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TERAKREDITASI

Microscopic Characterization of Keji Beling Extract (*Strobilanthes crispus* L.) As Herbal Medicine Studies

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ABSTRACT

Keji Beling (Strobilanthes crispus L.) is one of medicinal plants in Acanthaceae family. The leaves are the part that is often used as the herbal products such as; herbs, tea and the others. This study aims to determine the characteristics of the functional groups of organic compounds, mineral content and antioxidant activity in the leaf extract of Strobilanthes crispus L. using pottery. Test analysis in research using FTIR, SEM-EDX, XRF. The result of showed the presence of CH alkenes, CH aromatic, C-O alcohol/ether/carboxylic acid/ester, C-N amine, NO_2 Nitro compounds, O-H hydrogen bond alcohol /phenol and N-H amines/amides The mineral composition contained macro elements of Ca, K, P and S. While the identified micro minerals are Si, Fe, Mo, Sr, Mn, Ba, Cu, Zn, and Ni. The microscopic morphology of Strobilanthes crispus L. leaf extract is in the form of porous solid particels, thus it is to have high water solubility. The results of the antioxidant test using the DPPH method obtained an IC50 value of 19.20 ppm which is included in the group of very active antioxidants.

Introduction

One of the biodiversity possessed by Indonesia has the potential as a medicinal plant, namely Keji Beling (*Strobilanthes crispus* L.). *Strobilanthes crispus* L. is a plant that is included in Acanthaceae family. This plant is locally known as broken glass or vile shard. Several Keji Beling plants (*Strobilanthes crispus* L.) have been widely used as herbal medicine (Hamzah & Norfarizan-Hanoon, 2013). Almost all parts of the *Strobilanthes crispus* L .plant have traditionally been used for medicinal therapy. The traditional use of Strobilanthes crispus L. plant's parts such as leaves and stems can be done by boiling or brewing it which is consumed regularly as a herbal product either in the form of herbs or tea.

The content of Keji Beling compound (*Strobilanthes crispus* L.) will be responsible for the biological response, namely providing information on composition (type and content), with the aim to ensure the ingredients of a traditional medicine product. In these plants, the content of secondary metabolite compounds synthesizes metal oxide composites. Keji Beling (*Strobilanthes crispus* L.) has rich benefits and content of phytochemical compounds including potassium, sodium, calcium, silicic acid, salicylic acid, calcium carbonate crystals, alkaloides, saponins, flavonoids, polylenoid and tannins (Isnawati et al., 2004). These compounds can later act as a source of weak alkali and stabilizers for the formation of metal

oxide composites (Ashna et al., 2020). An active compound that can be developed in herbal medicine is the content of flavonoids.

Along with the development of times, nanotechnology is increasing rapidly so, it becomes an opportunity in the development of medicinal plants. Nanotechnology is an application in science and technology. In recent years, precious metal nanoparticles have been studied to see the unique, optical, electronic, mechanical, magnetic and chemical properties of different plant samples. The result and characteristics of the nanoparticles will respond differently to each plant (Saranya et al., 2016). Each plant gives a different result and microscopic characterization. Therefore, many references are needed related to the synthesis of particles with various types of plants (Wendri et al., 2017). It is important to know the mineral content in plant extracts because they have a role in activities in the body that will help in the prevention and treatment of diseases due to mineral deficiency. Plants use minerals as structural components in carbohydrates and proteins; organic molecules in metabolism (Soetan et al., 2010).

Information reporting the active compounds from the herbal ingredients Strobilanthes crispus L. using the boiling method using pottery is important to provide an overview of organic functional groups and mineral content as a scientific basis for herbal medicine. Based on the explanation above, it is necessary to characterize the active compounds contained in Keji Beling plant (*Strobilanthes crispus* L.) which are used as herbal tea beverage. The purpose of this study was to report the characteristics of the functional groups of organic compounds and mineral content in the leaf extract of *Strobilanthes crispus* L. using pottery which is used as an herbal tea drink that has antioxidants.

Materials and Methods

Preparation of leaf *Strobilanthes crispus L.* extract

The research material was *Strobilanthes crispus* L. leaves accompanied by 250 grams of stems, washed first until clean, then dried to air. After that the dried Keji Beling leaves are mashed by using a grinder with a speed of 25,000 rpm for 10 minutes until they become powder. Then the powder is sieved with a size of 200 mesh. The result of the sieve is simplicia. The stages of making *Strobilanthes crispus* L. leaf extract, namely 61 grams of simplicia powder, added 500 mL of distilled water in pottery. Furthermore, at a temperature of 800 C for 30 minutes. The submerging result of *Strobilanthes crispus* L. extract is filtered using filter paper. The next process is freeze drying for 2-3 days. The results of freeze drying are particles in form of solid powder and can be used for the characterization stage of *Strobilanthes crispus* L.extract particles.

Characterization of leaf *Strobilanthes crispus* L. Extract

1. FTIR (Fourier Transform InfraRed) Analysis

FTIR testing was carried out at the State University of Malang. Dry powder samples with water solvent were analyzed using a FT-IR (Fourier Transform Infra Red) spectrophotometer to determine the presence of Si-O and Al-O bonds, as well as the presence of Cu-O bonds on the catalyst. The sample was a solid 10 mg of *Strobilanthes crispus* L. tea then prepared with a mixture that from 1 mg sample and 100 mg KBr until homogeneous. After that the sample is forming in the form of a pellet and measurements are taken using FTIR spectroscopy merk Shimadzu, Type IR Prestige 21. Characterization is carried out in the wave number range 4,000 - 400 cm^{-1} (D et al., 2017). Furthermore, it appears in the spectra for analyzing of functional groups contained in these compounds.

2. SEM (Scanning Electron Microscope) Analysis

SEM test analysis was carried out by characterizing the sample to determine the crystallinity of the sample. The sample was placed in a sample holder coated with gold with a thickness of 10 nm then, the sample was homogenized with potassium bromide (Setyawan et al., 2018). IR spectra were recorded using SEM-EDX merk FEI, type inspect-S50 and samples observation were observed with a voltage of 20 kV. Samples were observed using SEM at magnifications of 500, 1,000, 5,000, and 10,000 times.

3. XRF (X-Ray Fluoresence) Analysis

The test sample of *Strobilanthes crispus* L. leaf was then measured using an XRF spectrometer test. Print out analysis data is the composition and mineral content data.

4. Antioxidant activity

The results of testing the antioxidant activity of leaf extract of Strobilanthes crispus L. which were developed in pottery with 6 concentration levels, namely 2, 5, 10, 20, 30 and 40 ppm with 3 times the square. First, the absorbance of the DPPH blank (2,2-diphenyl-1-picrylhydrazyl) was measured to calculate the % inhibition value and to obtain the absorbance value of the DPPH blank at a wavelength of 517 nm.

Results and Discussion

FTIR test analysis to see the specific peaks that exist at certain wavelengths, then the types of functional groups will appear in the *Strobilanthes crispus* L. Extract compound. Based on the FTIR spectrum data, the *Strobilanthes crispus* L. extract can for the spectrum pattern it can be seen in Table 1. Based on the microscopic results (Table 1) there is an O-H group of hydrogen alcohol/phenol so it is suspected that there are groups of flavonoid, polyphenol and phenol compounds and so have antioxidant activity.

At the peak of the 675-995 cm^{-1} wavelength shows that there is a CH alkene functional group, the peak wavelength of 690-900 cm^{-1} appears the C-H group of aromatic rings, while the functional group of C-O alcohol / ether / carboxylic acid / ester appears at the peak wavelength of 1,050-1,300 cm^{-1}, the peak of the wave 1,180-1,360 cm^{-1} contains C-N Amine / amine. NO_2 functional groups of nitro compounds at the peak of the wave 1,300-1,370 cm^{-1}, 2,850-2,970 cm^{-1} there are C-H Alkanes, O-H alcohol bonding Hydrogen / phenol at the peak of the wave 3,200-3,600 cm^{-1}, and for N-H amines / amides seen at the peak of the wave 3,300-3,500 cm^{1}. For the interpret the number data in Figure 1. On the measurement results in the spectra, it can be seen that the wave number area shows the vibrations which are very weak, weak, and sharp. All the functional group peaks of organic compounds had 24 peaks of functional groups of organic compounds extracts of *Strobilanthes crispus* L. which followed the FTIR spectrum pattern (Figure 1).

The characterization of mineral types using the SEM-EDX method aims to provide information related to the types of minerals contained in the Strobilanthes crispus L extract sample. To determine the content of both elemental and oxide content. Mineral is an inorganic material that has a certain chemical composition, provides specific physical properties and composition. ordered atoms (Julinawati et al., 2015). In this SEM-EDX test, you can see the appearance of the microstructure of the sample, grain size and composition and what phenomena occur in the oxidation process (Sujatno et al., 2017).

Table 1. Infrared Absorption Areas on Several Functional Groups of leaf *Strobilanthes crispus* L. Extract

No	Infrared Absorption Areas on Several Functional Groups of Keji Beling Extract	
	Wave Number (cm^{-1}) (Range)	Spectrum Interpretation
1	675-995	C-H Alkena
2	690-900	C-H Cincin Aromatik
3	1,050-1,300	C-O Alkohol/eter/Asam karboksilat/ester
4	1,180-1,360	C-N Amina/Amida
5	1,300-1,370	NO_2 Senyawa-senyawa nitro
6	2,850-2,970	C-H Alkana
7	3,200-3,600	O-H Alkohol ikatan hidrogen/fenol
8	3,300-3,500	N-H Amina/Amida

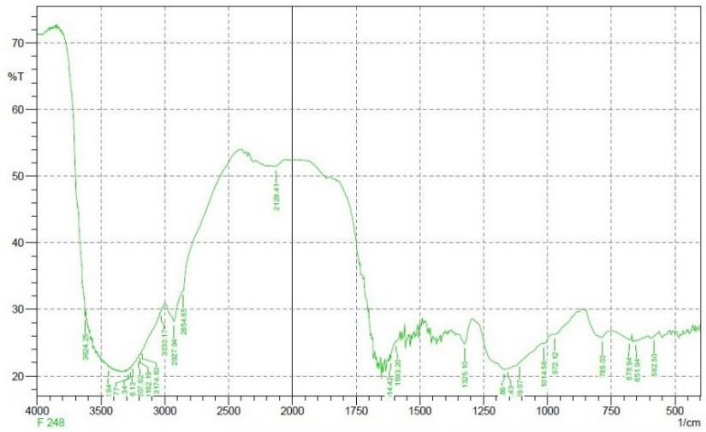

Figure 1. Spectra of FTIR Test Results of *Strobilanthes crispus* L. Extract

The results of characterization testing used SEM (Scanning Electron Microscopy) to see the texture, mophology, composition and crystallographic information of the particle surface of Keji Beling extract in form of dry solids. In the shape and morphology, the results varied depending on the sample of the nanoparticles from the plant extracts. The observations from SEM show at magnification of 500x, 1,000x, 5,000x, and 10,000 x are spherical in shape (Figure 2 and 3). Based on the results of Figure 2. (a) and (b) are the SEM morphology at 500X and 1,000X magnification and Figure 3. (1) and (2) are the SEM results with the 5,000X and 10,000X magnifications. From the figure, it shows the distribution of the samples produced with varying shapes, some are small and some are large on the submicron scale.

The characteristic morphology of the leaf extract of *Strobilanthes crispus* L. showed that the surface looked like an aggregate and was irregular, indicating that the shape of the particles was not similar and the surface was uneven. In small materials the shape are slightly oval and in large materials the shape is like a cracked irregular chunk that has a firm edge (Figures c and d). In addition, it appears that the distribution of elements in this study that is the largest peak in the elements C, O, Mg, Si, Cl, K, Ca, Mn, Fe, Cu, and Zn (Figure 4). The highest average percentages of elemental content for MgO, Al_2O_3, SiO_2, and CaO were 4.03% wt, 21.42% wt, 42.6% wt and 31.3%wt. The results of the analysis of *Strobilanthes crispus* L. leaf extract by boiling using earthenware showed the formation of crystals, this can be shown in the highest peak values, the higher the peak, the more crystals produced and the sharper crystal shape at the peak.

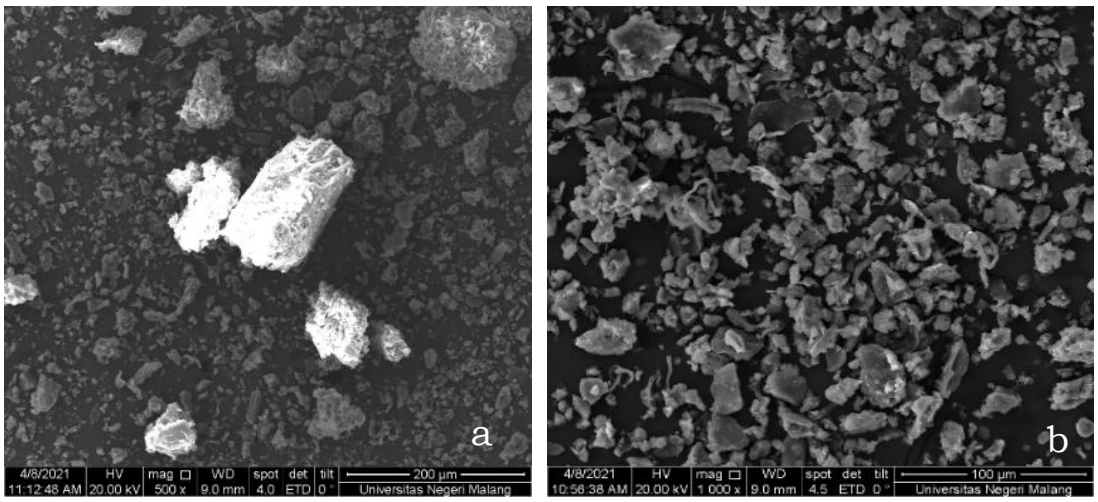

Figure 2. Photo of SEM Morphological Characterization of *Strobilanthes crsipus* L. Extract Material Code 500x (A) and 1,000x (B)

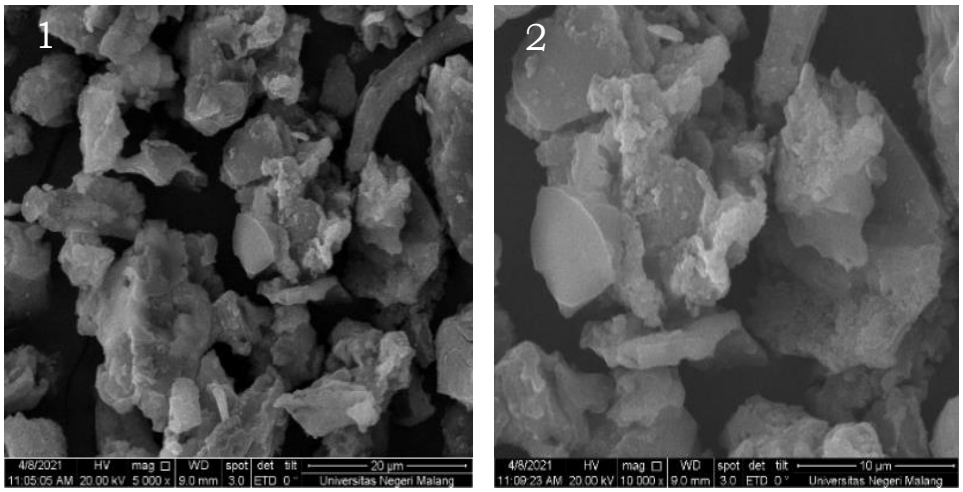

Figure 3. Photo of SEM Morphological Characterization of *Strobilanthes crsipus* L. Extract Material Code 5,000x (1) and 10,000x (2)

The results of XRF analysis showed the percentage (%) of types and mineral content which contained in *Strobilanthes crispus* L. extract sample. Based on the results in Table 2 shows that the mineral composition in the leaf extract of *Strobilanthes crispus* L. found 15 types of minerals consisting of macro and micro minerals, including Si, P, S, K, Ca, Ti, Mn, Fe, Ni, Cu, Zn, Sr, Mo, Ba, Re with successive levels of 21.5%; 0.60%; 2.1%; 17.5%; 45.2%; 0.1%; 0.51%; 2.25%; 0.064%; 0.14%; 0.08%; 0.77%; 8.6%; 0.3%; 0.2%.The result of cations that appear has function as substitution atoms or charge balancing cations. The most abundant amount of macro elements is Calcium (Ca), while from the micro elements there is Nikel (Ni).

Macro mineral needs are needed by the body in large quantities, while micro minerals are needed in small amounts. The identified macro minerals include calcium (Ca), potassium (K), phosphorus (P), and sulfur (S). As for the identified micro minerals, namely Silicon (Si), Iron (Fe), Molybdenum (Mo), Strontium (Sr), Manganese (Mn), Barium (Ba), Copper (Cu), Zinc (Zn), and Nickel (Ni).

According to research Suproborini et al. (2020) and Dali et al. (2017) the brewing results of *Strobilanthes crispus* L. leaves have anti diabetic and anti cancer benefits. According to the research results of Djamil et al. (2020) stated that the thick extract of keji beling leaves has the ability to inhibit the activity of the α-glucosidase enzyme, thus it has the potential to be developed for anti-diabetic herbal medicine as well.

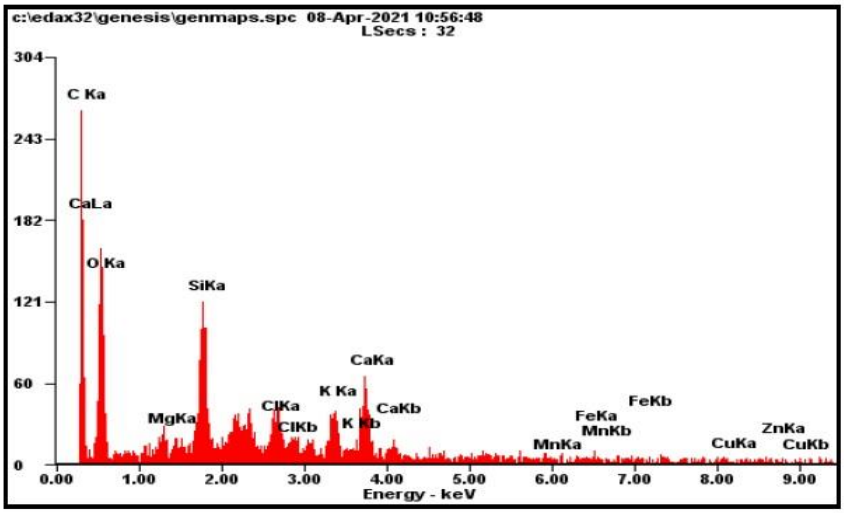

Figure 4. Microanalysis Report Using SEM-EDX of leaf *Strobilanthes crispus* L. Extract

Table 2. Element Types and Mineral Contents of leaf *Strobilanthes crispus* L. Extract

No	Element types and mineral contents of *Strobilanthes criscup extract*	
	Element type	Percentage of Elements (%)
1	Ca	45.2
2	Si	21.5
3	K	17.5
4	Fe	2.25
5	S	2.1
6	Mo	8.6
7	Sr	0.77
8	P	0.60
9	Mn	0.51
10	Ba	0.3
11	Re	0.2
12	Cu	0.14
13	Ti	0.1
14	Zn	0.08
15	Ni	0.066

Strobilanthes crispus L.contains vitamins C, B1, B2 and catechins so it produces high antioxidants (Adibi et al., 2017). These antioxidants can be a solution in the production of nanoparticles that are environmentally friendly (*green synthesis*) (Muliadi et al., 2015). Consumption of medicinal plants as therapeutic therapy is one form of prevention and medicinal treatment of disease. Some of these medicinal plants can be used as nutritional supplements and herbal teas. A medicinal plant can accumulate metals such as Mg, Fe, Zn, Co, Mo, and Ni for its growth (Nkuba & Mohammed, 2017). The elemental composition in *Strobilanthes crispus* L.leaf sample shows that there are 10 elements including Carbon (C), Oxygen (O), Magnesium (Mg), Chlorine (Cl), Silicon (Si), Calcium (Ca), Potassium (K), Sulfur (S), Aluminum (Al) and Iron (Fe). Usually the elements of carbon, oxygen, magnesium, chlorine, and calcium are seen in the leaves and stems of *Strobilanthes crispus* L (Fernandes & Sellappan, 2019).

The role of calcium minerals in the body is important in the growth of bones and teeth, preventing osteoporosis, helping the blood clotting process, stimulating insulin release (Siddiqui et al., 2014). In addition, it can maintain homeostatic balance, muscle contraction, stimulate the work of the nerves and brain (Amran, 2018). Potassium can play a role in the body to control muscle activity, maintain fluid and electrolyte water balance in the body, control insulin secretion from the pancreas, as a cofactor of protein synthesis activity, enzyme activity (Soetan et al., 2010). Phosphorus has an important role in the physiological process of the body. Together with calcium it can form bone and tooth skeletal tissue, as a component for detoxification, can overcome digestive disorders such as diarrhea, can increase the effectiveness of vitamins, namely vitamins riboflavin (B2) and niacin (B3), energy metabolism, maintain acid-alkali balance (Sulistyoningsih et al., 2017).

Micro minerals like as silicone minerals are derivation of silicic acid which are also needed for the body health. Silicone minerals can help to build collagen and bone mineralization, bind tissue and bone, act as anti-cancer and anti-diabetes, and also can prevent heart disease. The average silicon intake is around 20-30 mg / person / day (Boguszewska-Czubara & Pasternak, 2011). Molybdenum micro minerals can function for nitrogen fixation, cofactors in nitrate reduction and various enzymes (Soetan et al., 2010). The mineral zinc can play a role in glucose metabolism and reduce the risk of cancer (Siddiqui et al., 2014). Iron minerals in the body can help prevent anemia (Ridwan, 2012).

The use of *Strobilanthes crispus* L. herbal is able to increase potassium ions, thus it has the same potential as synthetic diuretics. The plant has important role in maintaining health which is influenced by solubility, thus it makes easier the body to absorb the needed minerals and nutrients. Secondary metabolites contained in *Strobilanthes crispus* L. such as alkaloids, flavonoids, and saponins can provide a synergistic effect with deuretics.

In the results of the antioxidant activity of the test compounds that have been carried out in this study, the IC50 value of 19.20 ppm was included in the class of very active antioxidants, so that it was able to ward off free radicals by 50% (Table 3). The smaller the IC_{50} value, the higher the antioxidant ability to ward off free radicals. Based on the antioxidant

results, it is shown that the extract of strobilanthes crispus L is used as a better natural source, and it is highly expected to be developed into a food supplement that is beneficial to health Antioxidant activity will increase accompanied by an increase in the hydroxyl group and will decrease in the presence of a glycoside group. This is because the role of the hydroxyl group found in the extract of *Strobilanthes crispus* L. can donate hydrogen atoms to free radicals, thus showing high antioxidant activity (Khalaf et al., 2008).

Potential development of secondary metabolites can be used as a prevention and treatment. Flavonoids are polyphenolic compounds consisting of two C6 groups (substituted benzene rings) connected by a three-carbon aliphatic chain. A flavonoid compound derived from catechins is excessively found in green tea, black tea and red wine. Phenolic compounds can have antibacterial properties and polyphenol compounds contained in *Strobilanthes crispus* L. can later be used as reducing agents. Thus, it is hoped that the Keji Beling (*Strobilanthes crispus* L.) plant can be used to synthesize nanoparticles, namely silver nanoparticles (Arifin & Ibrahim, 2018).

Table 3. Antioxidant Activity

Consenters' ppm	Absorbance	% Inhibition	IC_{50} ppm
Blanko	0.711	0	
2	0.5871	17.43	
5	0.4106	42.25	
10	0.3705	47.89	19.20
20	0.3158	55.58	
30	0.295	58.57	
40	0.216	69.64	

Alkaloid compounds are a large group and for flavonoids are the largest phenol groups in nature. The alkaloid groups include caffeine, theobrumin, and theophylline which are useful for diuretics. Flavonoid compounds can be useful for anti-inflammatory and diuretic. Flavonoid compounds have C2=C3 double bond configured with a C4 carbonyl group for a particular group, and the hydroxylation pattern in the catechol section is located in ring B, the methoxyl group. In addition, there are fewer saccharide bonds so that they can form high antioxidant properties (Arifin & Ibrahim, 2018). In addition, other secondary metabolites such as saponin compounds can stimulate kidney to work faster and increase the absorption of diuretics (Andriyanto et al., 2013). Chemical compounds were isolated and identified from the leaves of Keji Beling (*Strobilanthes crispus* L.*)* namely mono-terpenes. D-limonen. Limonen functions to improve blood circulation, relieve sore throat and cough, and can even inhibit the growth of cancer cells (Sulastri et al., 2021).

Conclusion

The results of the study concluded that the use of boiled *Strobilanthes crispus* L. leaf extract in pottery showed the presence of CH alkene, aromatic CH, CO Alcohol/ether/Carboxylic acid/ester, CN Amine/Amide, NO2 Nitro compounds, CH alkene, OH Hydrogen bonded alcohols/phenols, and NH Amines/Amides. While the types of minerals found 15 types of macro and micro minerals, namely Si, P, S, K, Ca, Ti, Mn, Fe, Ni, Cu, Zn, Sr, Mo, Ba, Re. In addition, the results of antioxidant activity showed very high.

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