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SCREENING OF ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES FOR SAFFLOWER WATER EXTRACTS TO INCREASE IMMUNITY DURING A PANDEMIC

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Abstract: Viral infection is the multiplication of viruses in the body. Viruses can reproduce with the help of a host. Viruses infect a host by inserting their genetic material into cells to duplicate their particles. Coronavirus is a type of virus. The coronavirus identified in 2019, SARS-CoV-2, has caused a pandemic of the respiratory disease called COVID-19. Screening of the antibacterial and antioxidant activity of safflower aqueous extract has to be carried out to seal immunity against the virus. The purpose of this study was to determine the antibacterial and antioxidant activity of Kasumba turate water extract. The method used in this study was an experiment consisting of the preparation of *Staphylococcus aureus* suspensions, the preparation of safflower aqueous extract, phytochemical screening of safflower aqueous extract, a study of the functional group of safflower aqueous extract using FTIR, and the determination of antibacterial and antioxidant activities. The results obtained show that the extract has physical characteristics of a brownish yellow color and the characteristic odor of Kasumba turate. A phytochemical study verified that the aqueous extract of safflower contains alkaloids, flavonoids, and tannins. Analyze FTIR for the expected presence of functional groups including -OH, =CO, and -NH. The best antibacterial activity under three conditions was shown at a 5% extract concentration with a solvent heating temperature of 90 ºC for 15 minutes which gave abroad an inhibition zone. Safflower aqueous extract indicated DPPH radical scavenging activity with an IC_{50} value of 965.33 mg mL^{-1}. Furthermore, safflower aqueous extract has the potential for antioxidant activity, but it has low antibacterial activity. However, this study supports making Safflower a natural colorant in food and recommends using Safflower as a tea or herbal drink that provides natural antioxidant effects during the pandemic.
Keywords: antibacterial; antioxidant; safflower

Abstrak: Skrining aktivitas antibakteri dan antioksidan ekstrak air kasumba turate dilakukan. Metode yang digunakan dalam penelitian ini adalah metode eksperimen yang terdiri dari preparasi suspensi *Staphylococcus aureus* as test bacteria, preparasi ekstrak air kasumba turate, skrining fitokimia ekstrak air kasumba turate, studi gugus fungsi ekstrak air kasumba turate menggunakan FTIR, penentuan aktivitas antibakteri, dan penentuan aktivitas antioksidan. Hasil yang diperoleh menunjukkan bahwa ekstrak memiliki ciri fisik warna kuning kecoklatan dan bau yang khas. Studi fitrokimia membuktikan bahwa ekstrak air kasumba turate mengandung alkaloid, flavonoid, dan

tanin. Analisis FTIR diharapkan adanya gugus fungsi yang meliputi -OH, =CO, dan -NH. Aktivitas antibakteri terbaik pada ketiga kondisi ditunjukkan pada konsentrasi ekstrak 5% dengan suhu pemanasan pelarut 90 ºC selama 15 menit yang memberikan zona hambat luas. Ekstrak air safflower menunjukkan aktivitas penangkapan radikal DPPH dengan nilai IC_{50} 1250 mg mL^{-1}. Selain itu, penelitian ini mendukung untuk menjadikan kasumba turate sebagai pewarna alami pada makanan dan merekomendasikan penggunaan kasumba turate sebagai teh atau minuman herbal yang memberikan efek antioksidan alami selama masa pandemi.
Kata kunci: antibakteri; antioksidan; kasumba turate

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Introduction

Viral infection is the multiplication of viruses in the body. Viruses can reproduce with the help of a host. Viruses infect a host by inserting their genetic material into cells to duplicate their particles. Coronavirus is a type of virus. The coronavirus identified in 2019, SARS-CoV-2, has caused a pandemic of the respiratory disease, called COVID-19. The coronavirus (SARS-CoV-2) in 2020 has spread all over the world. Based on available data, in Indonesia this case has reached 1.27 million cases, 1.08 million recovered, and 34,316 died (Worldometers, 2020). This case makes the public physical distancing. In addition, the government also urges the public to continue to pay attention to the Health protocol if they have activities outside their homes. Researchers are trying to find a vaccine for this virus and these efforts have certainly paid off. A vaccine for this virus has been found, the Indonesian government is trying hard to vaccinate all its citizens. However, the large number of residents and limited health personnel make the vaccine process take a long time. Therefore, other measures need to be taken to minimize the possibility of being infected with this virus. One way is to maintain and improve the body's immune system. This can be done by utilizing existing natural ingredients, including Curcuma (Aldizal et al., 2019), turmeric, galangal, ginger, lemongrass, lime, and others. From the results of the study, the composition of the plant is proven to have the potential to increase the body's immunity. Another natural ingredient from plants that may be able to increase immunity is kesumba turate (*Carthamus tinctorius* Linn). This plant has been proven to be used as a therapeutic agent for various diseases (Asgarpanah and Kazemivash, 2013). The use of Safflower a natural ingredient that improves health is increasing along with advances in knowledge about its chemical composition (Adamska & Biernacka, 2021).

The dried flower of safflower (the dried flower is the flower that is picked from the stalks of the safflower plant and dried at room temperature) from the Asteraceae tribe is a traditional medicinal plant that is empirically used by the

people of South Sulawesi for the treatment of measles which is given by brewing it with hot water to increase the patient's immune system or immune system. This plant can be used as an antimicrobial. The administration of these medicinal plants has been shown to inhibit the growth of *Salmonella pullorum* and *Escherichia coli* bacteria (Faridah & Febrianti, 2019). Previous research showed that a concentration of 0.5% safflower was able to inhibit the growth of *Salmonella pullorum* and *Escherichia coli* bacteria so this could have potential as an antibacterial (Khatimah, 2018). In addition, other studies also reported that safflower contains compounds that act as antibacterial (flavonoids) which can make milk more resistant and durable. These compounds can also function as natural dyes and preservatives in pasteurized milk (Faridah & Febrianti, 2019). According to Hamsidi et al. (2018), the content of ethanol extract of safflower dried flowers obtained from the maceration method includes flavonoids, anthraquinones, saponins, terpenoids, and tannins. According to Asgarpanah & Kazemivash (2013b), the aqueous extract of safflower contains flavonoids, phenylethanoid glycosides, coumarins, fatty acids, and steroids. From these ingredients, the biological activity of the aqueous extract is considered to be potential as an anticoagulant, vasodilating, antihypertensive, antioxidant, neuroprotective, immunosuppressive, anticancer agent with an inhibitory effect on melanin synthesis (Xie et al., 2016). In addition, the water extract of this plant also has the potential to treat cardiovascular disease. However, the phytochemical investigations reported are still lacking so attention needs to be paid more (Delshad et al., 2018). Safflower water extract is expected to have the potential to be used to improve the immune system during a pandemic.

Therefore, this study aimed to perform phytochemical screening of the aqueous extract of safflower. The aqueous extract was tested for its antibacterial activity at various concentrations, temperatures, and heating times to get the best activity presented in the form of an inhibition zone or a clear zone. In addition, the aqueous extract of safflower was tested for its antioxidant activity by determining the IC_{50}.

Methods

Materials and Instruments

The sample used in this study was safflower (*Carthamus tinctorius* L.) which was obtained from Bone Regency, South Sulawesi, Indonesia. The bacterium used in the antibacterial activity test was isolated from *Staphylococcus aureus* bacteria (collection of the Microbiology Laboratory of the Pharmacy Department of UIN Alauddin Makassar). In addition, this study also used several materials, including nutrient agar (NA) (Merck, Germany); Muller Hinton Agar (MHA) (Merck, Germany); blank antimicrobial susceptibility disks (Oxoid, New York); barium chloride ($BaCl_2$) (Merck, Germany); sulfuric acid (H_2SO_4) Emsure (Merck, Germany); ethanol (Merck, Germany); methanol (Merck, Germany);

DPPH (2,2-Diphenyl-1-picrylhydrazyl) (HiMedia, India) CAS Number 1898-66-4; ascorbic acid 1.00468.0000 (Merck, Germany); physiological sodium chloride (NaCl); distilled water one (Onemed, America). The instruments used in this study were the spectrophotometer Genesys 20 (Thermo Scientific, USA); Nicolet iS10 Fourier Transform Infrared (FT-IR) spectrophotometer equipped with smart iTR Attenuated Total Reflectance (ATR) Sampling Accessory (Thermo Scientific, USA); vortex mixer (Velp-arec, Italy); hotplate stirrer (Velp-arec, Italy); digital balance sheet (Kern, Germany); oven (Memmert, Germany); incubator (Heraeus, Germany); electric stove (Maspion, Indonesia); autoclave (Gea, China); laminar airflow (Esco Scientific, Singapore); micropipette with a scale of 100-1000 µL (Biorad, USA); micropipette with the scale of 1000-5000 µL (Watson, USA).

Preparation of Staphylococcus aureus suspensions

The culture of *S. aureus* was put into a test tube containing physiological sodium chloride (NaCl). Cultures included in NaCl were adjusted to the standard McFarland 0.5 with a concentration of 1.5×108 mL^{-1}. The number of bacteria that met the sensitivity test requirements was 10^5-10^8 mL^{-1}. The observations were made visually, if the turbidity of the bacterial suspension was the same as the McFarland standard, the bacterial suspension was ready for use (Carter, 1979).

Preparation of safflower aqueous extract

Dried forms of Safflower were purchased from the Bone regency (South Sulawesi, Indonesia). Considering that these herbal plants commonly are used to dissolve in boiling water, we also prepared them through this method. Safflower aqueous extracts were prepared with the various concentration of 1%; 2%; 3%; 4%; and 5% (*v:v*). The extracts were boiled at various temperatures of 60 ºC; 70 ºC; 80 ºC; 90 ºC; and 100 ºC. Maceration of Safflower was carried out at various times, they were 5 min; 10 min; 15 min; 20 min; 25 min. All test of various concentration was conditioned at 80 ºC for 15 min. All test of various temperature was conditioned at a concentration of 5% for 15 min. All test of various maceration time was conditioned at a concentration of 5% and 80 ºC. Furthermore, Safflower aqueous extracts were obtained at the condition.

Phytochemical screening of safflower aqueous extract

The content of secondary metabolites in the aqueous extract of safflower was tested using various reagents based on standard testing procedures (Harborne, 1996) (Kurkin, 2015) (Turgumbayeva et al., 2018) (Adamska & Biernacka, 2021b). The extract used to be tested is an extract that has a high concentration.

Study of a functional group of safflower aqueous extract using FTIR (Fourier-*transform Inform Spectroscopy)*

FTIR is used to analyze functional groups. FTIR with a Smart iTR Attenuated Total Reflectance (ATR) sampling device which works to eliminate

sample preparation so that the testing process is simpler. The sample was entered on the iTR device. Next, the analysis process was carried out.

Determination of antibacterial activities

Antibacterial activity was determined by the disc diffusion method against *Staphylococcus aureus* as a human pathogenic bacterium (Rıos & Recio, 2005). The inhibition against bacteria is thought to be one of the potentials of Safflower. This is based on the presence of secondary metabolites generated from the dye in Safflower. This study investigated the effect of Safflower on pathogenic bacteria. This was measured by knowing the clear zone produced by each Safflower extract. The measurement of the clear zone (inhibition zone) of bacteria was carried out with a calliper. The measurement of the zone of inhibition against pathogenic bacteria was not only used in the extract of Safflower but also in positive control and negative control. The positive control used was chloramphenicol, while the negative control was sodium chloride. The number of extracts to be tested for inhibition is 15 extracts of Safflower with different extraction conditions.

About 100 µL of *S. aureus* suspension was inoculated into Muller Hinton Agar medium in a petri dish. About 20 µL of safflower extract was dropped onto sterile disc paper. Furthermore, the paper disc is placed on the test medium which has previously been inoculated with the test bacteria. The incubation process was carried out at 37 ºC for 24 hours. The measurement of inhibition was carried out by observing the emergence of a clear zone using a calliper. In this test, chloramphenicol was used as a positive control (Salem et al., 2014) (K Khatimah et al., 2021).

Determination of antioxidant activities

Antioxidant activity testing was carried out using the 2, 2-diphenyl 2-picrylhydrazyl hydrate (DPPH) method. The tests with this method were determined based on (Hatano et al., 1988); (Moraes-de-Souza et al., 2008); (Salem et al., 2014); (González-Palma et al., 2016); and (Sun et al., 2020) with several modifications. DPPH reacts with an antioxidant compound that can donate hydrogen and reduce DPPH as a radical compound. The colour change happens from deep violet to light yellow, it was measured using an instrument. Safflower aqueous extracts were prepared at the various concentrations (1000 mgL^{-1}; 2000 mgL^{-1}; 3000 mgL^{-1}; 4000 mgL^{-1}; and 5000 mgL^{-1}). About 3 mL of the extract was mixed with 3 mL DPPH 0.4 mM in methanol. Absorbance was determined in a spectrophotometer at 517 nm after incubation for 20 min. The antioxidant activity was calculated by using the following equation (1). The blank solution as negative control contained 3 mL methanol and 3 mL DPPH 0.4 mM.

$$\% \ inhibition = \frac{C_b - C_s}{C_b} \times 100\% \quad \text{..... (1)}$$

C_b is the blank concentration; C_s is the sample concentration.

Result and discussion

Phytochemical study of Safflower aqueous extract

The aqueous extract of Safflower has physical characteristics of brownish yellow color and characteristic odor. The extract was tested for phytochemicals to detect the presence of secondary metabolites in it. The results can be seen in Table 1.

Table 1. Phytochemical study of Safflower aqueous extract

Test	Reagent	Result
terpenoid dan steroid	Lieberman	-
alkaloid	Wagner	+
alkaloid	Mayer	-
alkaloid	Dragendorff's reagent	+
flavonoid	H_2SO_4 p.a	+++
flavonoid	NaOH 10%	+++
tannin	$FeCl_3$	+++

Table 1 showed that the aqueous extract of safflower contains alkaloids, flavonoids, and tannins. The flavonoid content in the water extract of Safflower is very dominant compared to the others. Terpenoids and steroids were not found in the extract. This happens because the two compounds are nonpolar, while the solvent used is polar water. The difference in polarity resulted in the possibility of these two compounds not being extracted from the sample. Therefore, the water extract of Safflower does not contain terpenoids and steroids.

The results obtained in this study are similar to the results of several previous studies. Flavonoids are one of the main components found in Safflower flowers (Shirwaikar et al., 2019). Natsir (2018) suggested that the ethanolic extract of Safflower consists of alkaloids, flavonoids, and steroid/triterpenoid components. Hamsidi et al. (2018) reported that the ethanol extract of Safflower contains secondary metabolites including terpenoids, flavonoids, tannins, and several other phenolic compounds. Sabah dan Saleh (2015) reported that the ether extract of Safflower did not contain alkaloids, carbohydrates, glycosides, saponins, and tannins. However, the extract contains phenols, steroids, and terpenoids. While the flavonoid extract of Safflower only contains flavonoid compounds.

The presence of flavonoid compounds in the aqueous extract of Safflower is influenced by the presence of many hydroxyl groups. This group causes flavonoids to be polar. The more hydroxyl groups, the greater the solubility of the compound in water.

Functional groups analysis of Safflower aqueous extract

The aqueous extract of Safflower was subjected to FTIR analysis. This analysis is intended to prove the alleged presence of compounds based on phytochemical studies (Figure 1). These results showed the presence of a typical absorption spectrum. The spectrum is in the wavenumber 614.41 cm^{-1}; 1637.96 cm^{-1}; and 3452.90 cm^{-1}.

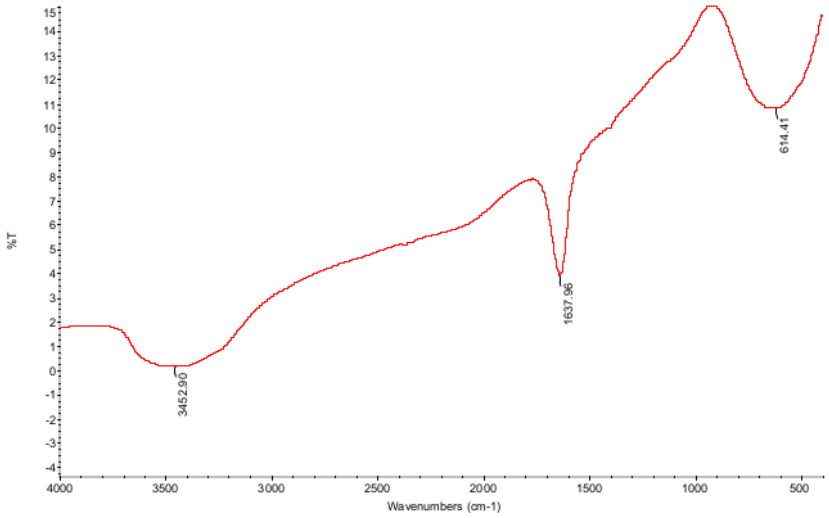


Figure 1. IR spectra of Safflower aqueous extract

The wavenumber of 1637.96 cm^{-1} which is located between 1600-1500 cm^{-1} is thought to be a C=O group because the peak is very sharp and distinctive. The 3500-3000 absorption region appears as a peak which is predicted as a broad -OH absorption at a wavenumber of 3452.90 cm^{-1}. These data indicate that there are compounds in the carboxylic acid group. In addition, the absorption of 3500 cm^{-1} showed the presence of the -NH group. Absorption at 1650 cm^{-1} indicates the presence of alkenes, while absorption at 1650-1450 cm^{-1} indicates the presence of aromatic compounds.

Antibacterial Activity of Safflower aqueous extract

The antibacterial activity test of the water extract of Safflower was screened based on three conditions/factors, namely variations in concentration, variations in temperature, and variations in immersion time. The value of the inhibition zone was evaluated to determine the activity of the Safflower extract in inhibiting bacteria. The clear zone of the colony is presented in Figure 2.

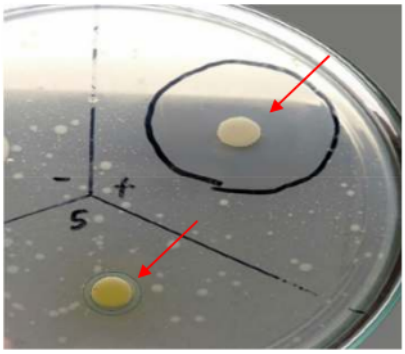


Figure 2. A clear zone of *S. aureus colony*

Table 2. Inhibition zone of *S. aureus* in various concentrations of Safflower aqueous extract

[C]	+	-	1%	2%	3%	4%	5%
IZ of *S. aureus* (mm)	16.76	0.00	2.00	2.54	2.90	2.98	3.70

Note: [C] is concentrations of Safflower aqueous extract; IZ is the inhibition zone of bacteria

Table 3. Inhibition zone of *S. aureus* in various temperatures of Safflower aqueous extract

T	+	-	60 °C	70 °C	80 °C	90 °C	100 °C
IZ of *S. aureus* (mm)	16.76	0.00	2.50	2.50	2.90	3.20	2.70

Note: T is the temperature of Safflower aqueous extract; IZ is the inhibition zone of bacteria

Table 4. Inhibition zone of *S. aureus* in maceration time of Safflower aqueous extract

MT	+	-	5 min	10 min	15 min	20 min	25 min
IZ of *S. aureus* (mm)	16.76	0.00	2.80	2.74	2.90	2.70	2.44

Note: MT is the maceration time of Safflower aqueous extract; IZ is the inhibition zone of bacteria

The aqueous extract of Safflower has little antibacterial activity. During this study, isolates with three factors could be controlled. Meanwhile, the growth of *S. aureus* isolates could be inhibited by the extract although its ability to inhibit was very small. The aqueous extract of Safflower with a concentration of 5% has a large inhibitory value among other concentration variations (Table 2), the aqueous

extract of Safflower at 80 ºC has a large inhibitory value among other temperature variations, and the aqueous extract of Safflower with immersion time of 15 minutes has a large inhibitory value among other time variations. However, this cannot be categorized as strong in inhibiting. The inhibitory value of this extract was very much different from the positive control (chloramphenicol as the standard antibacterial).

Based on these data, the optimum antibacterial activity under three conditions was shown at a 5% extract concentration with a solvent heating temperature of 90 ºC for 15 minutes. The extract under these conditions produced a broad spectrum among other extraction conditions, although the extract could not be categorized as a good antibacterial agent or a strong antibacterial agent. According to David dan Stout (1971), if the value of the inhibitory power of antibacterial substances is 11-19 mm, the antibacterial compound is classified as strong. Khatimah et al. (2021) stated that extracts of Safflower with various concentrations dissolved in boiling water at a temperature of 90 ºC within 15 minutes produced an inhibition zone in the bacterial growth medium. This indicates that the active substance in the extract acts as an antibacterial on *S. pullorum* and *E. coli* bacteria. The results of these studies are slightly different from the results obtained in this study.

Several studies used methanol extract, flavonoid extract, and non-polar solvent extract of Safflower in inhibiting bacterial growth. Karimkhani et al., (2016) have investigated the antibacterial activity of methanol extracts of four varieties of Safflower, namely IL111, Padide, Isfahan-28, and Mahali. Isfahan-28 has the best antibacterial activity among the four extracts. The minimum inhibitory concentrations against S. aureus and S. enterica serovar Typhi were 30 and 60 mg mL^{-1}, respectively. Meanwhile, oil extract and flavonoid extract of Safflower at a concentration of 1000 g mL^{-1} can inhibit the growth of S. aureus and E. coli bacteria. This extract showed better activity against Gram-positive than Gram-negative baºteria (Sabah dan Saleh, 2015). In a study by Abdel et al. (2018), methanol extract and aqueous extract of Safflower have a high potential to inhibit the growth of *S. aureus*, *E. coli*, *P. aeruginosa*, and *K. pneumonia* bacteria. Tayebeh et al. (2021) reported that Safflower extract had a higher sensitivity to *P. aeruginosa* and *K. pneumonia* than other test bacteria. The highest consistently inhibitory effect was observed in *K. pneumoniae*.

Based on several previous studies, methanol extract, ethanol extract, and non-polar solvent extract of Safflower can act well as antibacterial agents. However, this study reported that the aqueous extract of Safflower had poor antibacterial activity, although the water extract contained components of flavonoids, alkaloids, and tannins, all of which could inhibit bacterial growth. There are at least two possibilities into consideration why the aqueous extract of Safflower in this study has poor inhibition against the test bacteria. First, the inhibitory power of Safflower extract also varies, depending on the type of

bacteria (Abdel et al., 2018). Second, the quantity of these components is not strong enough to inhibit. Methanol, ethanol, and other polar solvents can extract more of these compounds than water solvents.

Antioxidant Activity of Safflower aqueous extract

The free radical scavenging activity of DPPH by the aqueous extract of Safflower was detected by a spectrophotometer (Singh dan Nimbkar, 2007). The results were calculated and presented as inhibitory concentrations (%) with a linear range of 1000-5000 ppm.

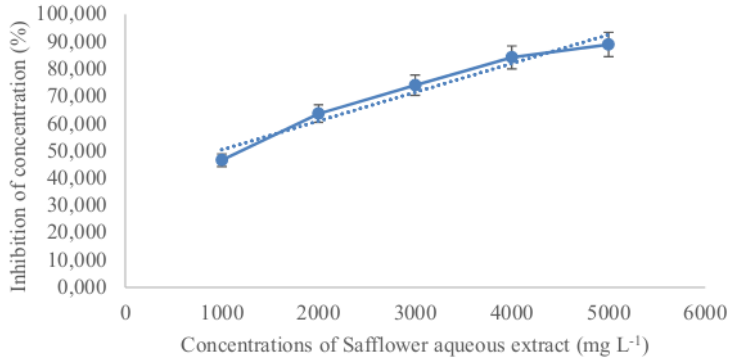


Figure 3. Graph of the relationship between the concentration of Safflower aqueous extract and the inhibitory concentration of Safflower aqueous extract

Figure 3 showed that the linearity value obtained is close to 1 with R = 0.9595. This means that the greater the concentration of the aqueous extract of Safflower, the greater the activity of the extract in inhibiting free radicals. The resulting regression equation is y = 0.0105x + 39.864. Based on this equation, the IC_{50} of Safflower in reducing free radical compounds is 965.3 ppm or equivalent to 965.3 mg L^{-1}. According to Yuan et al. (2009), Safflower extract showed DPPH radical scavenging activity with an IC_{50} value of 1250 mg mL^{-1}. Ebadia et al. (2014) reported that the IC_{50} value of the ethanol extract of Safflower in its activity against DPPH radicals was 1003.287-2387.432 mg L^{-1}. The results of the previous study were different from this study, the IC_{50} of the water extract of Safflower obtained was lower than in the previous study. The lower the IC_{50} value, the greater the antioxidant activity of a substance. This difference arose possibly due to the different types of extraction reagents used, extraction conditions, and plant varieties (Ozkan et al., 2021). This study also examines the ability of ascorbic acid to inhibit free radicals in the form of IC_{50}. The IC_{50} obtained was 1725.49 mg L^{-1}. This value is even higher than the IC_{50} water extract of Safflower.

Antioxidant activity depends on the number of phenols, flavonoids, and anthocyanins in a plant. This compound has a high ability to remove free radicals (Mazzei et al., 2020). In a study by (Hiramatsu et al., 2009), carthamin, which is a polyphenolic compound, was detected in Kasumba turate calyx. It has been reported that the carthamin content in this extract of Safflower correlates with the activity of DPPH radical cleavage.

Several studies have proven that Safflower extract contains phenolic compounds and high antioxidant capacity. Salem et al. (2011) reported that extraction with 2% water: acetone (v:v) had the highest polyphenol content in Carthamus tinctorius L. flowers with a value of 15.09 mg g^{-1} dry weight. Baydar dan Ozkan (2005) stated that the total content of phenolic compounds in the petals of *Carthamus tinctorius* L. with 80% water: methanol (v:v) was 9.06; 20.92; and 16.62 mg g^{-1} dry matter. In a study by (Karimkhani et al., 2016), the antioxidant capacity of the methanol extract of four different safflower varieties was investigated, and the values varied from 46.2 to 62.3 mg GAE/g dry matter. According to Ozkan et al. (2021), Safflower extract ranged from 5.33-14.11 mg TE/g. Ozkan et al. (2021) investigated the in vitro treatment of Safflower extract from three genotypes. The three types of extracts showed strong antioxidant activity. One of the three has the highest antioxidant activity, namely Remzibey-05. After being tested in vitro as to the mechanism of gastric digestion, the recovery rate decreased from 100% to 11-39%. Kim et al. (2015) who studied sausages have found that Safflower makes dishes reddish without the presence of nitrite.

The addition of nitrite to food also inhibits lipid oxidation and reduces the residual nitrite content. Thus, Safflower can be used as a natural colorant to give a reddish color to the desired food, for example in meat, especially in sausages because the main color ingredient is carthamin. Ozkan et al. (2021) reported that Arizona SC III and Dincer 5-18-1 Safflower species can be used as natural additives in food to obtain red, orange, and yellow colors and provide natural antioxidant effects. The results of this study are parallel with previous studies. Although carthamin has limited use in the food industry due to its low water solubility, most of it is used for chocolate production in countries, such as Japan and China (Ekin, 2005) (Emongor, 2010).

Conclusion

The components of secondary metabolites in the aqueous extract of Safflower are alkaloids, flavonoids, and tannins. The best antibacterial activity of the aqueous extract of Safflower can be seen in the best extraction conditions which produce a large zone of inhibition. The best extraction conditions for water extraction of Safflower is at a concentration of 5% water: Safflower aqueous extract (*v:v*) with a heating temperature of 90 ºC for 15 minutes. IC_{50} of aqueous extract of Safflower is 965.33 mg L^{-1}.

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