to reduce particle size using a blender to obtain simplicia powder, which was ready for further processing.

Extraction

200 g of purple cat whiskers simplicia was extracted using the boiling method with different boiling time variations for 10, 20, and 30 min. Simplisia was put into a pot with a capacity of 2 L, add 2000 ml of distilled water as a solvent. A pot containing simplicia and solvent is heated over a water bath. After that, it is heated until the water reaches a temperature of 90 °C, then the time is calculated for 10, 20, and 30 min after the temperature reached 90 °C. The temperature is maintained using thermometer. The filtrate is collected each time and filtered to get the residue. The extraction was repeated three times in 24 h, where the residue was extracted again by adding distilled water with a ratio of distilled water: simplicia (10:1).

Validation of analysis methods

Accuracy

Accuracy using the standard addition method is determined by the percentage of recovery (% recovery). Rosmarinic acid comparator with a concentration of 10 µg/ml was added to cat's whiskers extract with a 150 µg/ml concentration. Then, analyzed with HPLC, measurements were made three times a repetition. The area under the curve (AUC) was recorded from the combination of the cat's whiskers extract and rosmarinic acid (A), the area of the cat's whiskers extract (B), and the area of rosmarinic acid (C) [30, 31]. Requirements for acceptance of accuracy are recovery values in the 97-105% range. The formula calculates %recovery:

$$\% \text{ Recovery} = \frac{A - B}{C} \times 100\%$$

Precision

Precision is determined by the relative standard deviation percentage value (%RSD). The specified precision is repeatability precision [30]. A reference solution of rosmarinic acid was prepared with a concentration of 6 µg/ml to determine repeatability precision then injected into the HPLC system. The procedure was repeated six times at the same time. Requirements for precision acceptance are the value of %RSD ≤ 2%.

$$\%RSD = \frac{\text{Standard deviation}}{\text{Average test result}} \times 100\%$$

Linearity

Five variations of rosmarinic acid concentration were made with different concentrations, namely concentrations of 2, 4, 6, 8 and 10 µg/ml [32]. Then it is in78jected into the HPLC system. A calibration curve was made by plotting the area with the reference concentration of rosmarinic acid. Then analyzed is the calibration curve's regression equation and correlation coefficient (r) [30]. The acceptance requirement for linearity is the value of r≥ 0.9950.

Specificity

Specificity is determined by comparing the retention time between comparators and samples [33]. In this study, the comparator used was rosmarinic acid, and the sample used was the of water extract from a cat's whiskers.

Determination of rosmarinic acid levels using HPLC

Determination of rosmarinic acid levels in cat whiskers extract is determined based on the area. Determination of content refers to the method [30] with a modification of the 6 maximum wavelength of the UV detector, which is 340.6 nm. The HPLC system used is isocratic elution with the mobile phase being 0.1% formic acid: acetonitrile (60:40). The column used is the (Shimadzu Serial L201354 Japan) reversed-phase C18 column (Agilent Pursuit XRs 5 C18 150 x 4,6 nm) with a column temperature of 25 °C. Separation time was carried out for 30 min using a flow rate of 1 ml/min [32].

Preparation of standard solutions and sample solutions

The standard solution was prepared using 1 mg of rosmarinic acid (Sigma Aldrich) as a reference and then dissolved in 10 ml HPLC grade methanol (100 µg/ml mains solution). The mother liquor was diluted into five concentration variations, namely concentrations of 2, 4, 6, 8 and 10 µg/ml [32]. Then, the solution was filtered with a special 0.45 µm millipore filter. Then, the filtrate was put into the Eppendorf tube. The test sample solution was prepared using 150 mg dried extract (boiled water extract that has been evaporated) of the of cat's whiskers dissolved in 10 ml HPLC grade methanol (mother liquor I 15,000 µg/ml) and sonicated for 45 min [32]. The mother liquor I was diluted into mother liquor II 1,500 µg/ml, then diluted back into a sample solution of 150 µg/ml and filtered using a 0.45 µm millipore filter, then the filtrate was put into a 1 ml Eppendorf tube.

Determination of rosmarinic acid levels in cat whiskers

Content determination refers to the method [30] with a modified maximum wavelength of 340.6 nm [32]. 20 µl of the sample solution was injected into the HPLC system. Sample measurements were carried out in 3 repetitions (triplo). The area of the sample area that corresponds to the reference standard for rosmarinic acid is recorded. The level of rosmarinic acid in the cat's whiskers was calculated by interpreting the linear regression equation from the calibration curve of the reference solution.

RESULTS

The yield of the cat's whiskers water extract can be seen in table 1.

Table 1: Yield of purple variety cat's whiskers water extract

Water extract	Yield (% w/b)
10th min	10.00
20th min	12.45
30th min	15.60

The initial stage in carrying out levels using HPLC determines to validate the HPLC analysis method. This validation aims to provide certainty that the analysis results are valid, reliable and accountable and are by the objectives of the test. The validation process carried out includes accuracy, precision, linearity and specificity.

The method's accuracy is carried out to measure the closeness of the test results to the true value. Accuracy using the standard addition method is adding rosmarinic acid as a comparator to the aqueous extract solution of cat's whiskers. The standard addition method was chosen because the cat's whiskers extract sample is a natural product that has not undergone an isolation process until it is pure first, meaning that the sample still contains very diverse types of secondary metabolite content so that it is not known with certainty what compounds it contains. The accuracy value is determined by the percentage value of recovery (% recovery) which comes from the sample area and comparison. The percentage value of the recovery obtained is 100.79% (table 2, fig. 1). These results indicate that the analytical method used is appropriate because the results are from the literature, which states that the recovery requirements are 97-105% [34].

Table 2: Analysis method validation accuracy

Measurement	% Recovery (%)
1	100.76
2	99.70
3	101.91
mean±SD	100.79%±1.11

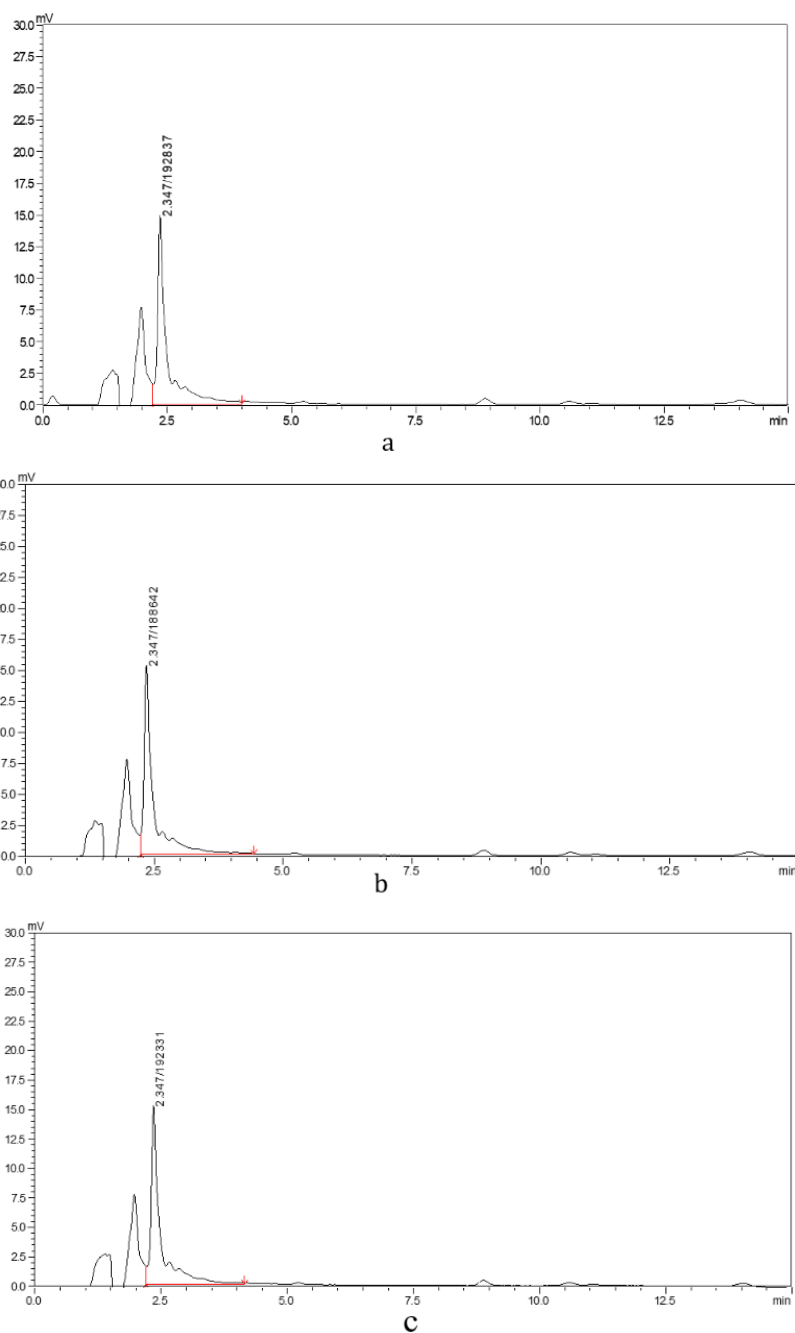Information: SD: Standard deviation, % Recovery: percentage of recovery

Fig. 1: Accuracy: chromatogram of extracts and standards (rosmarinic acid). a = replication 1; b = replication 2; c = replication 3

The method's precision is carried out to determine the suitability of the test if repeated. Precision measurements were made, namely repeatability. A reference solution of rosmarinic acid with a 6 µg/ml concentration was injected into the HPLC system. The procedure was repeated 6 times simultaneously [30]. The precision value is determined by the relative standard deviation (%RSD) percentage value, which comes from the area of the comparison area. The % RSD value obtained was 1.64 % (table 3, fig. 2). These results indicate that the method used in the analysis has a good and thorough repeatability value because the test results are from the literature stating that the requirements for good precision are %RSD ≤2% [30, 33].

Table 3: Precision validation of analysis methods

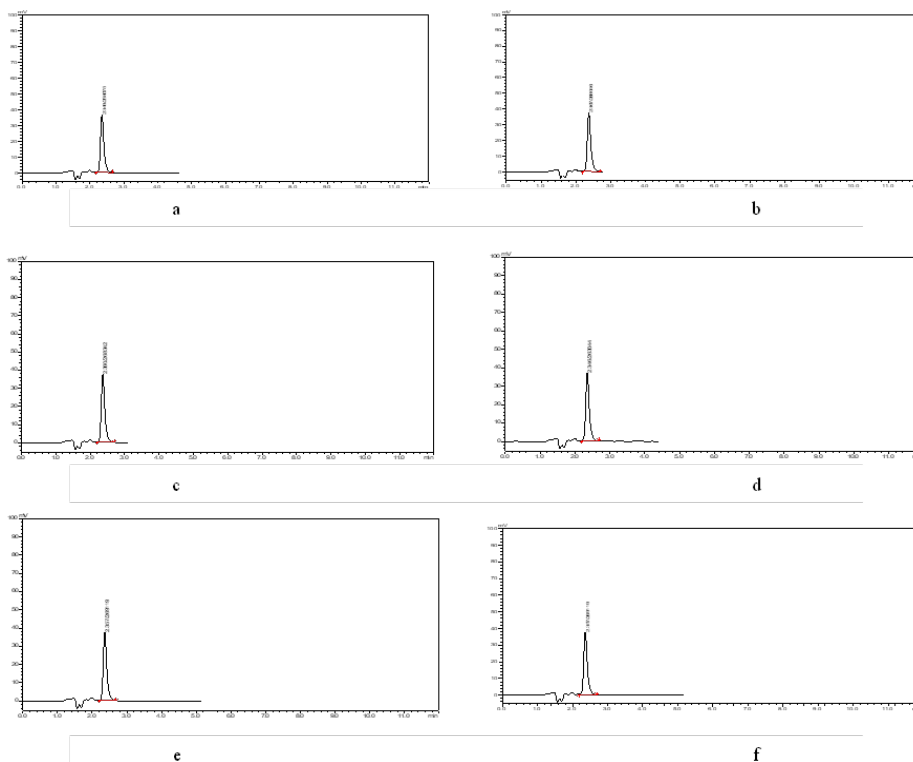| Concentration (µg/ml)	Area
6	258.075
6	268.046
6	268.432
6	263.044
6	269.119
6	262.825
Average±SD	264,923.5±4,347.41
%RSD	1.64%

Fig. 2: Precision: rosmarinic acid (RA) chromatogram. a = RA 6 ppm (replication 1); b = RA 6 ppm (replication 2); c = RA 6 ppm (replication 3); d = RA 6 ppm (replication 4); e = RA 6 ppm (replication 5); f = RA 6 ppm (replication 6)

The method's linearity was carried out to see a linear relationship between concentration and instrument response, in this case, the reference concentration of rosmarinic acid and the response of the HPLC instrument, namely the area value. Measurements were made by making 100 ppm rosmarinic acid as a reference base solution and then diluting it into five concentration variations, namely concentrations of 2, 4, 6, 8 and 10 ppm.

Variations in the rosmarinic acid reference concentration were analyzed using HPLC, and then each concentration was determined by the area. A calibration curve was created by plotting the area on the Y-axis and the concentration on the X-axis. The linear regression equation of the calibration curve for the comparison of rosmarinic acid is $y = 45.973x-3.784$ with a value of $R^2 = 0.9972$. The calibration curve for the comparison of rosmarinic acid can be seen in fig. 3.
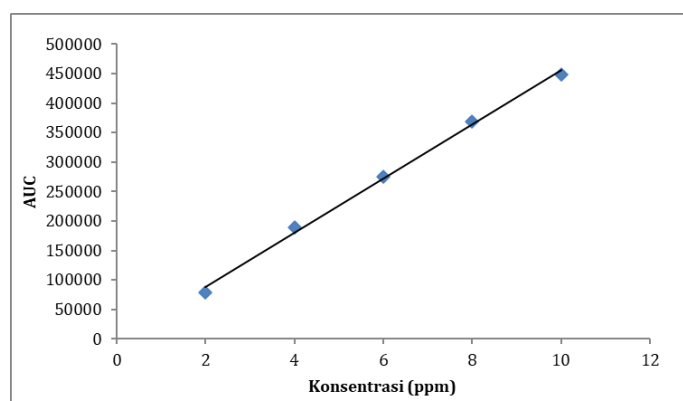

Fig. 3: Rosmarinic acid standard calibration curve, ▬■▬ Comparison curve of rosmarinic acid at a maximum wavelength of 340.6 nm, regression equation: $y = 45.973x–3.784$, r = 0.9986

The results of linearity measurements are the correlation coefficient (r) of 0.9986 (table 4, fig. 4). These results indicate that the reference concentration of rosmarinic acid and the area of the area are directly proportional, where the higher the concentration, the higher the area produced. This follows the literature, which states that the acceptance requirement for linearity is to have a value of r ≥ 0.9950[30, 33].

Table 4: Linearity validation method of analysis

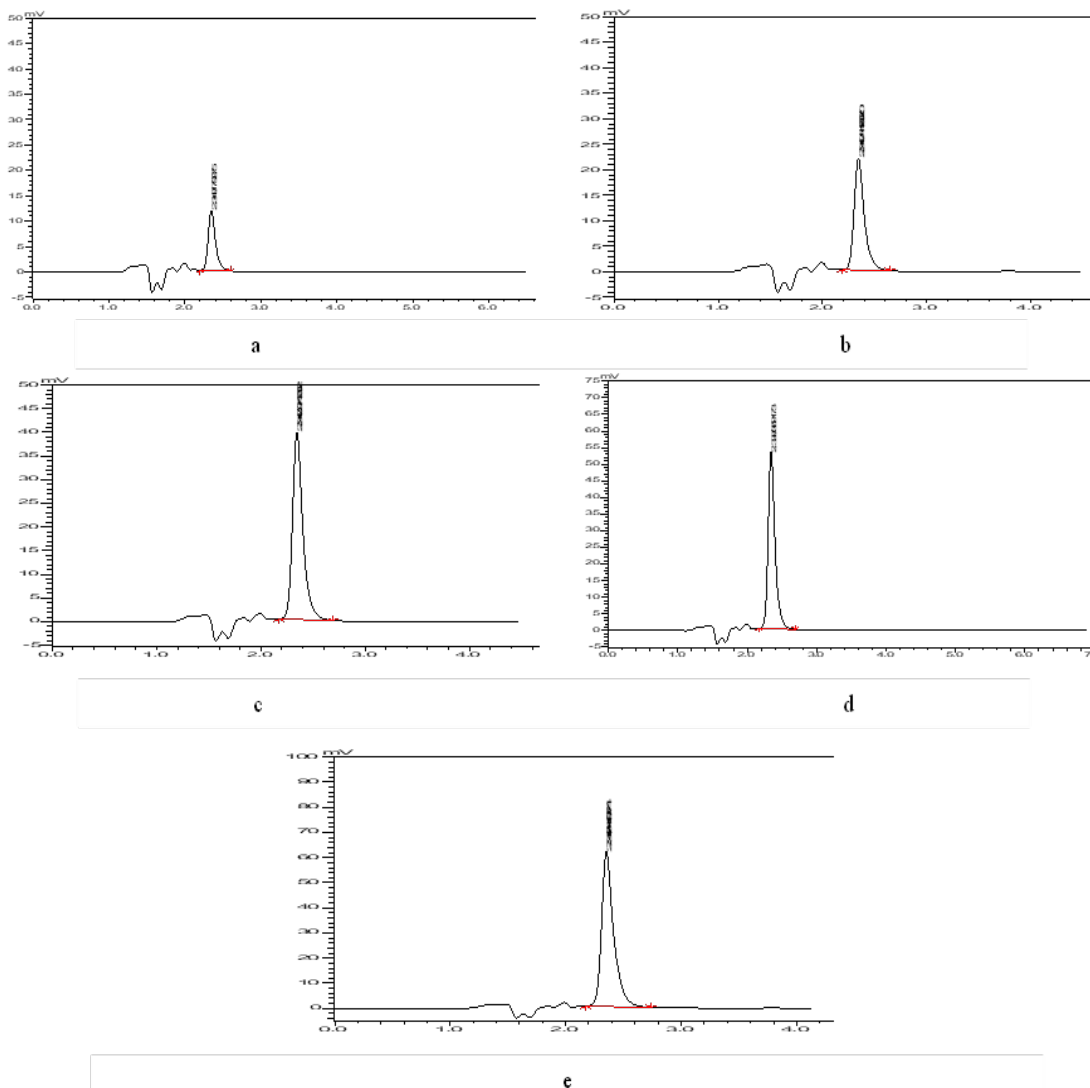| Linearity range	Regression equation	Coefficient of determination (R2)	Correlation coefficient (r)
2-10µg/ml	y = 45.973x-3.784	0.9972	0.9986

Fig. 4: Linearity: rosmarinic acid (RA) chromatogram. a = RA 2 ppm; b = RA 4 ppm; c = RA 6 ppm; d = RA 8 ppm; e = RA 10 ppm

The specificity of the method is carried out to ensure that the compound to be analyzed is measurable specifically despite the presence of other compounds in the sample. The specificity measurement was done by comparing the retention times between samples and controls under the same HPLC system conditions. The results of the specificity measurement were that the rosmarinic acid comparator had a retention time of 2.353 min and the cat's kumis water extract for 2.352 min (table 5, fig. 5). These results indicate that the analytical method is specific because it produces the same retention time between the two.

Table 5: Specificity validation method of analysis

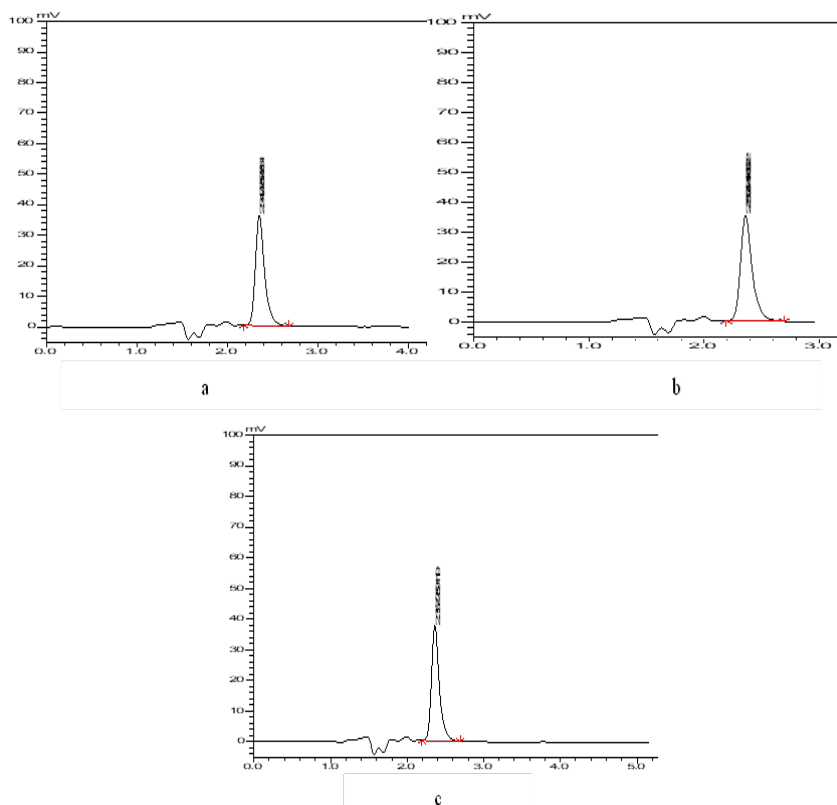| Measurement	Retention time (min)	
	Rosmarinic acid	Cat's whiskers water extract
1	2,349	2,351
2	2,353	2,350
3	2,357	2,354
Average±SD	2,353±0.004	2,352±0.002

Fig. 5: Specificity. rosmarinic acid (RA) chromatogram. a = RA 6 ppm (replication 1); b = RA 6 ppm (replication 2); c = RA 6 ppm (replication 3)

After validating the HPLC analysis method, the rosmarinic acid level was determined from the water extract of the of cat's whiskers. The water extract obtained from the decoction was the extract, which was boiled for 10, 20 and 30 min. Each boiling time was analyzed qualitatively and quantitatively using the HPLC instrument for three repetitions.

The qualitative analysis compared the retention time of water extract of cat's whiskers and rosmarinic acid. The results showed that the aqueous extract of the cat's whiskers had the same retention time as rosmarinic acid, which was 2.3 min (fig. 6). This shows that the water extract of the of cat whiskers contains rosmarinic acid compounds.
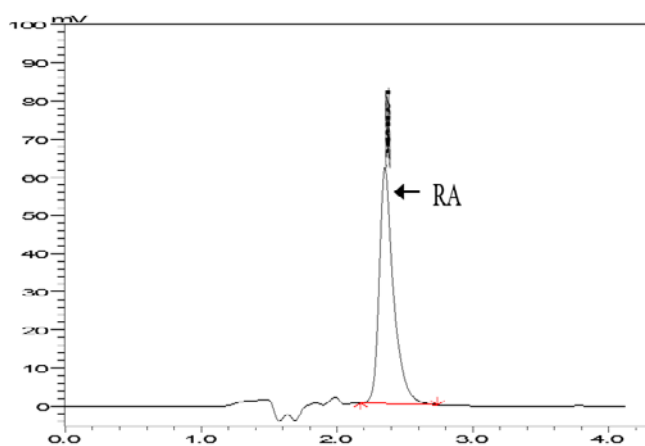

Fig. 6: Rosmarinic acid (RA) standard chromatogram

Meanwhile, quantitative analysis was carried out by determining the area of water extract of the cat's whiskers and then calculating the levels by interpreting the linear regression equation of the rosmarinic acid comparison calibration curve.

The results showed that the highest levels of rosmarinic acid were in water extract of cat's whiskers boiled for 20 min, 2.3209% w/w (table 6; fig. 7). The rosmarinic acid levels were higher when compared to previous studies. A study comparing rosmarinic acid levels in purple and white-purple varieties of cat whiskers reported rosmarinic acid levels in the ethanol extract of purple cat whiskers of 1.36% w/w [35]. Another study reported the highest levels of rosmarinic acid in the water extract: ethanol extract of kumis Whiskers leaves, namely 1.986% w/w or 19.861 mg/g [36], whereas in the water: methanol extract (50:50) the rosmarinic acid content was 0.283% w/w or 2.826 mg/g [10].

Table 6: Results of determination of rosmarinic acid levels

Measurement	Level of water extract of cat's whiskers min (% w/w)		
	10	20	30
1	2.0730	2.2883	2.1633
2	2.0792	2.3291	2.1686
3	2.0775	2.3453	2.1168
Average±SD	2.0766±0.003	2.3209±0.029	2.1496±0.028

Information: Sample: Water extract cat whiskers, SD: Standard deviation

The results of the calculation of rosmarinic acid levels were analyzed using SPSS software version 25 using one way ANOVA (Analysis of Variance) method. The requirements for using this method are that the sample data has a normal distribution and the variance data must be homogeneous. So that, before processing data with ANOVA, it is necessary to carry out normality and homogeneity tests. The normality test uses the Kolmogorov-Smirnov test method to see the distribution of the sample data. The result of the sample normality test is a significance value of 0.223 (table 7). These results are in accordance with the literature, which states that data values are considered to have a normal distribution if the significance value is greater than 0.05 [37].

Meanwhile, the Bartlett homogeneity test was carried out to see homogeneous data variants. The homogeneity test results, namely the significance value obtained, is the value of 0.070. These results follow the literature stating that the variance between data groups is considered the same or homogeneous if the significance value is greater than 0.05 [38].
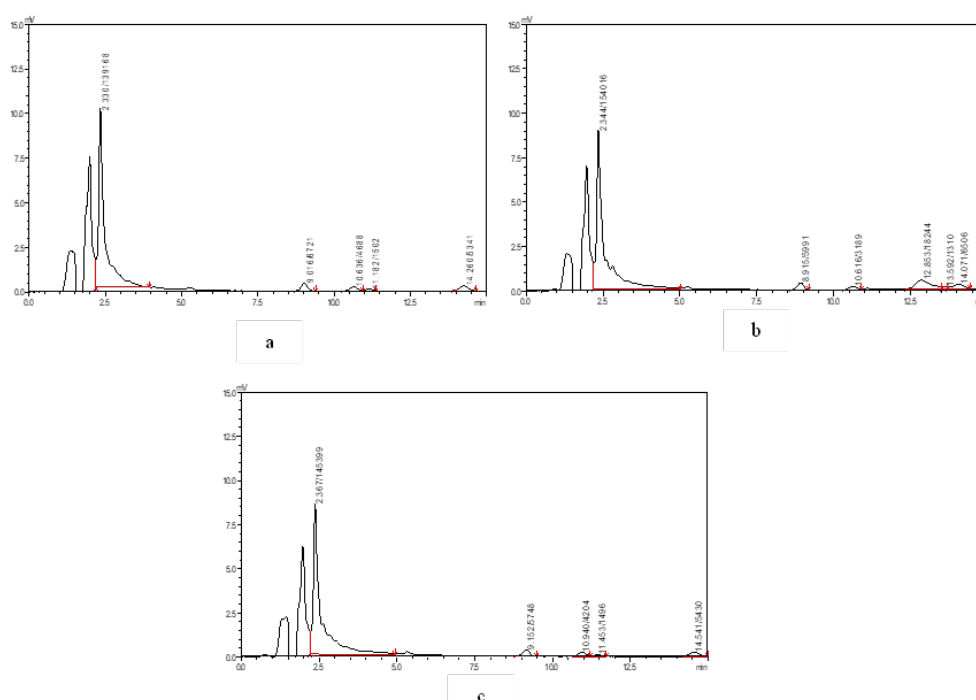

Fig. 7: Chromatogram of cat's whiskers water extract (150 ppm). a = 10th min, b = 20th min, c = 30th min

Table 7: Normality test results and homogeneity test

Method	Sig.
Normality test (Kolmogorov-Smirnov)	0.223
Homogeneity test (Bartlett)	0.070

Information: Sig.: significance value (p>0.05)

Based on the preliminary test results, it can be assumed that the sample data meets the requirements. Furthermore, data analysis was carried out using the one-way ANOVA method. The analysis aims to determine whether or not there are significant differences in cat's whiskers rosmarinic acid levels due to different treatments, namely boiling times for 10, 20 and 30 min. The p-value<0.05 was considered statistically significantly different in this analysis. The results of the significance value test, namely p = 0.000, showed that the rosmarinic acid levels had a significant difference because the p-value<0.05. These results illustrate the effect of boiling time on the levels of rosmarinic acid water extract of the of cat's whiskers [39]. The results showed at least a significant (significant) difference in one of the cat's whisker's rosmarinic acid levels with a boiling time of 10, 20 and 30 min. The next analysis stage is to carry out the Duncan test, which further explores which group of boiling times has a significant difference in value [39].

Based on the results of the analysis of Duncan's test showed that the rosmarinic acid levels resulting from minute boiling on the 10th was significantly different from the results of rosmarinic acid levels at boiling time of 20 and 30 min (table 8). The results of the analysis illustrate that there is an effect of boiling time (10, 20 and 30 min) on the levels of rosmarinic acid extract of of kumis cat water, and each boiling time showed statistically significant differences in rosmarinic acid levels.

Table 8: Duncan's test results

Component	Boiling time to the min (% w/w)		
	10	20	30
The average levels of rosmarinic acid (%)±SD	2.0766±0.003[a]	2.3209±0.029[b]	2.1496±0.028[c]

Information: [abc]Different letter notations indicate significant differences in rosmarinic acid levels at each boiling time of the purple variety cat's whiskers (p<0.05), SD: Standard deviation

DISCUSSION

Extraction by boiling method at 90 °C using distilled water as solvent. The reason for choosing this boiling method is that the compound to be extracted is a rosmarinic acid compound resistant to heat at that temperature. Previous studies compared the effect of 50, 70 and 90 °C temperatures on rosmarinic acid levels. The results stated that the highest levels of rosmarinic acid were at high temperatures of up to 90 °C [40].

The yield of water extract from the cat's whiskers increases with the longer extraction time as we can see in table 1. These results are in accordance with the literature, which states that the longer the extraction time, the higher the yield value produced. This is related to the contact time between the solvent and the compounds contained in the plant. The longer it is [41].

Determination of rosmarinic acid levels in cat whiskers is conducted on a purified sample that has been isolated. Because the sample has a very diverse type of secondary metabolite content, an instrument that has high analytical sensitivity is needed so that the instrument used is Liquid Chromatography High Performance (HPLC). The HPLC method has the advantages of high analytical sensitivity, fast, and good resolution and the column can be reused. At the same time, the disadvantages of HPLC are high costs and the detector used is less sensitive than gas chromatography detectors [42].

This research refers to previous research using an optimized HPLC system. Optimal conditions are needed for the best results [32]. The condition of the HPLC system used is isocratic elution with a modified UV detector with a maximum wavelength of 340.6 nm. The reversed phase C18 column is used with a column temperature of 25 °C. Separation time was carried out for 30 min using a flow rate of 1 ml/min [32].

The mobile phase, which was able to separate and analyze the rosmarinic acid compound in the of cat's whiskers extract, was formic acid 0.1%:acetonitrile (60:40) [32], the mobile phase is polar, and the selection of the mobile phase is based on the solubility of the rosmarinic acid compound which is polar so that rosmarinic acid can be eluted quickly with the mobile phase and results in a short retention time. Meanwhile, other compounds which are semi to non-polar will be retained longer in the C18 column, which is non-polar, so that they elude more slowly or leave the column longer than polar compounds. This is in accordance with the principle of HPLC, partitioning, where the separation is based on the solubility of the compound in the mobile and stationary phase [42].

Rosmarinic acid belongs to a class of phenolic compounds derived from caffeic acid [10]. As state in table 6, rosmarinic acid levels in cat's whiskers, boiled for 10 min, had the lowest level, 2.0766% w/w. The highest rosmarinic acid content was in the water extract of the of cat's Whiskers, which was boiled for 20 min and showed a yield of 2.3209% w/w, in line with the literature, which states that extraction time can affect the extract yield. The amount of extract will increase if the time boils longer [17]. Therefore, the levels of rosmarinic acid in the of cat's whiskers, which were boiled for 20 min, were greater than those of rosmarinic acid, which were boiled for a shorter time.

Meanwhile, the levels of rosmarinic acid in cat's whiskers which were boiled for 30 min, decreased to 2.1496% w/w. These results indicate that the boiling time is too long, and the rosmarinic acid levels are actually decreasing. This is in line with one of the Arrhenius theories, which states that if the temperature used is higher, then the rate of chemical reactions will increase [43]. This is related to the longer the cooking time for the cat's whiskers, the longer the plant will experience contact with high hot temperatures at 90 °C, that matter will loosen the intermolecular bonds of the compounds, causing collisions between the compound molecules to increase, resulting in a reaction rate in this case the rate of degradation will also increase so that the stability and levels of the rosmarinic acid compound can be reduced.

Besides that, The levels of the extracted rosmarinic acid phenolic compounds decreased also relates to other literature, which states that if the extraction time is too long, oxidation of phenolic compounds will occur due to too high oxygen exposure [16]. This oxidation process is one of the mechanisms for the decomposition of a compound (degradation) caused by the presence of oxygen. Therefore, the levels of rosmarinic acid in the of cat's whiskers which were boiled for 30 min, decreased.

Based on the analysis of rosmarinic acid levels of the, it can be determined that the most optimal boiling time for boiling the of cat's whiskers leaves is 20 min because it has the highest levels of rosmarinic acid when compared with the boiling time for 10 min and 30 min.

CONCLUSION

Boiling time greatly influenced the levels of rosmarinic acid in the purple variety of cat's whiskers. The results of rosmarinic acid levels boiled for 10, 20 and 30 min were 2.0766% w/w, 2.3209% w/w, and 2.1496% w/w. The optimal boiling time for extracting rosmarinic acid from the purple variety of cat whiskers is 20 min.

AUTHORS CONTRIBUTIONS

Fahrauk Faramayuda, Soraya Riyanti, Suryani, Shindi Juni Karmila, Ari Sri Windyaswari, Rizka Khoirunnisa Guntina experimented and wrote the manuscript.

CONFLICT OF INTERESTS

The authors have no conflicts of interest to declare. We certify that the submission is original work and is not under review at any other publication.

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