ANTIBACTERIAL ACTIVITY OF THE SOLASODINE OF *SOLANUM NIGRUM* AGAINST BACTERIAL ISOLATES FROM THE WOUNDS

Mohammed Abdul-Imam Almazini* Hammed Gadoh Abbas **, Amani Abdul-Amer**

[1]Department of Biology,College of Science,University of Basrah,Basrah,Iraq.

[2]Alfeha'a Hospital-Basrah City, Ministry of Health,Iraq

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ABSTRACT

In this study , 80 clinical samples collected from patients including wound, blood, urine and cerebrospinal fluid and isolates of Gram-positive and Gram-negative bacteria were identified . The inhibitory effects of ethyl alcohol extracts and solasodine compound of *Solanum nigrum* were tested on clinical isolates of the wound included: *Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli* and *Pseudomonas aeruginosa.*

The ethyl alcohol extract showed inhibitory activity on all tested bacterial *Spp* . All the activities were compared with a tetracycline.The Minimum Inhibitory Concentration (MIC) of the Solasodine were 8, 6, 4, 8 mg/ml respectively for *S. aureus , S. epidermidis , E. coli* and *P. aeruginosa* respectively .The results lend scientific credence to justify the use of the Solasodine compound against bacterial isolates of the wounds and some bacterial diseases.

INTRODUCTION

Traditionally, plants are used as source of treatment of diseases in different parts of the world(1,2). Presently, the use of plant extract as alternative form of medical treatment is enjoying great popularity since the late 1990.Earlier in the 1990 approximately one- third of people, surveyed in the United State used at least one unconventional therapy during the

previous year (3). The acceptance of traditional medicine as an alternative form of health care and the development of microbial resistance to the available antibiotics has led researchers to investigate the antimicrobial activity of medicial plants (4).

Rong Nightshades (*Solanum spp*.) are common agricultural weeds throughout the world (5). Solassodine is a water insoluble, steroidal alkaloid used as a raw material for steroid drug manufacture (corticosteroids) (6).

Because of the great importance of solasodine in the pharmaceutical industry, there are many publications concerned with the search for solasodine glycosides in various plants (7,8) . There is increasing problem in microbial resistance and increasing demand for the development of new antimicrobial agents, especially in hospital settings.

The Solasodine has not been thoroughly evaluated for antibacterial activity and there is no previous study on hospital strains of pathogenic microbes, we decided to study the effects of Solasodine and ethyl alcohol extract of *Solanum nigrum* on the bacterial organisms which are known to cause wound infection.

MATERIAL AND METHODS

Collection of plant materials:

Plant of *Solanum nigrum* was collected from gardens in the Basra University. The plant was identified at Basra University, Herbarium-Biology Department. Leaves of this plant were dried at 25C^{O} and then grounded with a blender (Coffee grinder Model A979)and stored at approximately 4C^{O} until required for use.

Bacterial strains:

The species of bacterial *Spp* were *Staphylococcus aureus, Staphylococcu epidermidis, Escherichia coli* and *Pseudomonas aeruginosa*. They were clinical isolated from patients at Al-Fuha'a Hospital in Basra City. The cultures of bacteria were maintained on nutrient agar slants at 4C^{O}, re- identified by biochemical tests (9,10) and subcultured on to nutrient broth for 24h prior to testing.

Ethyl alcohol extract of leaves :

Ten gram of dried ground leaves of *Solanum nigrum* were continuously extracted by soxhlet using 200ml of ethyl alcohol 95% solvent (polar solvent) for 24 hours at $40C^{O}$, and the extract dried by rotary evaporator at approximately $50C^{O}$, then kept at $-20C^{O}$ until time of use (11).

Extraction and purification of Solasodine:

The method for the extraction and purification of Solasodine was based on that of ,(12) as follows : 1 gram portions of the dried leaves of the plant, plant powder were gently shaken for 30 mine in 50 ml of 2% aq. Oxalic acid in an magnetic stirrer apparatus. Suspensions were vacuum filtered through (whatman no. 1 filter paper) with only the first 10 -25 ml of clear extract being collected. Tubes were gently heated to $75C^{O}$, 1ml of 60% NaOH was added, and the tubes were kept at room temperature overnight. Tubes were centrifuged at $20C^{O}$ for 10 min. at 3000rpm . The supernatants were decanted and the pellets dissolved in 5 ml of 0.5 M HCL and hydrolysed by refluxing at $100C^{O}$ for 90 min. samples were coaled briefly and made alkaline NAOH with 1ml 60 % .

Heating to $100C^{O}$ was resumed for 10min. to complete the formation of the Solasodine insoluble.

The extracted compound was crystallized by 80% methanol, dried at room temperature and obtained as the dried green-white powdered with weight 10.26 gram for each 50g from the extracted leaves (13). In addition, physiochemical of this compound compared with standard Solasodine were recorded in the Thesis of (14).

Antibacterial activity:

The antibacterial activity was done by utilizing the hole –in- plate bioassay procedure (15).Pure culture of the organisms were inoculated onto Muller-Hinton broth(MH) (Oxoid , England), incubated for 24h at $37^{o}C$, diluted with sterile nutrient broth to a density of 9×10^{8} cfu/ml equivalent to 3 MC – Farland standard . The suspension was used to streak of the surface of (MH) agar plates with sterile swab. Using a sterile cork- borer of 6mm diameter, four holes were made in to the set agar in Petri-dishes containing the bacterial culture. Concentration of 5-120 mg/ml of the extracts were poured in to the wells. Standard antibiotic (tetracycline 10 mg/ml) was used as reference or positive control. The plates then incubated

at 37^{o}C for 18-24 h . Antibacterial activity was recorded if the zone of inhibition was greater than 9mm. The significance of the difference of the antibacterial activity of the extracts were tested by one way analysis of variance (ANOVA) (16).

Determination of minimum inhibitory concentration (MIC):

The Solasodine compound showed significant activity ($p< 0.05$) was chosen to assay for MIC. MIC was determined by the standard method of (17). Nutrient broth was prepared and sterilized using autoclave. One ml of the prepared broth was dispensed in to the test tubes numbered 2-12 using sterile pipette. A stock solution containing 0.8g of the Solasodine in 10 ml of distilled water was prepared. Then 1ml of the solution was dispensed into each of the tubes numbered 1 and 2. subsequently, from tube 2, serial dilution was carried out and 1 ml from tube 2 was transferred up to tube number 10 and 1 ml from the tube 10 was discarded. Tube 11 was control for sterility of the medium and tube 12 for viability of the organisms. An overnight culture (inoculums) of each of the test isolates was prepared in sterile nutrient broth 1:100 (10^2 dilution of the broth). One ml of the dilution was transferred into each tube from tube 2 to tube 12 with exception of tube 11, to which another sterile nutrient broth was added. The final concentration of the solasodine in each of the test tubes numbered 1-10 after dilution were 80,000; 40,000; 20,000; 10,000; 5,000; 2,500; 1,250; 625; 312; 5 and 156.25 µg/ml , respectively. Tetracycline was used as control .

These tubes incubated at 37^{o}C for 24-48h and were examined . The last tube in which growth failed to occur was the MIC tube.

RESULTS AND DISCUSSION

The pure cultures of Gram-positive and Gram-negative bacteria were obtained from the patients of samples(80 samples) . These samples were identified by different biochemical tests (Table 1).

Table(1): The biochemical tests for bacterial isolates

Isolates	Number of isolates	Gram stain	IMVIC test				Oxidase	Urease	Coagulase test	Mannitol Fermentation test	Catalase test
			Production	Methyl Red	Voges proskauer	Utilizationo					
Staphylococcus aureus	80	+	ND	ND	ND	ND	ND	ND	+	+	+
Staphylococcus epidermidis	53	+	ND	ND	ND	ND	ND	ND	-	-	+
Escherichia coli	44	-	+	+	-	-	-	-	ND	ND	ND
Pseudomonas aeruginosa	75	-	-	-	-	+	+	-	ND	ND	ND

ND= Not Detected

Table (2) explain the types and numbers of bacterial isolates (80samples) . We noticed that *S. aureus, S. epidermidis, E. coli* and *P. aeruginosa* were predominant in the wounds samples .

Table (2): The types and number of clinical samples and microorganisms used for microbiological assessment(n= 80 samles)

Type of Sample	*S. aureus*		*S. epidermidis*		*E. coli*		*P. aeruginosa*	
	No.	%	No.	%	No.	%	No.	%
Wound	**44**	**55**	**25**	**31.2**	**15**	**18.7**	**48**	**60**
Blood	**10**	**12.5**	**9**	**11.2**	**3**	**3.7**	**13**	**16.2**
Urine	**4**	**5**	**1**	**1.2**	**8**	**10**	**6**	**7.5**

Ear	**2**	**2.5**	**6**	**7.5**	**2**	**2.5**	**4**	**5**
Eye	**5**	**6.2**	**8**	**10**	**0**	**0**	**4**	**5**
Feces	**13**	**16.2**	**2**	**2.5**	**10**	**12.5**	**0**	**0**
Vagina	**1**	**1.2**	**2**	**2.5**	**6**	**7.5**	**0**	**0**
CSF	**1**	**1.2**	**0**	**0**	**0**	**0**	**0**	**0**

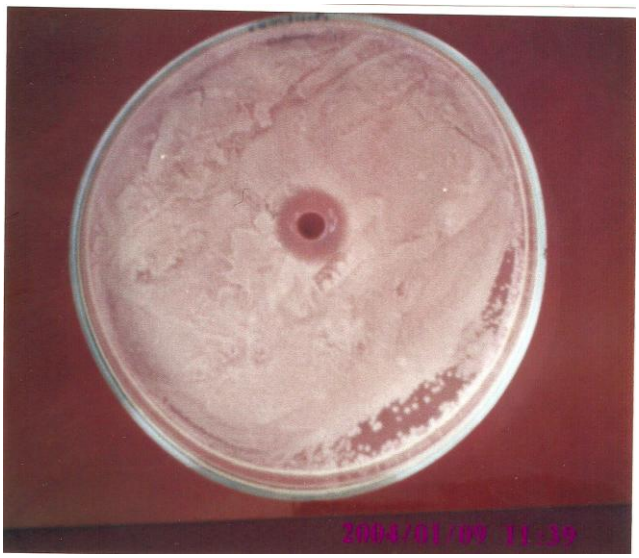
The results of the ***in vitro*** assays of antibacterial activity of the obtained ethyl alcohol extract and the Solasodine are shown in Table (3). The effect of ethyl alcohol extract of the leaves of *Solanum nigrum* possess significant ($p<0.05$) inhibitory activities against most the tested bacterial isolates (Table 3 and Figure 1) but the Solasodine possess higher inhibitory activities compared with ethyl alcohol extracts (Table 4 and Figure 2).

Table (3): Antibacterial activity of ethyl alcohol extract of *Solanum nigrum*

Material	**Extract concentration (mg/ml)**	**Zone of inhibition(mm)**			
		S. aureus	***S. edidermidis***	***E.coli***	***P. aeruginosa***
Ethyl alcohol extract	**10**	**-**	**-**	**-**	**-**
	30	**13.20±2.30**	**14.42±0.58**	**13.20±0.22***	**12.91±0.22**
	90	**27.10±20***	**27.30±0.06***	**28.40±0.62***	**28.50±1.00***
	120	**34.00±0.75***	**35.10±1.23***	**33.00±1.40***	**35.00±0.68***
Tetracyclin (positive control	**10**	**20.66±0.55***	**22.60±0.15***	**26.00±0.55***	**-**
Ethanol (negative control)		**-**	**-**	**-**	**-**

*= Significantly different from the control($p<0.05$) by using analysis values are mean± standard error of the mean. - = No activity.

Ethyl alcohol extract (30 mg/ml) against *S. aureus* Figure (1)



Ethyl alcohol extract (30 mg/ml) against *S. epidermidis* figure(2)

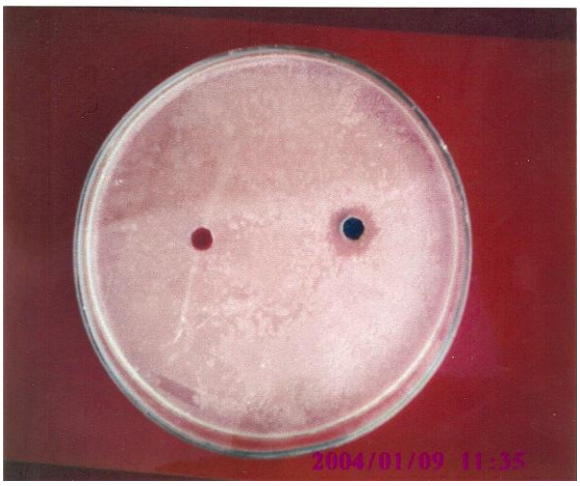
Figure(1) : The antibacterial activity of ethyl alcohol extract of *S .nigrum*

Table (4): Antibacterial activity of Solasodine of *Solanum nigrum*

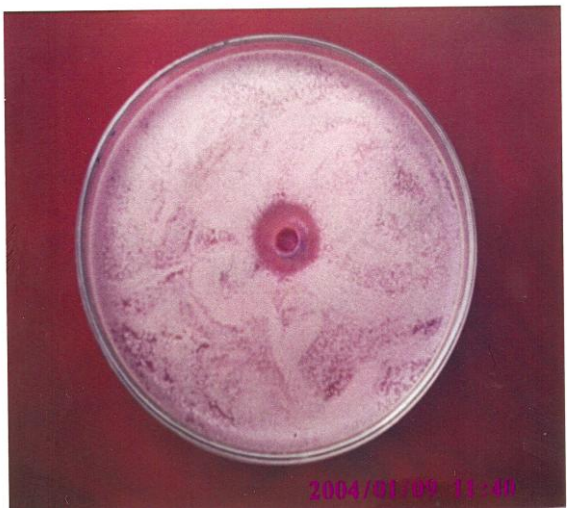
Material	Extraction concentration (mg/ml)	Zone of inhibition(mm)			
		S. aureus	*S. epidermidis*	*E. coli*	*P.aeruginosa*
Solasodine	10	8.71±0.60	13.60±1.22*	14.25±0.87*	10.00±2.60
	30	16.60±0.40*	17.40±0.33	19.64±1.20*	14.60±0.30*
	90	34.20±1.64*	35.00±0.22*	36.00±0.41*	36.66±1.77*
	120	42.07±0.82	44.00±1.11*	42.00±0.75*	45.73±0.33*
Tetracycline (positive control)	10	19.33±0.41*	2.00±0.25	25.10±0.50*	-
Ethanol (negative control)		-	-	-	-

* = Significantly different from the control($p<0.05$) by using analysis of variance.

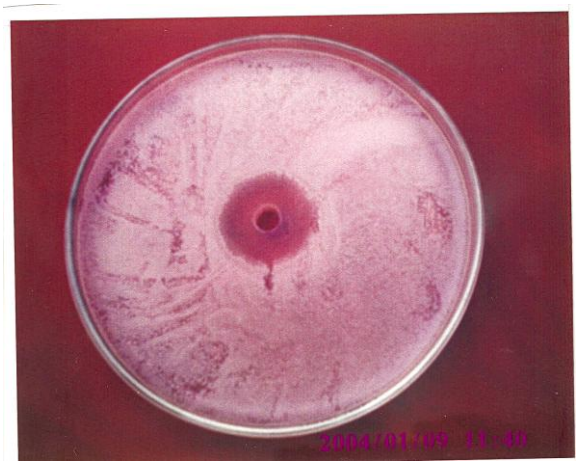
- = No activity. -
- Values are mean ± standard error of the mean.



Solasodine (30 mg/ml) against *S. aureus*



Solasodine (30 mg/ml) against *S. epidermidis* figure(4)



Solasodine (30 mg/ml) against *E. coli* figure (5)

Figure(2) :The antibacterial activity of the Solasodine of *Solanum nigrum*

The MIC of ethyl alcohol extract and Solasodine ranged from 14-20 and 4-8 mg/ml, respectively (Table 5). The Solasodine has the lowest MIC compared to ethyl alcohol extract and the control tetracycline.

Table (5): MIC values of alcohol extract and Solasodine on tested bacteria mg/ml.

Bacteria	Ethyl alcohol extract-concentration (mg/ml)	Solasodine-conc.(mg/ml)
S. aureus	**20**	**8**
S. epidermidis	**16**	**6**
E. coli	**16**	**4**
P. aeruginosa	**14**	**8**

The antimicrobial properties of this plant probably explain its traditional use for treating bacterial diseases. (18) indicate that the biological activity of many of the *Solanum* steroidal alkaloids may exhibit different pharmacological properties and the phytochemical screening results have shown the prescence of Alkaloids, phenols, Tannis and flavonoids, these classes of compounds have been reported with antimicrobial activity (14). In addition, the great importance of Solasodine in the pharmaceutical industry, there are many publications concerned with the search for Solasodine glycosides in various plants(7, 19).Therefore, these compounds (especially Solasodine)may be responsible for the antibacterial activity. The mechanism of action of this constituent may exhibit their action through inhibition of nucleic acid, protein and membrane phospholipids biosynthesis(20).

الفعالية التضادية للسولاسودين المعزول من نبات *Solanum nigrum* ضد العزلات الجرثومية المعزولة من الجروح

محمد عبد الامام المازني*، حامد جدوع عباس ،اماني عبد الامير**

قسم علوم الحياة ،كلية العلوم ،جامعة البصرة،البصرة ،العراق.

مستشفى الفيحاء،البصرة،وزارة الصحة،العراق.

الخلاصة

جمعت 80 عينة سريرية من الجروح، الدم، الادرار وسائل النخاع الشوكي ثم شخصت العزلات الجرثومية السريرية الموجبة والسالبة لصبغة كرام كما أختبرت الفعالية التثبيطية أو التأثير المثبط لمستخلصات الكحول الاثيلي ومركب السولاسودين المعزولة من نبات *Solanum nigrum* على العزلات السريرية المعزولة من الجروح والمتمثلة بـ *Staphylococcus aureus* و *Staphylococcus epidermidis* و*Escherichia coli* و *Pseudomonas aeruginosa* .

أظهرت مستخلصات الكحول الاثيلي تأثيرا تثبيطيا بينما السولاسودين أظهر تأثير تثبيطيا أعلى وعلى جميع الجراثيم المدروسة. جميع الفعاليات التثبيطية قورنت مع المضاد الحيوي القياسي التتراسايكلين. كان التركيز المثبط الادنى (MIC) للسولاسودين بتركيز 8 ، 6 ،4 ، 8 ملغم/مل في الجراثيم *S. aureus* و *S. epidermidis* و *E. coli* و *P. aeruginosa* على التوالي . هذه النتائج تشجع استعمال السولاسودين في المستقبل كمضاد لجراثيم الجروح وبعض الامراض الجرثومية.

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