STUDIES ON ANTIBACTERIAL ACTIVITY OF THE PLANT *Salicornia herbacea L*

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ABSTRACT

Alcohol extracts of the plant *Salicornia herbacea L*. were prepared, and found to be very effective against both Gram positive and Gram negative bacteria.

Cytotoxicit againsty human red blood cells, were studied and found that alcohol extract is not toxic, has a toxicity of 2500mg/ml.

Biological characteristics were studied by the measurement of minimum inhibitory concentrations on isolates of standard and pathological, Gram positive and Gram negative bacteria.

Minimum lethal dose that kill 50% of population (LD50) of Alcohol extracts were measured and found to be 6000mg/kg.

Stability of Alcohol extracts at different pH and temperature ranges were measured also.

INTRODUCTION

The resistance of microbes to antibiotics currently in use is one of the exciting events of life facing the world today, particularly as a continuing and growing progressively given the disappearance of antibiotics lethal weapon in the world of medicine because of the resistance which becomes absolute by bacteria.

The solution to this problem by the human person is an active search for new sources for the production of antibiotics effective and strong and honest were a few

toxic. Plants are strong candidate for such a task. Where the plant Kingdom is a source of many chemicals mainly products of secondary metabolism produced from specialized cells (5).

Most important of these materials which are pharmaceuticals are alkaloids and steroids, as well as milk plant such as rubber; there are other important materials that could be different chromosomes that can be extracted and used in manufacturing of many foodstuffs and medicines (14).

Aquatic plants are very important organisms in the plant Kingdom for the plant biotechnology for this reason the aquatic plants entered to many important industries, including food industry and fodder for easy access to and dried and used.

Salicornia herbacea L. (chenopodiaceae) has been used as a seasoned vegetable by living in coastal areas. It has been demonstrated that this plant stimulate cytokine production, nitric oxide release, and to show anti-oxidative effect (10).

Many of the materials produced under special circumstances, these materials can eliminate germs and also affect the growth of other plants either positively or negatively. There are many important antibiotics of plant origin such as Phytoalexines produced from a combination of high plants, as well as an important antibiotic Pisatin, produced from the plant Phaseolin which produced from the plant *Phasealous vulgaris* (9). Allicin, which is a compound of strong influence on gram negative and gram positive microorganisms, extracted from the plant Garlic through organic compounds (6).

The current study aimed to isolate effective substance act as antibiotic from *Salicornia* plant

because the importance of this plant and its normal and high growth is coastal areas submerged tidal waters in Shatt al-Basra and Khor Al-Zubair extended to the coast of Kuwait(7).

This plant is an important part of natural food for fish and shrimp in the Shatt al-Basra canal, especially the fish *Liza subviridis*, locally called, green Albiah fish(1 and 13). (3) stated that the fish *Beriophthalmous waltoni*, (locally called Abu Clambo) at Khor Al-Zubair feed largely on seeds and gestures of the plant *Salicornia herbacea L.* (2) also studied the chemical composition and fatty acids for their seeds.

Description of the study area

Shatt al-Basra canal is located between longitude 05″ 16′ 30°, latitudes 05 ″41′ 47° - 49′47°.

Total length of the channel 37 meters from the point of his meeting on the karmet ali River in the north even approaching the Supreme Khor Zubair south.

The presentation of the canal bottom 60 meters and 120 meters from the top. Depths of the canal ranging (6-10) meters. Shatt al-Basra canal opened in 1983. There is a regulator on the channel at a position 22 meters from the point of his meeting on karmet ali River. This regulator was destroyed during the Atlantic war 1991.

The water and salt tide of 25% advance north through the systematic cover for aquatic plants and taken from the tidal flat islands and breeding them. These plants during the decline of water in the case where the islands are being salted water channel and reach lengths of salinity in the nearly 10%.

MATERIALS AND METHODS

Culture media circles

1- Nutrient brooms N.B. (Difco).
2- Nutrient Agar N.A. (Difco).
3- Muller Hinton Agar M.H.A. (Difco).

These cultures were attended as instructed by the company manufactured and installed at each of them.

Method of extraction

Alcoholic extract of the plant Salicornia herbacea were prepared by mixing 5 grams of the dried powdered plant with 25 ml of ethyl alcohol (70%), then we leave the mixture with stirring by the magnetic stirrer (IKA-combimag) for 24 hours at the room temperature.

The filtrate were separated from the precipitate by centrifuge (Hirayama) in the speed of 3000 circle per minute for about 15 minutes, then the filtrate were concentrated to 5ml by the rotary evaporator (Rota vapor-RE, Buchi), at the room temperature (38-40 C°), then we leave it to be dried on glass watch or betray dish (Vladimir, 1983).

Measurement of minimal inhibitory concentrations (MICs):

For the measurement of minimal inhibitory concentrations of alcoholic extract of salicornia, we use a plate agar diffusion method on the culture media Muller Hinton Agar M.H.A. (Difco), different concentrations were used, from50 to 2000mg/ml against eight isolates of standard and pathological, gram positive and gram negative bacteria (11).

a- Initial screening against gram positive and gram negative bacteria:

Activity of alcoholic extract of salicornia were studied against eight standard and pathological isolates, this were done by a preparation of different concentrations from alcoholic extract of salicornia, arranged between 50 to1000 mg/ml. Specialized filter papers were immersed in these concentrations until saturation, then these papers were placed on the surface of betray dishes containing M.H.A. cultured by standard gram positive and gram negative bacteria and incubated for 24 hours at 37 C°, then the radiuses of inhibition were measured.

b- Measurement of the cytotoxicity against human red blood cells :

Cytotoxicity of alcoholic extract of the plant Salicornia herbacea against human red blood cells, were studied.

Different concentrations from alcoholic extract of salicornia, arranged between 50 to2500mg/ml, were prepared, other similar concentrations from standard Ox tetracycline antibiotic dissolved in Dimethylsulfoxide (DMSO) solution, also prepared.

1ml of blood dissolved in 20 ml of normal saline, were used.100µm from each concentration were added to each 2ml of blood mixture, the type of resulted mixture was studied, if the blood mixture becomes clear, the concentration is toxic or cause hemolytic effect on red blood cells (8).

c- Measurement of LD50 :

Minimum lethal dose that kill 50% of population (LD50) of Alcohol extracts were measured by the use of laboratory mice, type Albino-mice, from the strain of (BALB/C), males only were used, divided into five groups, 10 mice each. Control

group, injected by distilled water subcutaneously.

The five groups were injected by different concentrations (low and high concentrations), arranged between 500-6000. LD50 were measured after observation of treated animals for

72 hours, the number of died animals were recorded.

Statistical analysis was done by the use of probit analysis according to (4).

RESULTS

Initial screening against gram positive and gram negative bacteria:

Radiuses of inhibition were measured for alcoholic extract of salicornia against eight standard and pathological isolates (table no.1), these isolates are:

1- *Staphylococcus aureus* NCTC 6571.

2- *Staphylococcus aureus* ATCC 29213.

3- *Staphylococcus aureus* (clinical isolate multiresistance isolate).

4- *Escherichia coli* NCTC 5933.

5- *Pseudomonas auroginosa* NCTC 6750.

6- *Bacillus subtilis* PCI 219.

7- *Klebsiella pneumonia* ATCC 10031.

8- *Streptococcus pneumonia* ATCC 6308.

Table no.1: effect of alcoholic extract of salicornia: measurement of radiuses of inhibition against eight standard and pathological isolates.

Isolates	Concentration of alcoholic extract (mg/ml)				
	50	100	250	500	1000
1	2	3	5	10	13
2	2	3.5	5	9	13
3	1.5	3.5	4	8	14
4	0	1.5	3	5	6.5
5	0	0	3.5	5	8
6	4	5	10	11.5	14
7	1	1.5	3	5	7
8	2	3.5	4	7	11

Measurement of minimal inhibitory concentrations (MICs):

Minimal inhibitory concentrations of alcoholic extract of salicornia were measured against eight standard and pathological isolates (table no.2).

Table no.2: minimal inhibitory concentrations of alcoholic extract of salicornia against eight standard and pathological isolates, measured by mg/ml.

Test organisms	MICs mg/ml
1	50
2	50
3	50
4	200
5	500
6	75
7	250
8	250

Cytotoxicity against human red blood cells:

Cytotoxicity against human red blood cells, were studied and found that alcohol extract, even in high concentrations has no toxic or hemolytic effect on red blood cells, we can consider it as non toxic substance(table no. 3).

Table no.3: Cytotoxicity of alcoholic extract of salicornia against human red blood cell.

Compound	Concentration (p.p.m.)	RBCs toxicity after 1hr
DMSO	-	NT
alcohol extract of salicornia	50	NT
	100	NT
	500	NT
	1000	NT
	2500	NT

Measurement of LD50 :

Results of the statistical analysis by the use of probit analysis shows that alcohol extracts

of salicornia has no toxicity on laboratory mice, even in a high concentrations about 6000 mg/kg, so it's a safe and not toxic.

Conclusions:

It made clear the following:

1- The study on the effect of alcoholic extract of salicornia against eight standard and pathological isolates were reached, we can conclude that this extract is highly effective against gram positive bacteria, and have less activity against gram negative bacteria.

2- It made clear from the study of LD50 that the alcoholic extract of salicornia has no toxicity even at higher concentrations, It qualifies for human use in the future.

3- There is no activity for alcoholic extract of salicornia.

Allegations:

Protect our right in the studies of antibacterial activity of the plant Salicornia herbacea L. on gram positive and gram negative bacteria.

دراسة حول النشاط البكتيري المضاد لنبات.*SALICORNIA HERBACEA L*

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الخلاصة

تم تحضير المستخلص الكحولي للنبات *SALICORNIA HERBACEA L*. ووجد بانه فعالا جدا تجاه الجراثيم إيجابية وسلبية لصبغة كرام.

درست الفعالية السمية الخلوية للمستخلص تجاه كريات الدم الحمراء للانسان، ووجد أن المستخلص الكحولي غير ساما ،ويكون ساما عند جرعة 2500ملغم/ مل.

تم دراسة الخصائص البيولوجية بواسطة قياس التراكيز المثبطة للحد الأدنى على العزلات المرضية والقياسية السلبية والايجابية لصبغة كرام.

تم تحديد الحد الأدنى من الجرعة النصف المميتة (LD50) 6000ملغم /كغم.

كذلك تم قياس درجة الثباتية للمستخلص الكحولي ضمن مديات مختلفة من الدالة الحامضية والحرارة.

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