Comparison of *Suaeda Maritima* Extraction Methods and Analysis of Their Phytochemical Contents, Antioxidant, and Antibacterial Activities

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Abstract

Suaeda maritima, locally called *Alur* in Indonesia, is a water-based plant with high nutritional content commonly consumed as fresh vegetables or *urap*. Therefore, this study aims to determine the phytochemical content, antioxidant, and antibacterial activities of *S. maritima* extracts using two extraction methods: single maceration and ultrasonic-assisted extraction. Fresh *S. maritima* was analyzed for its proximate content and dried before the extraction. The dried *S. maritima* was extracted using three different solvents: methanol, ethanol, and acetone. The extracts were analyzed for the phytochemical compounds, total phenolics content, antioxidant activity (FRAP methods), and antibacterial activity. The results showed that fresh *S. maritima* comprised of moisture, ash, protein, fat, and carbohydrate were 86.74±0.58, 2.76±0.17, 1.08±0.33, 0.47±0.11, and 8.94±0.82%, respectively. It was also revealed that overall extracts, both in single maceration and ultrasonic-assisted extraction methods, contained alkaloids, flavonoids, phenolics, steroids, tannins, and saponins. The ethanol extract had the highest total phenolic contents among the other extracts, which was 6.20±0.27 mg GAE/g. The highest antioxidant activity was 85.04±4.01 mM $FeSO_4$/g, also found in ethanol extract. It was proven that there was a high relationship between the total phenolic content and antioxidant activity. However, the antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* on *S. maritima* was categorized as weak because its zone of inhibition was <5 mm. In conclusion, extraction methods produced similar results in total phenolic content and antioxidant activity. Differences in extraction methods did not significantly affect the *E. coli* and *S. aureus* antibacterial inhibition zone produced by *S. maritima* extracts.

Keywords: Antioxidants; Antibacterials; Phytochemicals; Total Phenolic; *Suaeda maritima*

Introduction

Suaeda maritima, belonging to the family Chenopodiaceae, is an annual herb that thrives in highly alkaline and saline moist soils of salt marsh mangroves (Nayak et al., 2018). *S. maritima*, locally called *Alur* in Indonesia, is a water-based plant having high nutritional content and is commonly consumed as fresh vegetables or *urap*. According to Pornpitakdamrong and Sudjaroen (2014), young leaves from *S. maritima* can be used as fresh vegetables or cooked with other vegetables to reduce their salty taste.

S. maritima has a high nutritional content. A previous study found that *S. maritima* comprised protein, lipid, carbohydrates, fiber, calcium, and beta-carotene content of 0.15±0.01% (w/w); 2.18±0.02%; 6.21±0.01%; 2,471.37±0.054 mg/100g; 3.46±0.04%; and 3,545.16±0.093 mg/100g, respectively (Sudjaroen, 2012). In several countries, such as India and Thailand, *S. maritima* has been studied for pharmaceuticals (Patra et al. 2011; Ravikumar et al., 2011) and food applications (Pornpitakdamrong & Sudjaroen, 2014). *S. maritima* leaves can be used to prevent hepatitis and for antivirals (Ravikumar et al., 2011). In addition, Patra et al. (2011) also showed that leaf and stem extracts from *S. maritima*, using several solvents, had high sources of natural antioxidants with moderate antimicrobial activity. In addition, Nayak et al. (2018) also found that the n-hexane extract of *S. maritima* had antibacterial activity against four Gram-positive (*Bacillus subtilis, Staphylococcus haemolyticus, S. aureus, Enterococcus faecalis*) and six Gram-negative

(*Escherichia coli, Citrobacter sp., Klebsiella pneumoniae*, *K. pneumoniae* carbapenemase-producing *K. pneumoniae*, *Pseudomonas* sp., and *Stenotrophomonas maltophila*) bacteria.

In Indonesia, *S. maritima* is only found in coastal areas. However, it grows all year round. Pornpitakdamrong and Sudjaroen (2014) also stated that it was only a few foods made from *S. maritima* in Thailand and it indicated that *S. maritima* is not as popular as other types of vegetables from plants such as white popinac, ivy pumpkin, and others. Several bioactive compounds in plants, as herbal medicines, can scavenge free radicals and potentially be a source of natural antioxidants (Shah et al., 2013).

Research on *S. maritima* related to the antioxidant and antibacterial content of its extract in Indonesia, especially using ultrasonic extraction, has not been conducted. Osorio-Tobón (2020) reported the different between conventional and alternative extraction methods. Conventional methods are often used as a baseline for comparison. Alternative methods, such as microwave-assisted extraction (MAE), pressurized liquid extraction (PLE), supercritical fluid extraction (SFE), and ultrasonic-assisted extraction (UAE) are often more efficient. These methods can extract more phenolic compounds in less time using safer solvents. Therefore, this study aimed to determine the phytochemical content, antioxidant, and antibacterial activities of methanol, ethanol, and acetone extracts of *S. maritima* using two methods: single maceration and ultrasonic-assisted extraction.

Materials and Methods

Materials

Fresh *S. maritima* was collected in March-May 2022 in Karangantu-Banten, Indonesia, with a coordinate point of -6.027107,106.160893 (Figure 1). The samples were packed in a plastic bag and transported to Politeknik Ahli Usaha Perikanan Jakarta by driving for three hours.

Extraction Methods

S. maritima was washed with tap water and drained. The whole plant of fresh *S. maritima* was dried in a hot air oven at 40°C until the moisture content reached $<$10% (Afifah & Niwat, 2020) and until the water content remained at 6.91% (Syafitri et al., 2014). The dried *S. maritima* was ground using a grinder machine into a powder form. After that, the dried *S. maritima* was extracted with two treatments: single maceration (SM) and ultrasonic-assisted extraction (UAE). Each sample was extracted using three different technical-grade solvents: methanol (99,99%), ethanol (99,99%), and acetone (99,99%) (Bratachem®, Indonesia).

SM method refers to the research of Ramadhani et al. (2020) and Widyasanti and Al-Ghifari (2016) with modifications in the ratio of samples and solvents and used only single-step extraction. The dried plant of *S. maritima* (100 g) was soaked in 600 mL of solvent (methanol, ethanol, or acetone) at a ratio of 1:6 (%, w/v). The dried plant was soaked for three days using an orbital shaker (Thermo Scientific®) with 150 rpm rotation (carried out the same for all treatments). After that, the filtrate was filtered and evaporated using a rotary evaporator (Heidolph®) at 40 ºC for each solvent. The extracts were analyzed for phytochemical screening, total phenolic content (TPC), antioxidant activity, and antibacterial activity.

UAE method of the dried plant was conducted following Hartanti et al. (2021) with modifications. The dried plant of *S. maritima* (30 g) was soaked in 180 mL of solvent (methanol, ethanol, or acetone) at a ratio of 1:6 (%, w/v). The mixtures were extracted for 20 minutes at 45 ºC with a frequency of 60 Hz using a water-bath ultrasonic instrument (Elma Ultrasonic LC 60 H, 230 V/H, 1.3 A). The filtrate obtained was filtered using Whatman filter paper No.41 and evaporated with a rotary evaporator (Heidolph®) at 40 ºC for each solvent. The extracts were analyzed for phytochemical screening, total phenolic content, antioxidant activity, and antibacterial activity.

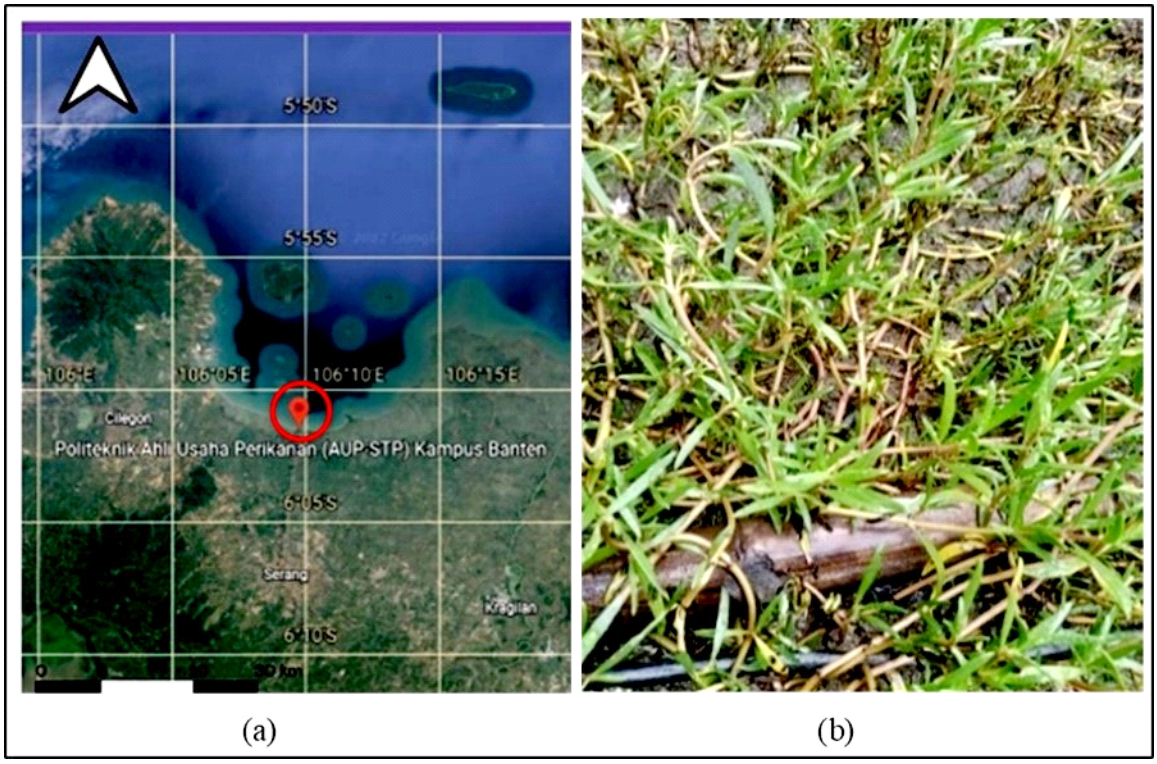

Figure 1. Sampling location of *S. maritima* in Karangantu-Banten, Indonesia (a), fresh *S. maritima* used in this study (b).

Proximate Analysis

Proximate analysis included testing for moisture content using gravimetry method according to SNI 1971:2011 (Badan Standarisasi Nasional, 2011), ash content using gravimetry method according to SNI 2354.1-2010, protein content using Kjeldahl method according to SNI 2354.4-2006 (Badan Standarisasi Nasional, 2006), and lipid content using soxhlet extraction method according to SNI 2354.3-2017 (Badan Standarisasi Nasional, 2017).

Phytochemical Screening

Phytochemical screening of extracts was carried out on several bioactive compounds, which were alkaloids, flavonoids, phenolics, steroid-terpenoids, saponins, and tannins, following the methods of Permadi et al. (2018) and Ningsih et al. (2020).

Alkaloids

Alkaloids screening was carried out by weighing 0.5 g of sample extract, putting it into a test tube and adding 10 drops of sulfuric acid 2 N (Merck®, Germany). The mixtures were reacted with several reagents: Dragendorf, Mayer, and Wagner. Positive results showed a red or orange precipitate on the Dragendorf reagent, a yellowish-white precipitate on the Mayer reagent, and a brown precipitate on the Wagner reagent (Permadi et al., 2018).

Flavonoids

Flavonoids screening was carried out by taking 0.5 g sample extract and putting it into a test tube. The samples were added with 10 mL of hot water (96±2 ºC). After that, 1 mL of hydrochloric acid (Merck®, Germany) and 0.05 g of magnesium powder (Merck®, Germany) were added. Positive results showed a yellow-red or orange solution (Permadi et al., 2018).

Phenolics

Phenolics screening was carried out by weighing 0.5 g of sample extract and then adding three to four drops of 1% iron (III) chloride (Merck®, Germany). A change in color from bluish-black to dark black (blue or blue-purple) indicated the presence of phenolic compound in the extract (Ningsih *et al*., 2020).

Steroids and terpenoids

The steroid and terpenoid screening tests were carried out by taking 0.5 g sample extract, then adding 2 mL of chloroform (Bratachem®, Indonesia), 10 drops of glacial acetic acid (Bratachem®, Indonesia), and three drops of concentrated sulfuric acid (Merck®,

Germany). A blue-to-green color change indicated a positive steroid result, while a brownish-red to purple color indicated a positive terpenoid result (Permadi et al., 2018).

Tannins

Tannin screening was carried out by weighing 0.5 g of the sample extract and then adding 10 mL of hot water (96±2ºC). The mixtures were dripped (three to four drops) with 1% iron (III) chloride. A positive reaction showed a green-black color solution (Ningsih et al., 2020).

Saponins

Saponins screening test was carried out by putting 0.5 g of the extract in a test tube, adding 10 mL of hot water, and mixing the solution. A positive result was shown by the stable foam in 10 minutes (Permadi et al., 2018).

Total phenolic content

The total phenolic content (TPC) was determined using the ISO 14502-1 (2005) method, which used gallic acid (Sigma Aldrich®, USA) as a standard. Samples (1.0 mL) were added with 10% v/v Folin-Ciocalteu reagent (5.0 mL) (Sigma Aldrich®, USA) and 7.5% w/v sodium carbonate solution (4.0 mL) (Merck®, Germany). The mixtures were incubated for 1 hour at room temperature (27±2 °C) and the absorbance was measured using a spectrophotometer (Cecil CE3021®) at 765 nm and using distilled water as blank. Total phenolic content was carried out three times and reported as gallic acid equivalents (GAE) in mg/g dry weight samples.

$$\text{Total phenolic content (mg GAE/g samples)} = \frac{\text{[C] (µg/ml) x V extract (ml) x dilution factor}}{\text{W samples (g) x \%dry matter x 1000}}$$

where C is the sample's concentration calculated from the standard curve, V is the extract volume, W is the weight of samples.

Antioxidant activity assay

Antioxidant activity was carried out using the FRAP (*Ferric Reducing Antioxidant Power*) method. Antioxidant activity of the FRAP method referred to Afifah and Niwat (2015) using ferrous sulfate as standard. FRAP stock standard solution (1000 ìM) was made by weighing 0.0139 g of ferrous sulfate (Merck®, Germany) then diluted in 50 ml of distilled water. After that, the stock solution was made into several concentrations, namely 1,000, 500, 250, 125, and 62.5 ìM. Standard solution and samples (400 µl) were mixed with 2.6 mL FRAP reagent. The mixtures were incubated at 37 ºC for 30 minutes. Absorbance was measured using a spectrophotometer (Cecil CE3021®) at 595 nm and used water as a blank. Antioxidant activity was carried out three times and expressed as mmol FeSO4/g dry weight samples.

$$\text{FRAP (mmol } FeSO_4\text{/g samples)} = \frac{\text{[C] (µmol/ml) x V extract (ml) x Dilution factor}}{\text{W samples (g) x \%dry matter x 1000}}$$

where C is the sample's concentration calculated from the standard curve, V is the extract volume, W is weight of samples.

Antibacterial activity assay

Antibacterial activity assay was conducted using disc-diffusion and well-diffusion methods following Niswah (2014) with slight modifications in bacterial suspension density. Methanol, ethanol, and acetone extract of *S. maritima* were used as samples. *Escherichia coli* ATCC 11775 and *Staphylococcus aureus* ATCC 25923 were provided by the Research Center for Marine and Fisheries Product Processing and Biotechnology, Ministry of Marine Affairs and Fisheries, Republic of Indonesia, Jakarta, Indonesia. Antibacterial testing was carried out three times.

Antibacterial activity was measured by disc-diffusion method and well-diffusion method. For disc-diffusion method, sample extract solution (60%) was put in a blank paper disc and soaked for 24 hours. The negative controls were the solvents (methanol, ethanol, and acetone) and the positive controls were chloramphenicol, which were also dissolved in methanol, ethanol, and acetone. The controls were soaked in a blank paper disc for 24 hours. Bacterial suspension with a density of 1×10^8 cells/mL (1 mL) was put into a test tube containing 15 mL of liquid Tryptone Soy Agar (TSA) media (Oxoid®). The bacterial suspension was measured by converting the absorbance value of the suspension to McFarland standard. The mixtures were then aseptically poured into a sterile petri dish and allowed to solidify. The sampel extracts, also the positive and negative control paper disc (6 mm), were placed on the agar plate. They were then incubated at 37 °C for 24 hours. The clear area around the discs showing no bacterial growth was measured using a caliper and its antibacterial potential was determined. Antibacterial activity was classified into four categories, namely weak activity (zone of inhibition <5 mm), moderate (zone of inhibition 5-10 mm), strong (zone of inhibition 10-20 mm), and very strong (zone of inhibition >20 mm).

For well-diffusion method, bacterial suspension with a density of 1×10^8 cells/mL (1 mL) was put into a sterile petri dish, then added 15 mL of liquid TSA media. The petri dish was shaken clockwise and counterclockwise 5 to 10 times so that the media and suspension were mixed and allowed to solidify. Two wells (6 mm) in each petri dish were made in the solid agar. Sample extract solutions (60%) were added to each well. The solutions containing positive control, chloramphenicol, dissolved in methanol, ethanol, and acetone, respectivey, and negative controls (solvents) were also added to the wells. All petri dishes were incubated at 37 °C for 24 hours. The clear area around the wells showing no bacterial growth was measured using a caliper.

Data analysis

All data were analyzed using factorial design with two parameters: extraction methods (SM and UAE) and three types of solvents (methanol, ethanol, and acetone). Subsequently, the data obtained were statistically analyzed using *Microsoft Excel* and SPSS IBM 25 *Univariate-ANOVA* with the Duncan advanced test ($P<0.05$).

Results and Discussion

Chemical properties of Suaeda maritima

The chemical properties of *S. maritima* are shown in Table 1. The protein and lipid content in *S. maritima* from Karangantu were 1.08% and 0.47%, respectively. Previous study from Sholehah and Suryawati (2016) report that *S. maritima* originating from the Madura coastal area comprised of protein 1.68% and fat 0.45%. The moisture content, total ash, and carbohydrates have different values from Pornpitakdamrong and Sudjaroen (2014) on *S. maritima* from Thailand due to the samples' locations. Behr et al. (2017) different salinity levels in the water source could affect the composition of phytochemicals.

Table 1. Chemical content of *S. maritima*

Proximate	Composition (% wet base)	Reference (% wet base)
Moisture Content	86.74±0.58	91.22**
Ash	2.76±0.17	1.59**
Protein	1.08±0.33	1.68*
Lipid	0.47±0.11	0.45*
Carbohydrate	8.94±0.82	4.85**

* Sholehah and Suryawati (2016)
** Pornpitakdamrong & Sudjaroen (2014)

Phytochemical Properties of Dried Plant Extracts

The phytochemical content in dried plant was analyzed qualitatively. The phytochemical screening method was carried out by observing color alteration using a color reagent (Minarno, 2015). Flavonoids were the most abundant class of compounds in most *S. maritima* due to their significant visible color change. The phytochemical compounds on methanol, ethanol, and acetone extracts of SM method and of the ultrasonication method are shown in Table 3.

Table 3. Phytochemical contents of single maceration method extracts

Secondary Metabolites		Single maceration			Ultrasonic-assisted extraction			Response Observation
		Methanol	Ethanol	Acetone	Methanol	Ethanol	Acetone	
Alkaloids	Dragendorf	+	+	+	+	+	+	Orange-reddish precipitate
	Mayer	-	-	-	-	-	-	Yellowish-white precipitate
	Wagner	+	+	+	+	+	+	Brown-yellow precipitate
Flavonoids		+	+	+	+	+	+	Yellow/orange, red/cockroach
Phenolic		+	+	+	+	+	+	Bluish-black
Tannins		+	+	+	+	+	+	Blackish green
Steroids/Terpenoids		+	+	+	+	+	+	Steroids (blue-green), Terpenoid (brownish-purple)
Saponins		+	+	+	+	+	+	Stable foam/foam

Notes:
(+) means positive result for the presence of the indicated secondary metabolite
(-) means negative result for the presence of the indicated secondary metabolite

Phytochemical screening showed that *S. maritima* extracts contained alkaloids, flavonoids, phenols, steroids, tannins, and saponins compounds in SM and UAE methods (Table 3 and Table 4). According to Septiana and Asnani (2012), differences in extraction methods do not affect the qualitative assessment of the phytochemical content in plant extracts, such as flavonoids, saponins, terpenoids, or other compounds.

Alkaloid screening of extracts showed positive results in Dragendrof and Wagner reagents yet it showed negative results in Mayer reagents. Alkaloid was found in *S. maritima* because 2 out of 3 reagents were positive. Alkaloid compounds were found in *S. maritima* from Bhitarkanika, India (Patra et al., 2011). Alkaloid compounds for plants function as toxic compounds to protect plants from insects or herbivores (pests and diseases) and as mineral bases to maintain ion balance (Rohyani & Suripto, 2015). According to Julianto (2019), flavonoids have an effect as antioxidants. Phytochemical screening of *S. maritima* from India also showed that methanol, ethanol, acetone, and water extracts contained flavonoids (Patra et al., 2011).

Polyphenol and tannin tests also showed positive results. Patra et al. (2011) also found them in *S. maritima* from India. Phenolic compounds consist of several groups, namely polyphenols, phenolic acids, phenylpropanoids, flavonoids, and tannins (Julianto, 2019). Phenolic compounds are the main components that produce antioxidant activity (Sedjati et al., 2017).

Terpenoid/steroid screening showed positive results. Steroids were also found in *S. maritima* extracts from India (Patra et al., 2011). Steroids are a group of triterpenoids containing a pyropene perhydrophenentrena cyclopenane nucleus consisting of three cyclohexane rings and one cyclopenane ring (Nola et al., 2021). Steroids in plants have shown cholesterol-lowering and anticancer effects (Nasrudin et al., 2017). Saponin compounds showed positive results. Saponins are compounds in the form of glycosides that are widespread in higher plants and some marine animals. They are diverse compounds in structure, physicochemical properties, and biological effects (Addisu & Assefa, 2016). The positive impact of saponins is widely used for human interests because saponins have broad activities such as antibacterial, antifungi, the ability to lower cholesterol in the blood and inhibit the growth of tumor cells (Yanuartono et al., 2017).

Total Phenolic Content of Suaeda maritima *Extract*

The test result of the total phenolic content (TPC) of *S. maritima* extract is shown in Figure 2. The TPC in SM method was higher than in the UAE method with ethanol. The TPC of the methanol extract using SM method was 2.26±0.50 mg GAE/g, while in UAE method was 1.01±0.27 mg GAE/g. The TPC of the ethanol extract using the SM method was also higher than UAE method, 6.20±0.27 and 4.24±0.86 mg GAE/g, respectively. On the other hand, extraction methods did not significantly affect TPC in the acetone solvent, which was 0.13±0.04 (SM) and 0.18±0.09 mg GAE/g (UAE). Costa-Becheleni (2024) found that the other halophytes extract namely *Suaeda edulis* and *Suaeda esteroa* had the phenolic content 0.15698-0.20713 mg GAE/g (in methanol solvent). Lestari et al. (2018) mentioned that the TPC of gayam leaf extract (*Inocarpus fagiferus*) was greater in ethanol extract (313.704 mg GAE/g) than methanol extract (273.913 mg GAE/g).

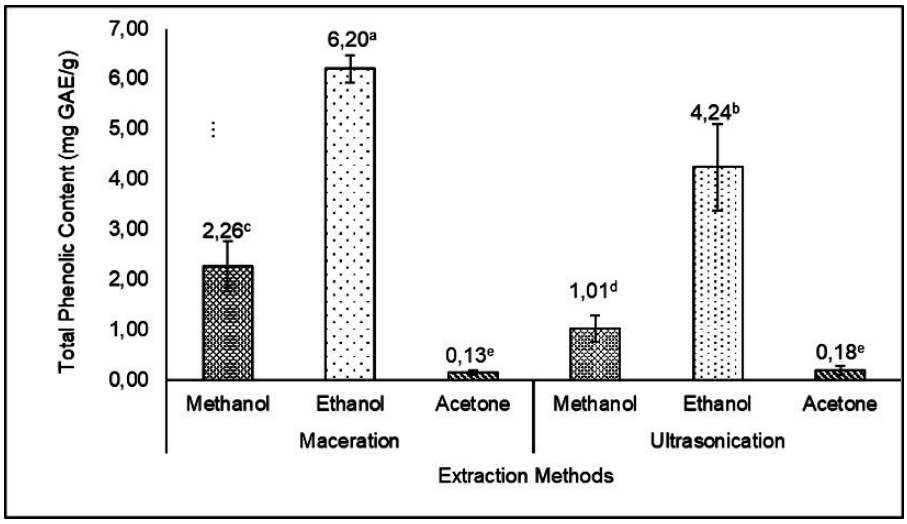

Figure 2. Total phenolic content of *S. maritima* extracts. Note: Different superscript letters on the column indicate significant differences (N = 3).

TPC with SM method was higher because the soaking period in SM method was longer, which was 72 hours, while the UAE method only took 30 minutes. The longer the soaking time, the more effective the extraction results due to the diffusion of the solvent into the sample was better. It was similar to the findings of Hasnaeni et al. (2019), who stated that the phenolic yield with the maceration method was higher because the soaking time was longer than that of other methods, so the compounds extracted optimally. However, Osorio-Tobón (2020) mentioned that the UAE method (an hour extraction time) effectively extracted the phenolic compounds using moderate temperatures, short extraction times, and solvents generally recognized as safe. It was not in line with this study because the extraction time (30 minutes) was not enough for the solvent to penetrate and have a mass transfer, producing lower extraction yields.

In line with the statement of Mottaleb and Sarker (2012), the solvent polarity used in extraction must be equal or very close to the polarity of the active compound so that the compounds are extracted into the solvent and produce a high yield. In addition, ethanol is a polar solvent capable of dissolving almost all compounds in the sample. The polarity of ethanol can dissolve phenols better so that the phenolic content in the extract is higher (Moein & Moein, 2010).

Antioxidant Activity of Suaeda maritima *Extract*

Bioactive compounds in plants could potentially be antioxidant, including flavonoids, phenolic compounds, tannins, steroids, and triterpenoids (Manongko et al., 2020). FRAP antioxidant activity of *S. maritima* extract is presented in Figure 3.

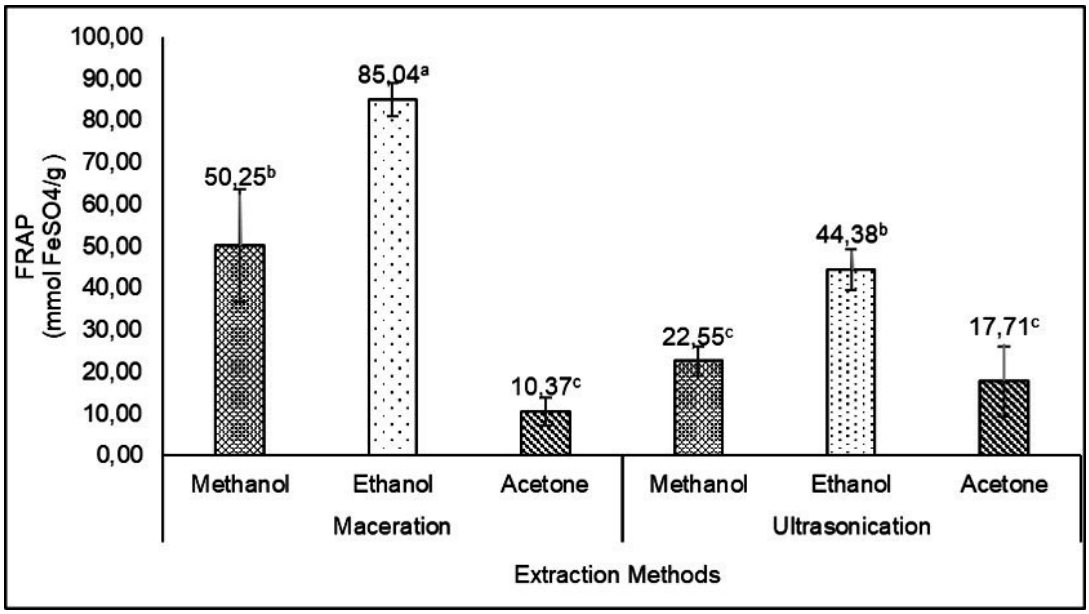

Figure 3. FRAP antioxidant activity of *S. maritima* extracts. Note: Different superscript letters on the column indicate significant differences (N = 3).

Based on univariate-ANOVA analysis with Duncan test, it was found that different extraction treatments and solvents significantly affected the antioxidant activity value ($P<0.05$). It can be seen in Figure 3 that SM method produces the best effect on the antioxidant activity of *S. maritima* compared to the UAE method. Different solvents also contributed to the resulting antioxidant activity value ($P<0.05$). The Pearson correlation analysis also showed a relationship between the total phenolic content of the sample extract and the antioxidant activity ($R^2=0.924$), as shown in Figure 4. The higher the total phenolic content produced, the higher the antioxidant activity. The statement applies to our findings that the highest antioxidant activity was found in the ethanol extract with the highest TPC. Similar finding from Kalaivani and Mathew (2010) showed a positive correlation between the antioxidant activity test and the total phenolic content of plant material.

Different extraction methods and solvents affect antioxidant activity differentl(Sayuti, 2017). Mohammed et al. (2020) found that the maximum antioxidant activity of *S. maritima* was found in the ethyl acetate extract, while thmethanol extract had the lowest activity. Compounds with antioxidant activity from *S. maritima* tend to dissolve more in polar solvents because the highest FRAP value was found in ethanol extracts. It is due to the solubility principle of like dissolve like, which means that a compound will dissolve in solvents of the same nature (Kemit et al., 2017).

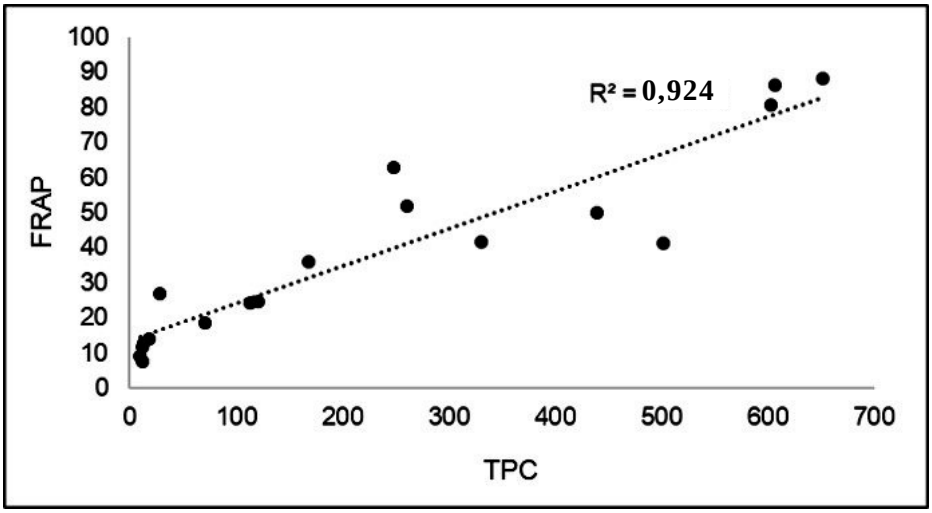

Figure 4. Correlation between TPC and FRAP based on Pearson Correlation Analysis.

According to Alhabsyi et al. (2014), polar solvents such as methanol and ethanol are more effective for antioxidant extraction from plant materials. Flavonoids are potent antioxidants soluble in water and have a strong anticancer activity (Okwu, 2001). Tannins are polar compounds, so they dissolve in polar solvents (Ikalinus et al., 2015). Moreover, compounds that usually have antioxidant activity are phenols that have a hydroxyl group (–OH) and an alkyl group (-OR) (Wirawan, 2016).

Aguilera et al. (2011) found that the antioxidant activity of phenolic compounds is directly related to chemical structure, such as the degree of glycosylation and the number and position of hydroxyl groups associated with the carboxyl functional group. Phenolics make an important contribution to antioxidant activity because they have activities that help to bind free radicals and chelate metals. Phenolic compounds also have the ability to donate hydrogen atoms or electrons to free radicals to form stable intermediates. These compounds bind to free radicals, decompose oxidation products, and chelate metal ions (Diniyah & Lee, 2020).

Antibacterial Activity of Suaeda maritima *Extracts*

Antibacterial compound can inhibit the growth and reproduction of a bacterium while controlling the growth of harmful bacteria (Putri et al., 2014). According to Pangestuti et al. (2017), compounds that act as antibacterial in *Sargassum* sp. are flavonoids, saponins, tannins, and phenols. According to Rahmawati et al. (2014), the diameter of the clear zone measuring <5 mm iweak, 5-10 mm moderate inhibition, and 10-20 mm strong inhibition. In this study, antibacterial testing was carried out against *E. coli* and *S. aureus* using disc diffusion method and the well diffusion method. As a report of Putri et al. (2023), disc diffusion and well diffusion methods are two commonly used methods in antimicrobial susceptibility testing to measure the effectiveness of antimicrobial agents against microorganisms. Both methods have the same basic principle, which is to detect the ability of antimicrobial agents to inhibit the growth of microorganisms, but with slightly different procedures. The well diffusion method involves creating wells (small holes) in an agar medium that has been inoculated with the test microorganism. A solution of the antimicrobial agent is then dropped into the well. After incubation, the inhibition zone (the zone free from microorganism growth) around the well is measured to determine the antimicrobial activity of the tested agent. The disc diffusion method involves placing paper discs that have been soaked in a solution of the antimicrobial agent on the surface of an agar medium that has been inoculated with the test microorganism. The antimicrobial agent will diffuse out of the disc into the agar. After incubation, the inhibition zone around the disc is measured to determine the antimicrobial activity of the tested agent.

Disc-diffusion method

The diameter of the antibacterial inhibition zone of *E. coli* disc-diffusion method on the extract of *S. maritima* is presented in Table 5 and described in Figure 5. Differences in extraction methods did not significantly affect the *E. coli* antibacterial inhibition zone produced by *S. maritima* extracts (P>0.05). However, different solvents significantly affected the *E. coli* antibacterial inhibition zone (P<0.05). Based on Table 5, it can be seen that ethanol and acetone produce the antibacterial inhibition zones for *E. coli* in the disc-diffusion method. Still, their antibacterial activity is relatively weak (<5 mm). The ethanol and acetone extract of *S. maritima* provided a bigger

inhibition zone diameter because their solvents probably have the closest polarity level to the phytochemical compounds that act as bacterial inhibitors.

Table 5. Inhibition zone of *S. maritima* extracts against *E. coli* and *S. aureus* by disc-diffusion method

Extraction Methods	Inhibition Zone Diameter (mm)					
	E. coli			*S. aureus*		
	Methanol	Ethanol	Acetone	Methanol	Ethanol	Acetone
Single maceration	0.00±0.00[b]	2.50±0.13[a]	1.77±0.29[a]	0.00±0.00[b]	2.72±0.71[a]	0.00±0.00[b]
Ultrasonic-assisted extraction	0.00±0.00[b]	2.18±0.90[a]	1.98±0.74[a]	0.00±0.00[b]	2.56±0.64[a]	0.00±0.00[b]
Control (+)	11.84	12.80	16.44	10.60	10.10	8.72
Control (-)	-	-	-	-	-	-

Note: Different superscript letters on the same column indicate significant differences (N = 3).

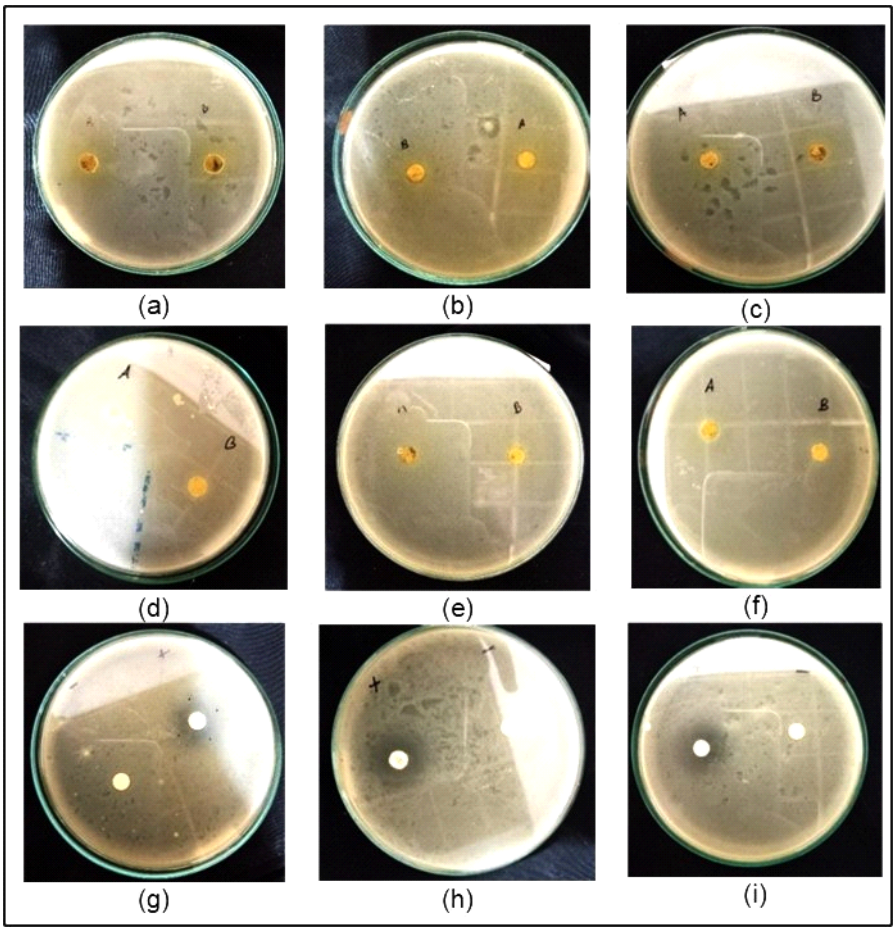

Figure 5. Zone formation of the solvents, extracts, and positive control against *E. coli* by disc-diffusion: methanol (a), ethanol (b), acetone (c), methanol with extract (d), ethanol with extract (e), acetone with extract (f), positive control in methanol (g), ethanol (h), and acetone (i).

Al-Azzawi et al. (2012) discovered that compounds that are efficacious as antibacterials are alkaloids, saponins, tannins, terpenoids, and steroids. Based on our results of phytochemical screening, it was also known that *S. maritima* contained alkaloids, flavonoids, phenolics, steroids, saponins, and tannins. Acetone extract of Indian's *S. maritima* was more effective against *Escherichia coli* bacteria than other extracts with an inhibition zone ranging from 9-14 mm (Patra et al., 2011), while our findings showed only 1.77 mm. Based on the phytochemical screeening, *S. maritima* in the previous study contained almost similar compounds such as alkaloid, carbohydrates, cardiac glycosides, tannin, and phenolic. However, the antibacterial compounds in this study might be lower in concentration compared to the previous study due to the different extraction methods used. They extracted the *S. maritima* overnight at room temperature with a ratio of dried sample to solvents 1:3, while our ratio of dried sample to solvents was 1:6 in 72 hours.

Each phytochemical compound has different effects on antibacterial mechanisms. According to Rijayanti (2014), alkaloids can interfere with the constituent components of peptidoglycan in bacterial cells, so that the cell wall layer cannot be formed completely, causing the death of the bacterial cell. Flavonoids have a mechanism of action by inhibiting nucleic acid synthesis, inhibiting the cytoplasmic membrane's function, and inhibiting bacteria's energy metabolism (Manik et al., 2014). Saponins have a working mechanism by interfering with the permeability of the bacterial cell membrane, resulting in damage to the cell membrane and causing the release of various vital components from inside the bacterial cell, such as proteins, nucleic acids, and nucleotides (Kurniawan & Aryana, 2015). While, steroids can damage lipid membranes, which can cause leakage in bacterial liposomes (Madduluri et al., 2013).

Antibacterial activities of the positive control showed that chloramphenicol dissolved in acetone, methanol, and ethanol produced inhibition diameters of 11.84 mm; 12.80 mm; and 16.44 mm, respectively. The negative control (methanol 96%, ethanol 96%, and acetone 95%) did not produce a clear zone, meaning that the solvent had no antibacterial activity and did not interfere with the bacterial activities of the extracts and positive control. According to Kumayas et al. (2015), the absence of negative control inhibition zones for gram-negative and gram-positive bacteria means that the solvent does not influence the inhibition but is due to the activity of the compounds in these plants.

In addition, the inhibition zone and zone formation of *S. maritima* extracts against *S. aureus* are shown in Table 5 and Figure 6, respectively. SM and UAE methods did not affect the resulting inhibition zone (Pe"0.05). On the contrary, different solvents significantly affected the *S. aureus* antibacterial inhibition zone (Pd"0.05). Table 5 shows that the ethanol extract shows weak antibacterial activity against *S. aureus* (<5 mm), while the methanol and acetone extracts were negative.

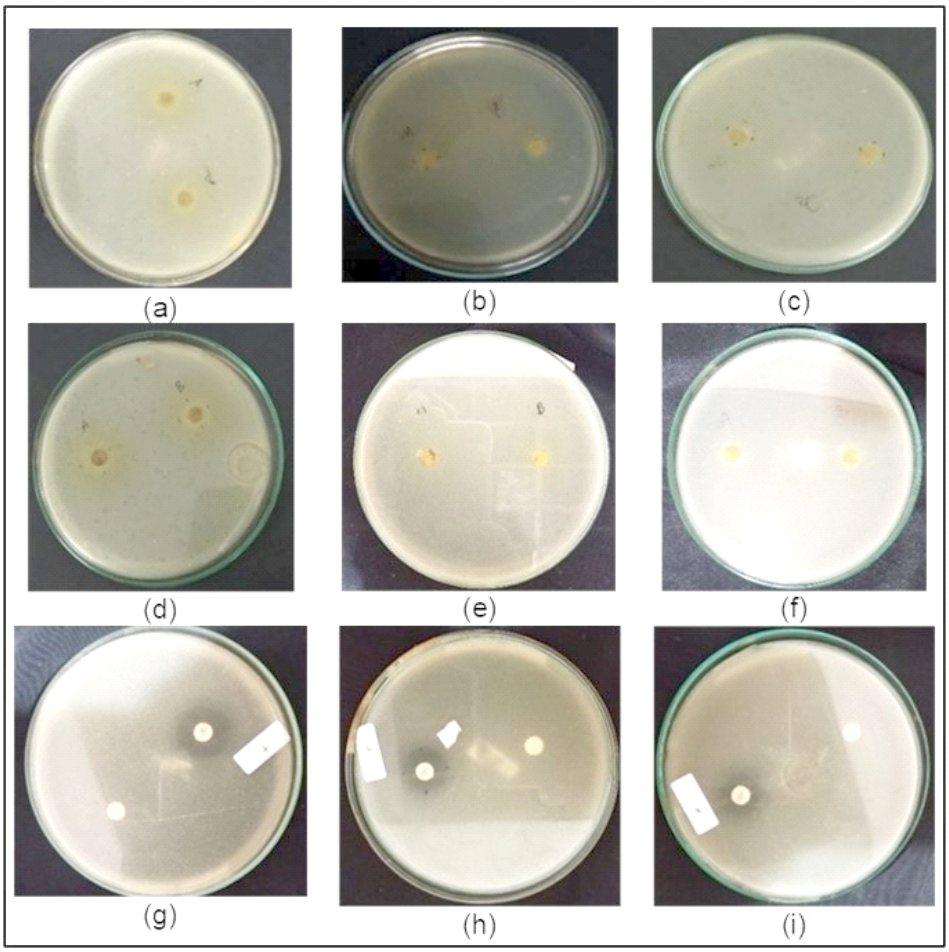

Figure 6. Zone formation of the solvents, extracts, and positive control against *S. aureus* by disc-diffusion: methanol (a), ethanol (b), acetone (c), methanol with extract (d), ethanol with extract (e), acetone with extract (f), positive control in methanol (g), ethanol (h), and acetone (i).

The ethanol extract showed an antibacterial effect against *S. aureus*, because the phytochemical compounds have the same polarity as ethanol (Kemit et al., 2017). It is also in line with Shakthi et al. (2024) that *Suaeda monoica* extract (in concentration 50-100 µg/mL) against *S. aureus* of 10 mm. While Patra et al. (2011) showed that *S. maritima* extracts (methanol, ethanol, acetone, and water) from India had no antibacterial activity against *S. aureus*. The difference in the content of metabolite compounds can affect antibacterial activity. In this study, the phytochemical analysis results showed the content of alkaloids, flavonoids, phenolics, steroids, tannins, and saponins in the *S. maritima* extracts. The bioactive compounds found in plants could affect the antibacterial activity (Lestari et al., 2016).

Well-diffusion method

The inhibition zone and zone formation of *S. maritima* extracts against *E. coli* by the well-diffusion method are shown in Table 6 and Figure 7, respectively.

Table 6. Inhibition zone of *S. maritima* extracts against *E. coli* and *S. aureus* by well-diffusion method

Extraction Methods	Inhibition Zone Diameter (mm)					
	E. coli			*S. aureus*		
	Methanol	Ethanol	Acetone	Methanol	Ethanol	Acetone
Single maceration	0.00±0.00^{b}	0.00±0.00^{b}	6.02±2.95^{a}	0.00±0.00	0.00±0.00	0.00±0.00
Ultrasonic-assisted extraction	0.00±0.00^{b}	0.00±0.00^{b}	5.41±1.77^{a}	0.00±0.00^{b}	1.45±1.40^{a}	0.00±0.00^{b}
Control (+)	8.70	9.40	11.38	5.13	6.50	7.36
Control (-)	-	-	-	-	-	-

Note: Different superscript letters on the same column indicate significant differences (N = 3).

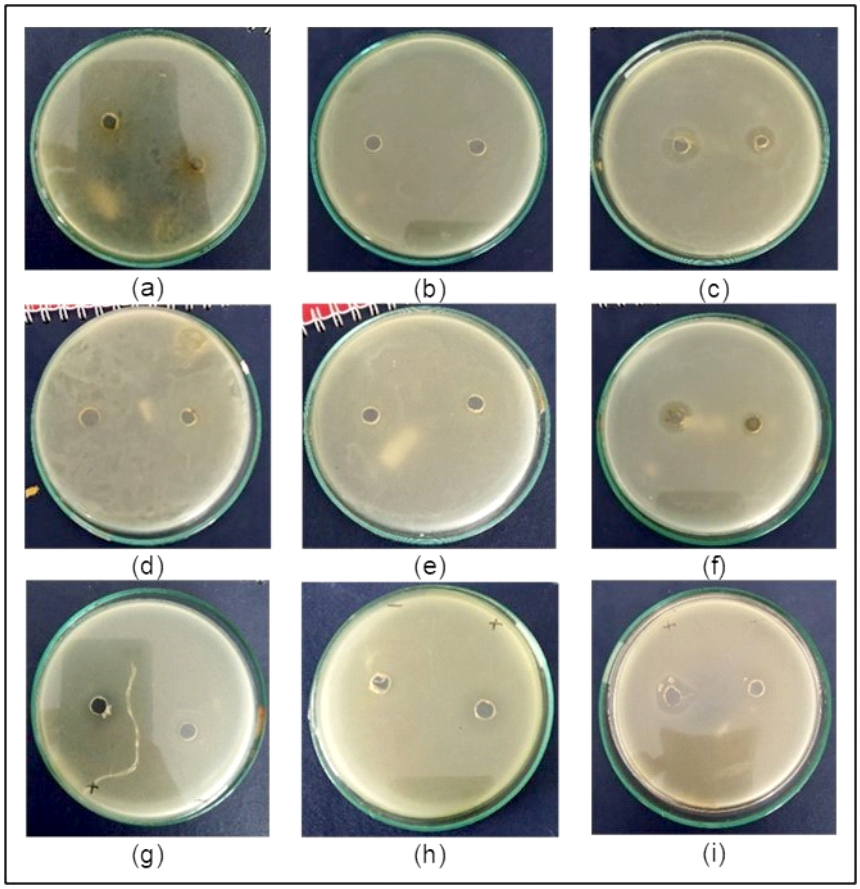

Figure 7. Zone formation of the solvents, extracts, and positive control against *E. coli* by well-diffusion method in methanol (a), ethanol (b), acetone (c), methanol with extract (d), ethanol with extract (e), acetone with extract (f), positive control in methanol (g), ethanol (h), and acetone (i).

The antibacterial inhibition zone of *E. coli* by the well-diffusion method showed similar results to the antibacterial inhibition zone by disc-diffusion methods in that the solvent had significantly affected the inhibition zone. Yet, the extraction methods had no effect on it. However, in Table 7, acetone extract shows the widest inhibition against *E. coli*. Alamsyah et al. (2014) showed that semi-polar solven like acetone provide hydrophilic and lipophilic properties. Therefore, optimum polarity and maximum antibacterial substances are obtained. The results were almost similar to those of Patra et al. (2011), who found that the acetone extract of *S. maritima* has a more effective antibacterial activity against *E. coli* than other solvents (methanol, ethanol, and water), but the resulting inhibition zone is different. Antibacterial activity is still relatively weak because the inhibition zone is <5 mm. However, our results showed that semi-polar compounds, such as alkaloids and steroids, had antibacterial activities. Harborne (1998) states alkaloids can be dissolved in semi-polar and polar solvents. Alkaloids and their synthetic derivatives are used as basic medicinal materials for analgesics, antispasmodics, and antibacterials (Okwu, 2001). Madjid et al. (2020) state that steroids and triterpenoids are non-polar compounds. It was in line with this study that based on phytochemical screening, acetone extract of *S. maritima* contained alkaloids and steroids (both in SM and UAE extracts) and play a role in antibacterial activities.

The inhibition zone and zone formation of *S. maritima* extracts against *S. aureus* by well-diffusion method can be seen in Table 6 and Figure 8, respectively. *S. maritima* extract did not show antibacterial activity against *S. aureus*, except for the ethanol extract of ultrasonication methods. It was almost similar to the finding of Patra et al. (2011) that *S. maritima* extracts (methanol, ethanol, acetone, and water) did not show antibacterial activity against *S. aureus*.

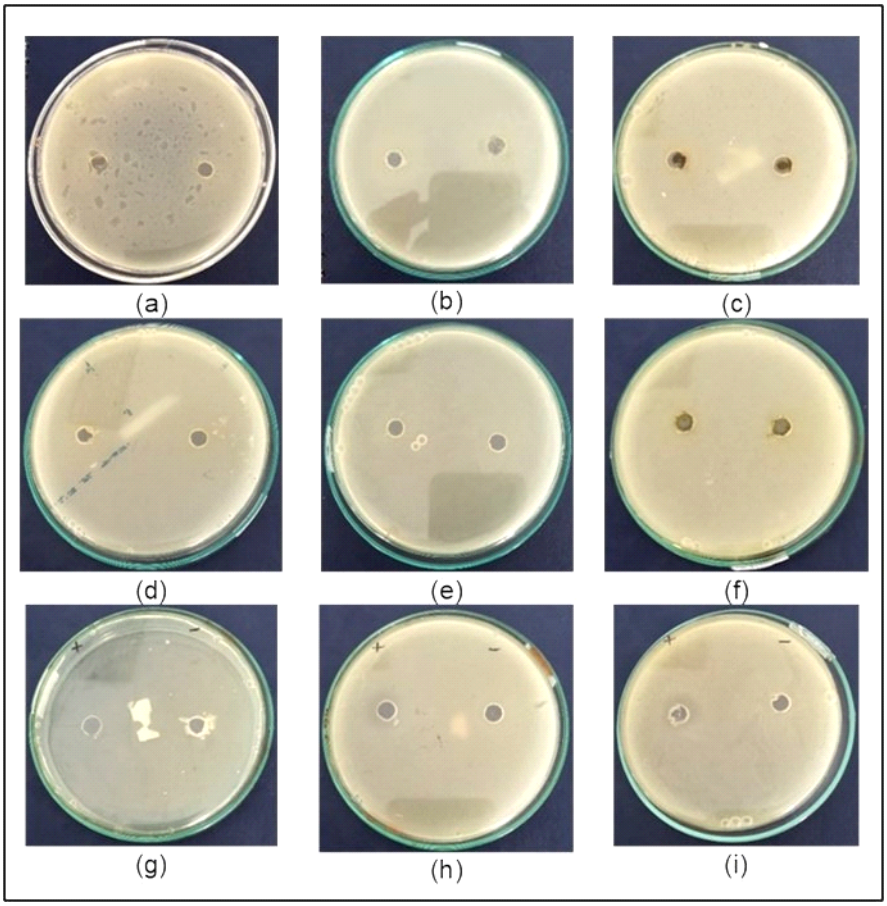

Figure 8. Zone formation of the solvents, extracts, and positive control against *S. aureus* by well-diffusion method in methanol (a), ethanol (b), acetone (c), methanol with extract (d), ethanol with extract (e), acetone with extract (f), positive control in methanol (g), ethanol (h), and acetone (i).

Conclusion

The phytochemical class of compounds identified in *S. maritima*, both in SM and UAE extract, were alkaloids, flavonoids, phenolics, steroids, tannins, and saponins. Ethanol extract of *S. maritima* had the highest total phenolic content and antioxidant activity from the SM or UAE method. It also was found that there was a high correlation between the total phenolic content and antioxidant activity in *S. maritima* (R^2=0.9242). Differences in extraction methods did not significantly affect the *E. coli* and *S. aureus* antibacterial inhibition zone produced by *S. maritima* extracts. However, the ultrasonic-assisted extraction method provided a wider inhibition zone against *S. aureus* by well-diffusion method in ethanol extract, while the others did not show antibacterial activity. The antibacterial activity of *S. maritima* ethanol extract against *E. coli* and *S. aureus* was categorized as weak since its clear zone was <5 mm.

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