

Extraction of glycoside compound from *Salicornia europae L.* and evaluation of its chemical properties and antibacterial activity

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Abstract:

Saponin glycoside compound isolated from *Salicornia europae L.* (Salicornioideae:Chenopodiaceae), that proved by using specific test (haemolysis test,foam test and libermann bruchard test).TLC was used to detect the purity of this compound which give one spot,also IR-spectrum,UV-spectrum,Melting point,solubility were also used to detect the chemical properties of this compound.The antibacterial activity of extracted glycoside was examined against two clinical isolates[*Escherichia coli* and *Staphylococcus aureus*] using disk diffusion method.Cork Poorer method was used to determine the minimum inhibitory concentration (MIC) for the extracted glycoside.The study showed that the MIC values of the compound was 35,35,55 V/V% ,against clinical isolates *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* respectively.

Key words: Glycoside, Saponin, *Salicornia europae L.*, Minimum inhibitory concentration, Infrared spectrum, UV spectrum

Introduction:

The Chenopodiaceae is a large family of mostly perennial herbs; most of them are adapted to saline soil and live in salt marshes or arid saline soil. It includes about 1300 species ranged from annual herbs to trees. Many of the species are somewhat weedy and occur near habitation (Kumar *et al.*, 2009).

The Salicornioideae are among the most salt-tolerant land plant and frequently occur in saline areas associated with coastlines, tidal floodways,

and salt lakes. The Salicornioideae sub family comprises approximately 15 genera and 80 species. (Radwan *et al.*, 2007).

Salicornia species contain many compound such as triterpenoid sapogenins (saponin glycosides), coumarins,phenolic compounds,alkaloids, flavonoids lipids,protein contents,amino acids, mucilage,gum and the seeds are rich in oil (Kumar *et al.*,2009, Rhee *et al.*,2009).

Saponins, glycosides were widely distributed in the plant kingdom, include several group of compounds characterized by their structure which

contain a steroidal or triterpenoid aglycone and one or more sugar chains, the presence of saponins has been reported in more than 100 families of plants, and in a few marine sources such as star fish and sea cucumber (Ozlem and Giuseppe, 2007). Many pharmacological activities have been reported to saponins such as antibacterial, antifungal, antiviral, hepatoprotective, anti-inflammatory and anti-ulcer effects (Soetan *et al.*, 2006).

Saponins have detergent or surfactant properties because they contain both water-soluble and fat-soluble components. They consist of a fat-soluble nucleus, having either a steroid or triterpenoid structure, with one or more side chains of water-soluble carbohydrates (Cheeke, 1998). The present study deals on the saponin of the *Salicornia europaea* L. as on antibacterial activity of saponin against *E.coli*, *S.aureus* and *P.aeruginosa* (Alexander *et al.*, 2009).

Materials and Methods:

Materials:

Salicornia europaea L. obtained from Khor al-zubair (Classified and cultured in marine science center by Dr. Kadijaa Kadeem), Different media [Nutrient agar (Difco), Nutrient broth (Difco) and Muller-Hinton agar (Difco)] were used in this study. Clinical isolate obtained from college of pharmacy.

Methods:

Extraction and isolation of glycoside:

1-Powdered plant (stems and leaves) was extracted with boiling alcohol (ethanol) for 30 min. to stop the enzymatic activity.

2-Distill water was added to dilute alcoholic extract then leave for 10 min.

3-Alcoholic extract was then treated with lead acetate solution to precipitate tannins, proteins, coloring matter and other non-glycosidal parts.

4-The precipitate was filtered. HCL (5%) was added to filtrate to precipitate excess lead as lead chloride and removed by filtration.

5-Solution was filtered; the filtrate was concentrated, purified by crystallization. (Ahmad, 2007)

Hot extraction (Decoction) for seeds and other part (stems, leaves):

In a large flask the ground material is extracted by water with heating to boiling (Heinrich *et al.*, 2004).

Identification of extracted glycoside:

To detect the purity of glycoside, (TLC) technique was used. R_f value was detected using three mobile phase (butanol: acetic acid: water 4:1:5), (butanol: acetic acid: water 4:1:2), (butanol: acetic acid: water 2:1:2). The spots were detected by iodine vapour.

Solubility:

The solubility of the extracted glycoside was tested in distilled water and alcohol (ethanol and methanol).

Melting point:

Melting point was measured using melting point | Smp3 | Stuart equipment.

Ultraviolet Spectrum:

Pye-Unicam SP 8-100 Spectrophotometer (in college of science) was used in the range (200-800) nm by using ethanol as solvent.

Infrared Spectrum:

Powder of extracted glycoside specimen was mixed with KBr salt, using a mortar and pestle, and compressed into a thin pellet. Infrared spectra were recorded as KBr pellets on a Shimadzu FTIR-8400 S Shimadzu-Japan 2002 (in college of science) between 4000–500 cm^{-1} .

Chemical tests:

Molish test, Benedict test (before and after hydrolysis), haemolysis test, foam test, Libermann-Burachard test (Ahmad, 2007) were used for qualitative analysis.

Antibacterial activity

Two clinical strains bacteria [*Escherichia coli*, *Staphylococcus aureus*] were used with compared with aqueous extraction of seeds and (stems,leaves) using disc diffusion method (Bauer *et al.*,1966). The inhibition zones were measured.

Determination of minimal inhibitory concentration (MIC):

Cork poorer method (Parekh and Turk,2007), was used to detect the (MIC) using different concentrations [95 , 85 , 75 , 65 , 55 , 45 , 35 , 25 , 15 , 10 and 5 V/V %] of extracted glycoside against three clinical bacterial strain [*Escherichia coli* , *Staphylococcus aureus* , *Pseudomonas aeruginosa*] .

Results:

The glycosidal compound extracted from *Salicornia europaea* L. appeared as white crystal, and it appeared as pure molecule with only one spot on TLC technique table (1).

Solubility:

The solubility of extracted glycoside appeared soluble in three solvent: water, ethanol and methanol.

Melting point:

The extracted glycoside decomposed at 180°.

Ultraviolet spectrum:

microbial concentration 10^6 CFU/ml and the antibacterial activity of extracted glycoside was

Figure (1) showed that the extracted glycoside has one peak, the highest absorption in wave length (316 nm).

Infrared spectrum:

Figure (2) showed the infrared spectrum of the extracted compound, the most important bands in this figure are, stretch band C=C in the position (1631.67cm^{-1}) and broad band between ($3020\text{-}3556\text{cm}^{-1}$) which covered -OH,-CH which found in the molecules as well as OH from the KBr disk.

Chemical tests:

Table (2) illustrate that it is positive for molish test, benedict test (before and after hydrolysis), haemolysis test, foam test, libermann burchard test.

Antibacterial activity:

Table (3) and Figure (3) showed the extracted glycoside and aqueous extract of seeds appeared active against *E. coli*.However only the extracted glycoside showed highest activity against *E. coli*, *S. aureus*.

The minimum inhibitory concentration (MIC):

Table (4) illustrated the MIC values; this result showed the highest activity of the glycoside was recorded against gram positive bacteria than gram negative bacteria.

Mobile phase	R _f
butanol:acetic acid:water 4 : 1 : 5	0.8
butanol:acetic acid:water 4 : 1 : 2	0.9
butanol:acetic acid:water 2 : 1 : 2	0.87

Table (2): Chemical tests for detection the presence of saponin glycoside

Chemical tests	Results
Molish test	(+)purple ring
Benedict test(before hydrolysis)	(+)orange precipitate
Benedict test after hydrolysis of extracted glycoside	(+)orange precipitate
Haemolysis test	(+)RBC's ruptured (examined under microscope)
Foam test	(+)foam persist for 2min.
Libermann Bruchard test	(+)violet ring

* + refer to Positive result

Table (3): Antibacterial activity of extracted glycoside and aqueous extract of seeds and (stems, leaves) measured in millimeter (mm)

Bacterial Isolates	Extracted glycoside Inhibition zone	aqueous extract (seeds) Inhibition zone	Aqueous extract (stems,leaves) Inhibition zone
<i>E. coli</i>	20	10	0
<i>S. aureus.</i>	22	0	0

Table (4): Minimal inhibitory concentration (MIC)

Bacterial isolates	MIC(V/V%)	Inhibition zone(IZ)mm
<i>S. aureus</i>	35	22
<i>E. coli</i>	35	20
<i>P. aeruginosa</i>	55	18

* mm refer to millimeter

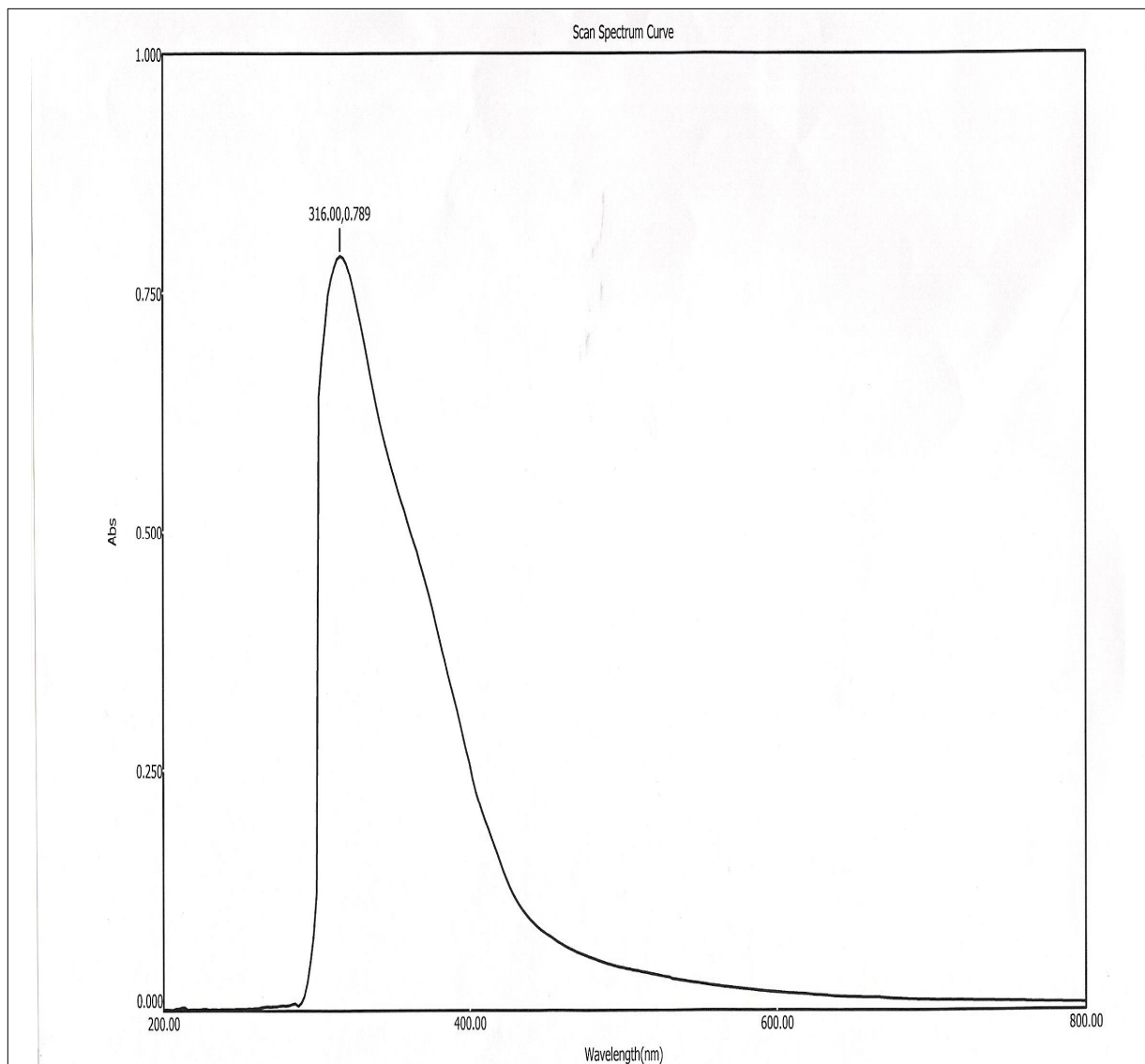


Figure (1): Ultraviolet spectrum for extracted glycoside

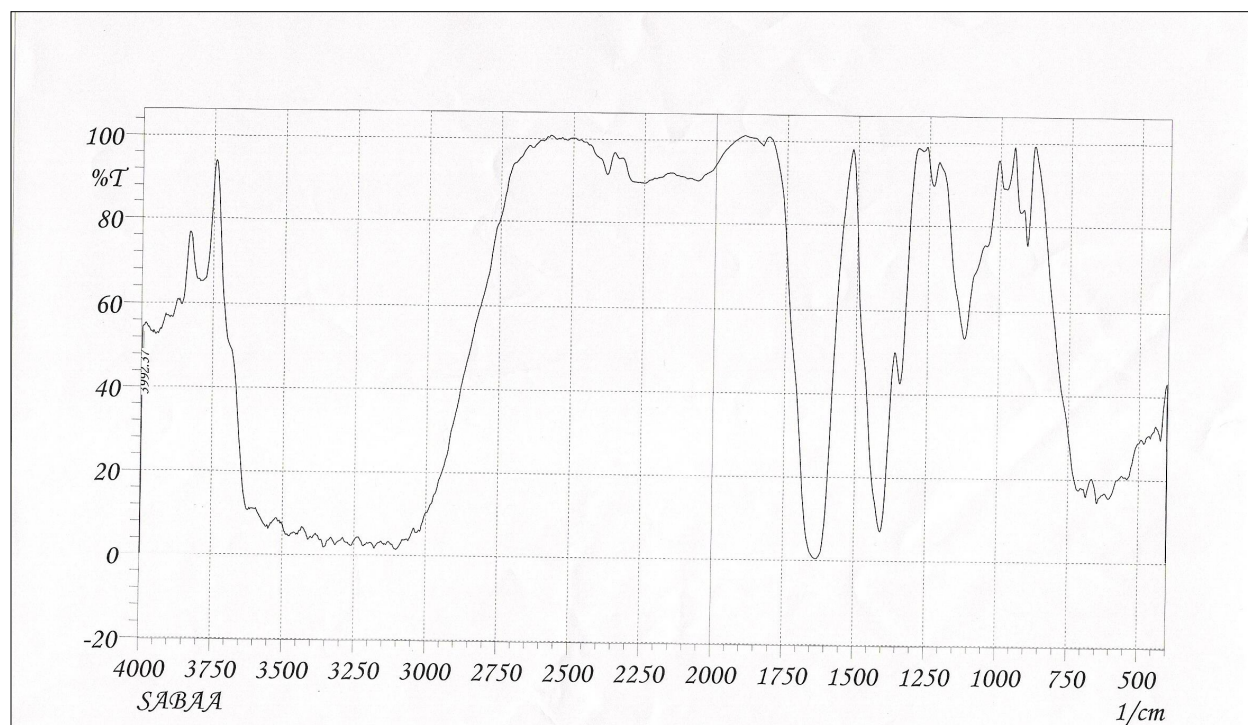


Figure (2): Infrared spectrum for extracted glycoside

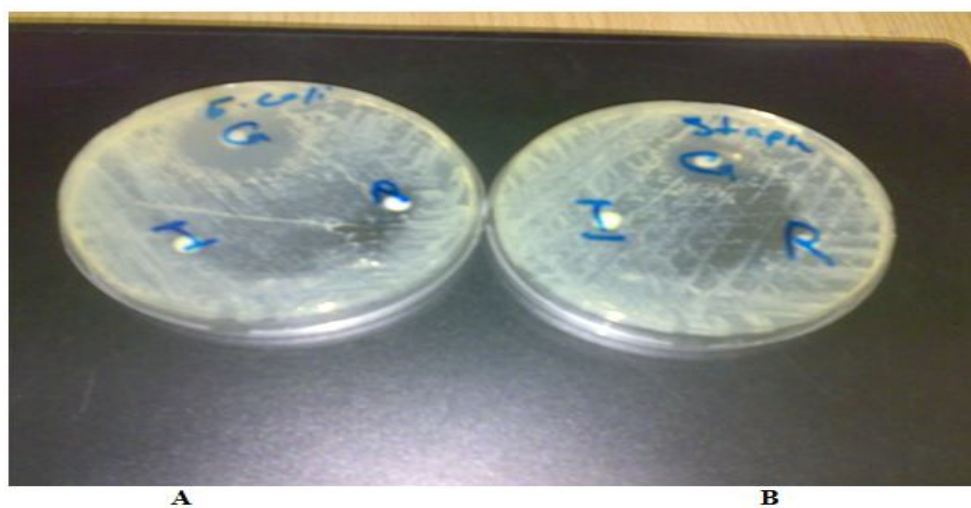


Figure (3): Antibacterial activity of extracted glycoside against two clinical isolates (A- *E. coli* B- *S. aureus*)

- * G refer to extracted glycoside
- * R refer to aqueous extract of root
- * I refer to aqueous extract of (stems&leaves)

Discussion:

The glycoside compounds differ in physical properties from chemical structure, because of that there's no one method to extracted glycoside , to extract saponin we use ethanol and water because water, alcohols (methanol, ethanol) and aqueous alcohols are the most common extraction solvents for saponins depending on readily soluble of saponin in this solvents (Ahmad ,2007; Deore *et al.* ,2009 and Sondnia,2005) .The purity of extracted glycoside was detected by TLC technique and appeared this compound pure with one spot(Table 1). TLC on normal and reversed phase is mostly used technique for separation and determination of large number of saponins. Silica gel is a preferred stationary phase while mobile phase consists of chloroform-methanol-water or butanol-acetic acid –water.

Solubility of saponins is also affected by the properties of the solvent and affected by temperature, composition, and pH .While water, methanol, ethanol and aqueous alcohols are the good solvents for saponins (Deore *et al.*, 2009).

The extracted glycoside melt with decompose at 180° , this result in agreement with many saponin glycoside melt with decompose such as saponin extracted from soy bean (Burrell and Walter, 1934).

The UV-spectrum (Figure 1) showed one peak in the wave length (316 nm) , this band resulted from electronical transmission type $\pi-\pi^*$ according to existing double bond in its structure from type $C=C$, the appearance of this band give us evidence the existing of unsaturated aromatic compound (Lambert *et al.*,1987). From infrared spectrum (Figure 2) illustrate important bands in extracted glycoside, which is stretch band $C=C$ in the position (1631.67cm^{-1}) and broad band between ($3020-3556\text{cm}^{-1}$) which covered $-OH,-CH$, The appearance of these bands agreement with the bands for saponin glycoside extracted from many plant such as *Nigella sativa*, *Terminalia Brownii* (Elbandy *et al.*, 2009 and Kareru *et al.*, 2008).

The results in this study showed that glycoside contain sugar part and that proved by molish test which is general test for all carbohydrate compounds, and as we know glycoside is carbohydrate compound because contain sugar part in its structure (Martin *et al.*,1981 and Claus *et al.*,1980), the type of this sugar part is reducing sugar and that proved by Benedict test before and after hydrolysis which is specific test for reducing sugar, glycosides compounds consist of glycon part and aglycon part. the glycon part (sugar part) consist of reducing sugar monosaccharide or disaccharide ,some glycosides contain more than one saccharide group, possibly as disaccharide or trisacchride.Upon proper conditions of hydrolysis,for example mineral acids and heating one or more of the saccharide groups can be removed from such compound, resulting in glycosides of simpler structure, because of that we use Benedict test before and after hydrolysis to prove that the extracted glycoside contain reducing sugar in its structure (Clause *et al.*,1980 and Dandekar,2004).

We used three diagnostic tests (haemolysis test, foam test and libermann bruchard test) for detection the type of extracted glycoside as saponin glycoside.

Saponin ruptured red blood cell because saponins are hemolytic, probably as a result of their interactions with steroids, especially cholesterol, the amount of cholesterol in the membrane has been shown to be important for this interaction. However, the absence of cholesterol in membranes does not inhibit pore formation by some saponins. The sugar side chains of saponins also have activity (Bachran *et al.*, 2006).

Due to the presence of a lipid-soluble aglycone and water-soluble sugar chain in their structure (amphiphilic nature), saponins are surface active compounds with detergent, wetting, emulsifying, and foaming properties.

As a consequence of their surface-active properties, saponins are excellent foaming agents, forming very stable foams. Yucca and Quillaja extracts are used in beverages, to provide the

foamy "head." Because of their surfactant properties, they are used industrially in mining and or separation, in preparation of emulsions for photographic films, and extensively in cosmetics, such as lipstick and shampoo. Quillaja bark has been used as a shampoo in Chile for hundreds of years, and Native Americans used yucca to make soap, the ability of forming foam test proved by foam test (Cheeke, 1998 and Deore *et al.*, 2009). Libermann bruchard test proved the presence of steroidal moiety which is aglycon part of saponin.

Saponins are glycosides containing one or more sugar chains (glycone part) on a triterpene or steroid aglycone skeleton hence classified into two groups steroidal and triterpenoidal saponins. Aglycone backbone of saponin is also called as a sapogenin. Their structural diversity is reflected in their physicochemical and biological properties, which are exploited in a number of traditional and industrial applications (Deore *et al.*, 2009).

Table(3),(4) and Figure(3) showed the antibacterial activity and the minimal inhibitory concentration of the extracted glycoside, in table(3), Figure(3) illustrate the highest antibacterial activity of extracted glycoside against clinical isolate comparative with the aqueous extraction of seeds and (stems, leaves), the main biological activity ascribed to saponin is their membrane permeabilizing property, the main actions are considered changes in membrane permeability and pore formation (Bachran *et al.*, 2006 and Noudeh *et al.*, 2010). The extracted glycoside also shows high antibacterial action. Table(3), Figure(3) against gram positive bacteria than Gram negative bacteria and that could be attributed to the fact that Gram negative bacteria are protected against most of the antibiotics, detergents and chemicals by their outer cell-wall, the outer layer of Gram negative bacteria cell-wall was made of lipopolysaccharide and protein, it covers a very few layers of as compared with gram positive bacteria in which the outer layer cell-wall was made of peptidoglycan only and does not contain lipoproteins (Parekh, 2007).

The lower activity of aqueous extract for (stems, leaves) and seeds because the aqueous extract is a mixture of many compounds not one pure compound beside decoction method used direct heating to boil and that may destroy some active compound like glycoside, nucleoside, proteins (Heinrich *et al.*, 2004). The activity of aqueous extract of seed against clinical isolate *E. coli* in comparative with *S. aureus* could be attributed to the presence of oils in it (Kumar *et al.*, 2009).

Table(4) showed MIC by using (V/V%) concentration, because saponin glycoside effected when drying and storage by moisture, light and heat. The complex structure of saponins may undergo chemical transformations during storage or processing which in turn may modify their properties / activity. The glycosidic bond (between the sugar chain and the aglycon), and the interglycosidic bonds between the sugar residues can undergo hydrolysis in the presence of acids/alkali, due to hydrotherolysis (heating in presence of water) or enzymatic/microbial activity resulting in the formation of aglycones, prosapogenins, sugar residues or monosaccharide depending on the hydrolysis method and conditions (Deore *et al.*, 2009), also the herb which contain saponins must storage airtight bags and away from heat and light (Madan *et al.*, 2009). The use of (V/V%) in MIC is related with the other material such as propolis which is resinous safer from photo-degradation because of that we use (V/V%) concentration (Fernandes *et al.*, 1995).

Acknowledgments:

I would like to thank Dr. Hatim A. Jasim (Department of Pharmaceutical chemistry, College of Pharmacy, University of Basrah, Iraq) and Dr. Bahjet A. Sayeed (Department of Chemistry, College of Education, University of Basrah, Iraq). For help me in the reading of infrared spectrum and ultraviolet spectrum.

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استخلاص مادة كلايكوسيدية من نبات الساليكورنيا *Salicornia europaea* L. وتقييم صفاته الكيميائية وفعاليتها ضد- بكتيرية

سبأ علي محمد الفضل

فرع الكيمياء الصيدلانية - كلية الصيدلة - جامعة البصرة

المستخلص:

تم عزل احد الكلايكوسيدات الصابونية من نبات *Salicornia europaea* L. وشخص المركب باستخدام الاختبارات: (Haemolysis test, foam test and libermann bruchard test) كما حددت نقاوة المركب باستخدام تقنية كروماتوغرافيا الطبقة الرقيقة، كذلك تم استخدام طيف الاشعة تحت الحمراء وطيف الشعاع فوق البنفسجية ودرجة الانصهار واختبار الذائبية لتشخيص الخصائص الكيميائية لهذا المركب. أختبرت الفعالية ضد-بكتيرية للكلايكوسيد المستخلص ضد العزلتين: *Escherichia coli* و *Staphylococcus aureus* كذلك استخدمت طريقة الحفر لتحديد التركيز المثبط الأدنى للكلايكوسيد المستخلص وظهرت النتائج أن التركيز المثبط الأدنى للبكتريا السريية المستخدمة كان (35,35,55 V/V%) *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* على التوالي.