



Antibacterial activity of different crude extracts of *Suaeda maritima* used traditionally for the treatment of hepatitis

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ABSTRACT

Traditionally, plants and their products are used as a folk medicine for the treatment of curable and incurable diseases for a long time in many areas of the world. However, there is a lack of systematic study of the antibacterial activity of *Suaeda maritima* (*S. maritima*). The present study is to estimate the antibacterial activity of various extracts of newly discovered species *S. maritima* plant which is collected from Oman. The methanol extract was prepared by the Soxhlet method and it was fractionation by different solvents to give different crude extracts. The antibacterial activity was assessed by using the agar disc diffusion method in which the extracts at different concentrations were applied to the disc by putting them in sterile filter paper of about 6 mm. The agar discs were incubated for 24 h and measure the inhibition zone of antibacterial activity. The range of the inhibition zone was between 7 and 12 mm. Overall all concentrations of each extract of *S. maritima* showed inhibition activity against the applied bacterial strains. The results found that the hexane extract at all concentrations was possessed the highest activity against the used two Gram-positive and two Gram-negative bacteria strains. In conclusion, the highest antibacterial activity hexane extract from the *S. maritima* could be used for the treatment of infectious diseases. In addition, the observed antibacterial activity of the selected plant could be due to the presence of high contents various group bioactive compounds.

1. Introduction

The issue of antimicrobial resistance to commonly used antibiotics has contributed to the need for the discovery of newer and alternative compounds for the remediation of drug-resistant diseases. As the earth's population rise, the need for usage of medicines worldwide increases gradually. Currently, pharmaceutical companies have prepared a variety of new antibiotics in the past three decades, however, an observed resistance has been noted against these medicines by microorganisms. Medicines have several sources in which they get their extractions, including plants, animals, and microorganisms (Bhat, 1995; Patil et al., 2019). Among these, plants have the capability, easy renewability compared to animals and other organisms. These plants which are medicines can also be used as flavonoid, food, perfumes or other certain spiritual activities (Pope, 1969). Insufficient drug supplies, excessively high cost of treatments, side effects of many synthetic drugs and increased resistance to many of recently used drugs for infectious diseases led to the elevated assurance on plant use of materials as a source of medicines for the sake of better human lifestyle (Amaral et al., 1998).

Antimicrobials from plant extracts have been used to treat infectious diseases for at least 2000 years. One of the natural known antimicrobials *Penicillin* discovered in 1928 was successfully used for the treatment of various microbial infections. The main function of an antimicrobial agent is to inhibit growth or kill the microorganisms. A microbial agent can be classified according to their functional abilities. Antimicrobials medicines are classified according to their action or act towards a specific microorganism. For example, an agent used against bacteria known as antibiotics, and antifungals used against fungi group (Laxminarayan et al., 2013). One of the major sources of antimicrobial components are plants, animals and marine extracts. Leaf, fruits, and vegetables can be used for the treatment therapies. Although most of the antimicrobials are known for being a pharmaceutical, wide range of non-pharmaceutical compounds as chemical and natural can be used as antimicrobials. Acids found in food products like lactic acid and its salts are a clear example of these types of antimicrobials. Recently, scientists put more effort in the discovery of more antimicrobial agents from plants and other natural sources. As the important compounds extracted from natural sources are highly used for the treatment of many of

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Fig. 1. Plant picture.

today's contagious diseases (Smith et al., 1998).

The *Suaeda maritima* (*S. maritima*) plant is an important medicinal plant found mainly in Oman. It belongs to the subfamily *Suaedoideae* in the family *Chenopodiaceae*. The *Suaeda* genus contains about 110 species (Schütze et al., 2003). Some common species are *Suaeda esteroa*, *Suaeda salina*, *Suaeda vera*, *Suaeda nigra* (Bassett and Crompton, 1978; Nayak et al., 2018). They have been mostly found in Golf countries such as Serbia, India and including Oman. Locally, it is called *Sueda* (Fig. 1). Most common names of this genus are seepweeds and sea-blites for their common existence in coastal salt-flats and tidal wetlands. It is a flowering plant mostly known to grow better in salty environments like coastal salt marshes and seashores. The selected plant species have the ability to tolerate high amounts of sodium chloride (salts) in its habitat and usually accumulating high levels of ions in its shoots (Hall and Flowers, 1976; Eswaraiah et al., 2019). The plant is a yellow-green shrub with fleshy, succulent leaves and green flowers, rarely seen to be grown annually. The flowers are bisexual having 5 petals and 5 stamens. It is in flower from July to October, and the seeds ripen from August to October. The leaves of this plant are mostly changing and simple in shape. The species is considered hermaphrodite (having both male and female organs) and is pollinated by wind. They are self-fertile, grow in length up to 0.3–1 m tall, and have green leaves having either green/red stems (Tanji, 1990). The plant has been found with different chemical constituents such as n-tetradecanyl dihydrocaffeate, n-nonanyl-n-octadec-9-enoate and n-hexadecanyl dihydrocaffeate (Muthazhagan et al., 2014). Tiny flowers can be seen also developing from inflorescences which grow out of the base of the leaves near the stem. Traditionally, the selected plant use in the treatment of hepatitis (Abbasi, 2018). It has been reported for its antimicrobial activity, antiviral, hepatoprotective and antioxidant activity (Patra et al., 2011; AL-Abbasi, 2018). Most of the studied on this plant were made in Pakistan, India, China and the United States of America (Harborne, 1998). Due to the medicinal importance, the selected plant is now cultivated commercially in different countries like the USA and Australia (Sage, 2016). In Oman, no previous history of studies has been found or reported regarding the new species of *S. maritima* species. Due to the medicinal value and traditional use which makes researchers in Oman more interested to work on the selected new plant species especially on antibacterial activity. Therefore, the main goal of this current study is to prepare different extracts from the newly discovered *S. maritima* plant species which has the highest activity, and to determine its antimicrobial effectiveness against several Gram (+ and -) bacteria strains.

2. Materials and methods

2.1. Chemicals

In this experiment, different solvents were used in the process of extraction such as butanol, methanol, ethyl acetate, hexane and DCM which were obtained from the Sigma Aldrich Company located in Germany. Other fundamental chemical reagents used in the experiment like DMSO were obtained from BDH, UK. The plastic Petti discs were

collected from the local market in Oman.

2.2. Bacterial strains

In this research, several bacteria strains were used as microorganism such as two Gram-positive: *Staphylococcus aureus* (*S. aureus*) and *Streptococcus pneumoniae* (*S. pneumoniae*) and two Gram-negative: *Escherichia coli* (*E. coli*) and *Klebsiella pneumoniae* (*K. pneumoniae*) bacteria strains. All cultured bacterial strains were obtained from the College of Arts and Sciences, University of Nizwa, Nizwa.

2.3. Sample collection

The *S. maritima* plant sample was collected from Sur, Ash Sharqiyah, Northeastern Oman. It was collected on Sunday, December 2nd 2018 at 5 p.m. The aerial parts of the selected plant were then to be carried and separated from the roots and held in a plastic bag to be moved to the Research Laboratory, University of Nizwa.

2.4. Sample processing

After the process of collecting samples was done the samples were being washed clearly with water to remove any waste, then dried at room temperature under a fan at normal temperature for a couple of days until they became fully dried. After being dried, the samples were ground into coarse powder by using a kitchen blender.

2.5. Preparation of crude extracts

Methanol (1500 ml) was used for the process of extraction from the dry coarse powder sample (274.74g) by using a Soxhlet extractor for 40 h. Then methanol containing extract was evaporated at 22 °C by rotary evaporator, the result was 2 g of the plant sample was separated from the methanol extract and kept in a vial. After that, the extract was dissolved in distilled water (200 ml) and well mixed. This mixture was then transported into a separatory funnel to proceed with fractionation method. In this method, several solvents like hexane, dichloromethane (DCM), ethyl acetate, butanol, and water were used for a volume of 30 ml each. The separatory funnel was continuously shaken for 20–30 min. The extract was then weighed and separated into the different tube and the mother solvent was evaporated by rotary evaporator under pressure at 24 °C (Afaf et al., 2014; Slusarenko et al., 1998).

2.6. Antibacterial activity

The antibacterial activity of the selected plant extracts was measured by using the slightly modified agar disc diffusion method against the selected available microorganisms at varying concentrations. Four concentrations (2, 1, 0.5 and 0.25 mg/ml) were used in this experiment (Huaag et al., 2011; Hile, 2004; Rehab and Hossain, 2016). As a standard, levofloxacin was used for positive control. From each extract (hexane, butanol, methanol, dichloromethane, ethyl acetate, and water) of 10 mg was taken in 10 ml test tube. Each extract, 5 ml of dimethyl sulfoxide (DMSO) was added. Different prepared concentrations were labeled by their concentrations. The serial dilution technique was followed to prepare the 2, 1, 0.5 and 0.25 mg/ml. The levofloxacin (1 mg) as control was prepared by using DMSO (3 ml). Filter papers 6 mm size was prepared by using a punch machine and placed in each concentration of each extract. The discs were prepared manually and various bacterial strains were applied using a cotton bird. Then, the filter paper 6 mm discs were soaked with different concentration of samples and applied to the agar disc. All the disc plates were incubated at a temperature of 37 °C for 24 h. The inhibition was recorded after 24 h incubation by calculating the diameter of the growth inhibition zones in (mm), and the antibacterial activities were determined.

Table 1
The various crude extracts of *S. maritima* plant.

Extract	Crude extracts (gm)
Hexane	4.37 ± 0.10
Ethyl acetate	5.65 ± 0.15
Dichloromethane	5.94 ± 0.19
Methanol	7.29 ± 0.62
Butanol	6.18 ± 0.45
Water	7.61 ± 0.98

3. Results and discussion

Recently, pharmaceutical companies have prepared a variety of new antibiotics in the past three decades, however, an observed that resistance has been noted against these medicines by microorganisms. Recently, continuous causes and factors of infections in the world keep growing. This has caused more cases of both morbidity and mortality for many people. However, science has not stopped from discovering new procedures and methods to stop these type of infections to spread. Plants which were used in the past for their traditional benefits are now put under consideration. Most of the therapies found in today's healthcare institutions involve plant extracts or their active constituent. Even in nowadays developing countries, basic health needs to be required in many cases the use of traditional methods. Uses like antimicrobial agents as an example, has brought a huge boost to the healthcare community. As such agents can be used to treat daily contagious diseases like viral and bacterial infections. Medicines have several sources in which they get their extractions, including plants, animals, and microorganisms (Bhat, 1995). Among these, plants have the capability, easy renewability compared to animals and other organisms. Those plants which are medicines can also be used as flavonoid, food, perfumes or other certain spiritual activities (Pope, 1969). Insufficient drug supplies, excessively high cost of treatments, side effects of many synthetic drugs and increased resistance to many of recently used drugs for infectious diseases led to the elevated assurance on plant use of materials as a source of medicines for the sake of better human lifestyle (Amaral et al., 1998). In the Sultanate of Oman, researchers are focusing more on the discovery and exploring of new medicines which are mainly extracted from plants or natural resources. A plant called *S. maritima* still remains an undiscoverable type of medicinal plant in Oman. Although it is seen in some regions of the country, some of its antimicrobial benefits worldwide, however, still remains hidden in the Omani communities.

Table 2
Antimicrobial activity of various crude extract *S. maritima* sample from Sur.

Bacteria strains	Extract Conc. (mg/ml)	Hexane (mm)	Dichloromethane (mm)	Ethyl acetate	Butanol	Methanol	Water
<i>E. coli</i>	2	11 ± 0.13	9 ± 0.08	8 ± 0.07	9 ± 0.10	11 ± 0.19	9 ± 0.10
	1	10 ± 0.96	8.15 ± 0.16	7.5 ± 0.11	8 ± 0.21	8 ± 0.10	9 ± 0.71
	0.5	10 ± 0.19	7 ± 0.34	7 ± 0.30	7.2 ± 0.64	7.5 ± 0.77	7 ± 0.34
	0.25	9 ± 0.43	6.5 ± 0.90	6 ± 0.47	nd	7 ± 0.53	6.8 ± 0.67
	Control	38 ± 0.12	19 ± 0.47	30 ± 0.55	30 ± 0.43	27 ± 0.68	24 ± 0.76
<i>K. pneumoniae</i>	2	10 ± 0.55	9 ± 0.32	8 ± 0.10	8.2 ± 0.10	10 ± 0.17	9 ± 0.10
	1	9.8 ± 0.91	7 ± 0.84	7.5 ± 0.18	7 ± 0.42	8.2 ± 0.15	8 ± 0.15
	0.5	9 ± 0.08	7 ± 0.10	7 ± 0.69	7 ± 0.19	7 ± 0.09	7.5 ± 0.59
	0.25	8.5 ± 0.81	6.75 ± 0.15	7 ± 0.23	7 ± 0.90	7 ± 0.65	6 ± 0.41
	Control	36 ± 0.10	32 ± 0.19	25 ± 0.12	22 ± 0.12	20 ± 0.29	27 ± 0.39
<i>S. aureus</i>	2	9.15 ± 0.11	7.5 ± 0.71	10 ± 0.87	11 ± 0.44	8.2 ± 0.44	6 ± 0.10
	1	9 ± 0.21	7 ± 0.58	9 ± 0.29	7 ± 0.27	7.8 ± 0.13	nd
	0.5	7 ± 0.31	nd	7.15 ± 0.13	7 ± 0.35	7 ± 0.69	nd
	0.25	7 ± 0.82	nd	nd	7 ± 0.16	7 ± 0.10	nd
	Control	40 ± 0.49	34 ± 0.14	33 ± 0.08	39 ± 0.11	20 ± 0.25	35
<i>S. pneumoniae</i>	2	10 ± 0.34	9.75 ± 0.19	nd	10 ± 0.08	8 ± 0.92	nd
	1	10 ± 0.44	9 ± 0.10	nd	8 ± 0.42	7 ± 0.78	nd
	0.5	9.5 ± 0.25	9 ± 0.57	nd	7 ± 0.13	7 ± 0.54	nd
	0.25	7 ± 0.10	7 ± 0.39	nd	nd	6.8 ± 0.10	nd
	Control	34 ± 0.09	39 ± 0.42	35 ± 0.10	25 ± 0.77	18 ± 0.59	27 ± 0.17

nd = not detectable.

Some of them are the uses of the selected plant to making of glass and soap (Patra et al., 2011). They have also been discovered to have some effect against hepatitis. According to the World Health Organization (WHO), 80% of people rely on medicinal plants/herbs in their primary health care needs (Lai and Joy, 2004). Previous studies on several species belong to this family's plant has been done in India and several characteristics were determined like the diameter of subshrub (1–1.5 mm) and seeds diameter (1.5–2 mm) (Nezhad et al., 2009). In conclusion, around 21,000 species of plants have the probability of being used as medicinal plants (Green et al., 2011). Therefore, the main goal of this current study is to prepare various extracts from the aerial parts of *S. maritima* which has the highest activity, and to determine its antimicrobial effectiveness against several Gram (+and -) bacteria strains.

3.1. Crude extract

The sample is collected from Sur, Asharqiyah, Oman and process the sample by standard protocols. The methanol extract was prepared by using a Soxhlet method. Then, it was dissolved in water and prepared hexane, dichloromethane (DCM), ethyl acetate, butanol and water by fractionation method. The yield of the prepared extract is presented in Table 1.

3.2. Antibacterial activity

Antimicrobial agents are used worldwide to treat different types of diseases nowadays. Many of antimicrobial agents are plants and other natural sources. In Oman, many rare plants are grown and known for their beneficial activity either medicinal, biological or industrial. Some need to be extracted and some don't. While on the other hand, plants which grow in rare regions or which is not famous for any use to the community might be actually beneficial. As the problem may be the lack of knowledge or insufficient sources and materials to conduct a proper investigation of these types of plants. One of these plants in Oman is *S. maritima* plant species, as studies revealed that there was no history of official research on the selected plant. This present study actually focuses on exploring this plant and to determine its antibacterial activity from its aerial parts. The antibacterial activity of the crude extract was determined by the agar gel diffusion method. In our experiment, four concentrations were used against two Gram-positive and two Gram-negative bacterial strains. After incubation the zone of inhibition was

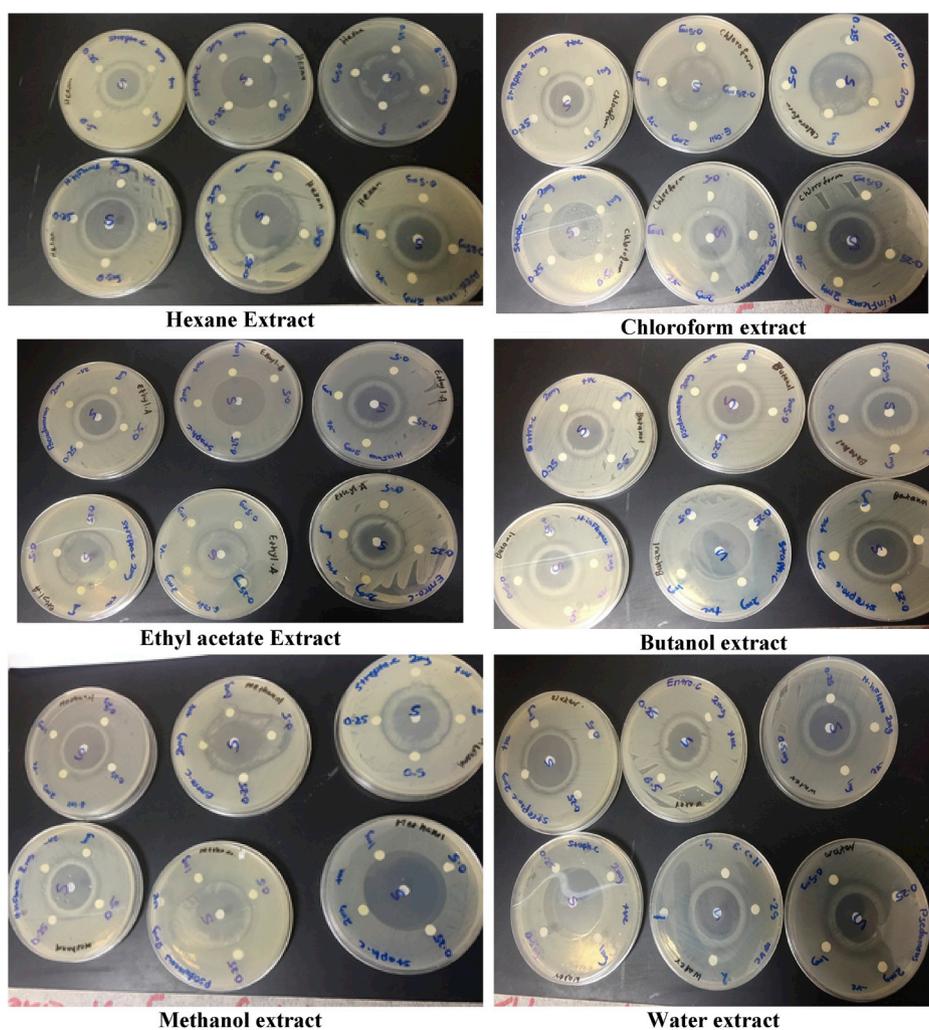


Fig. 2. Inhibition zone of each extract against Gram (+and -) bacterial strains.

measured manually is presented in Table 2. Two Gram-positive *S. aureus* and *S. pneumoniae* and 2 g-negative *E. coli* and *K. pneumoniae* bacterial strains were used (Dorman and Deans, 2000; Tirupathi et al., 2011; Kiran et al., 2009; Kuete, 2010). Among them, the hexane extract had the highest antibacterial property, and that was assessed from their results of inhibition zone which was done using the agar disc diffusion. The average zone of inhibition range was from 7 to 11 mm (Table 2 and Fig. 2). In our experiment, DCM extract gave the highest inhibition against *S. pneumoniae* at the concentration of 2 mg/ml. Most of the concentration of DCM extract gave moderate inhibition against the gram-positive and gram-negative bacterial strains. However, two concentrations such as 0.5 and 0.25 mg/ml did not give any activity against *S. aureus*. The ethyl acetate extract of the selected plant gave moderate inhibition against the applied gram-positive and gram-negative bacterial strains except at the concentration 0,25 mg/ml against *S. aureus*. However, all prepared concentrations of ethyl acetate extract did not give any inhibition against *S. pneumoniae*. All concentrations of butanol extract were given moderate inhibition except for *E. coli* and *S.*

pneumoniae at the concentration of 0.25 mg/ml. The methanol extract also showed moderate to high inhibition against all gram-positive and gram-negative bacterial strains. The water extract also showed positive inhibition against the applied bacterial strains except for *S. aureus*. The water extract at the concentrations of 0.25, 0.5, and 1 mg/ml did not show any activity against *S. aureus*. The same concentrations of water extract did not give any activity against *S. pneumoniae* (Table 2). The MIC of the crude extracts from the newly discovered plant species was determined by plotting square root diameters of the zones of inhibition against log concentrations. The highest MIC values were found in the hexane and DCM extracts and the lowest MIC was in the water extract (Table 3). The previous studies on antibacterial activity of this plant showed that our result is relatively low compared to the reported values (Dorman and Deans, 2000; Al-Abd et al., 2015; Delaporte et al., 2004; Manivannan et al., 2011; Scherrer and Gerhardt, 2018). In our experiment, antibacterial result is low because due to the procedure of extraction is different from them. In addition, in Gulf countries, the weather is hotter than India. Therefore, due to the weather condition,

Table 3
Minimum inhibition concentration (MIC) of various crude extracts of *S. maritima* species.

Bacteria strains	Hexane (µg/ml)	Dichloromethane (µg/ml)	Ethyl acetate (µg/ml)	Butanol (µg/ml)	Methanol (µg/ml)	Water (µg/ml)
<i>E. coli</i>	251.12	230.65	237.44	249.24	252.44	220.98
<i>K. pneumoniae</i>	229.45	218.45	222.13	198.90	226.16	218.76
<i>S. aureus</i>	201.89	121.41	232.98	231.56	188.32	–
<i>S. pneumoniae</i>	266.42	276.87	–	210.67	181.89	–

the chemical compounds in the prepared extract might be decomposed that is why our experimental result is low compared to other reports.

4. Conclusion

S. maritima is considered as a medicinal plant and traditionally used to treat hepatitis. The selected plant showed antibacterial activity against several pathogenic Gram-positive and Gram-negative bacteria strains. In conclusion, among the six extracts of the selected plant, the hexane extract displayed significant antibacterial activity against the applied bacteria strains. The results indicated that the *S. maritima* plant can be used as a medicine for the treatment of certain infectious diseases.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bcab.2019.101383>.

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