

# Study the Effect of the Alkaloid Compound Isolated from Leaves of Solanum Nigrum L. on Some Bacterial Isolates

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**Summary:** Alkaloid compound was isolated from Solanum nigrum L. leaf as crystal white greenish powder at (38°C). The chemical and physical properties were studied by using thin layer chromatography (TLC), IR-spectrum, Ultraviolet-visible spectrum, melting point (mp). The isolated alkaloid compound is shown high antibacterial activity against types of standard strains of bacteria (Staphylococcus aureus NCTC 6571 Staphylococcus aureus clinical strain, and Escherichia coli NCTC 5933).

**Keywords:** Solanum nigrum L. leaves, TLC, Alkaloid compound, antibacterial activity

## Introduction

Solanaceae also called nightshades is a cosmopolitan family. Members of this family are used as food (e.g. Solanum tuberosum L. (potato), Lycopersicon esculentum L. (tomato), Solanum melongena L. (eggplant)) and drugs (e.g. Solanum nigrum L. and Atropa belladonna). However, the species are distributed throughout the world. This family is an important source of almost 300 alkaloids [2]. Alkaloids based on the steroid nucleus tolerance to many bacterial and fungal diseases has been reported. When applied to surfaces of plants they inhibit the growth of bacteria and fungi. [3] Ethnomedicinal values of other Solanum species have been reported. Leaf of Solanum torvum

### Plant collection and authentication

The leaves of *S. nigrum* were collected in March, 2010 within the gardens of Basrah University and

**Plant preparation and extraction.** Estimation of crude alkaloid in dry leaves: 100 mg of finely powdered material and 40 ml of 95% ethanol are refluxed in a 100ml flask for 30 min. The extract then filtered, and then the residue is washed twice with 5 ml of ethanol. The washings are added to the original filtrate and transferred into a 50 ml standard flask, then added ethanol 95% adjusted to the mark. 5 ml of this solution is pipetted into a test tube and ethanol completely removed by evaporation on a water bath. Then we treated the residue with 3 ml of 1N NaOH, and then

is used for the treatment of wound infections, coughs, sore throat while [5]. Alkaloids based on the steroid nucleus are not very widely distributed being restricted to plants, Solanum and Veratrum species [2]. Parts of this genus are generally known to contain the alkaloids, solanine, or solasodine, or both [5]. Ethnomedicinal values of other Solanum species have been reported. Leaf of Solanum torvum is used for the treatment of wound infections, coughs, sore throat while Solanum elaeagnifolium is reported to have diuretic, purgative properties and active in the treatment of venereal diseases and leprosy [5].

### Materials and Methods

Authenticated by Prof. Abdulla H. Al-Beaty, Department of Biology.

We added acetic acid and the contents transferred to a 25 ml standard flask, the volume being to the mark of 10 ml with water. One ml of this solution is equivalent to 1 mg of dry material [7]. Then we finely recrystallized the dry powder by methanol 80% and stay to dry in room temperature.

### Identification of alkaloid

**I- Preliminary qualitative test:** The chemical family of the isolated compound was implemented using several tests such as a- Dragendorff test [7]

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b- Wagner Reagent[10]

c-Mayer's test [10].

2-Thin layer chromatography (TLC)

To determine the purity and relative to front ( $R_f$ ) of isolated compound, a thin layer chromatography was carried out for (45min) on glass plates (2x9cm) in a saturated chamber of the mixture of (chloroform : methanol) (0.5 : 9.5). The glass plate was dried and the spot which appeared was developed with UV-lamp at (336-200nm), iodine vapor

3- The determination of melting point : Melting point electro-thermal is used for the determination of melting point of the isolated compounds.

4 -Spectroscopy:

A-Inferred spectrum FT-IR spectrum of the isolated compound was recorded with (FT-IR 8400S SHIMADZU- Japan) in the college of Science Chemistry Department, University of Basrah.

B- Ultraviolet and visible spectra : ultraviolet and visible spectrum of the isolated compound was carried out in the College of Science. Department of Chemistry, by using ethanol as and the spectrum recorded with the Spectroscan 80D. UV-vis spectrophotometer UK .

5- Antibacterial activity

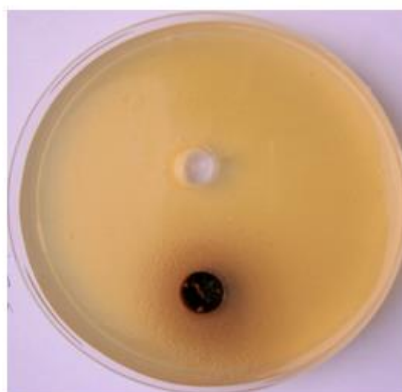
Agar diffusion method [5] was used to determine the antibacterial activity of the isolated compound (30.000 $\mu$ g/ml) against types of reference strains of gram positive and gram negative bacteria (Staphylococcus aureus NCTC 5671) and (Escherichia coli NCTC 5933), Staphylococcus aureus clinical strain which are tested using plate of Muller-Hinton agar. The antibacterial activity was defined as the clear zone of growth inhibition [6],[1].

6-The preparation of alkaloid compound:

500mg/ml (0.5mg of alkaloid compound soluble into 1ml DMSO) as stock solution.



*Staphylococcus aureus*  
NCTC 6571



*Staphylococcus aureus\**



*Escherichia coli* NCTC 5933

Fig (1) Antimicrobial activity of Alkaloid compound Solanum nigrum L. (500 $\mu$ g) against two standard species of Bacteria with \*clinical strain Staphylococcus aureus

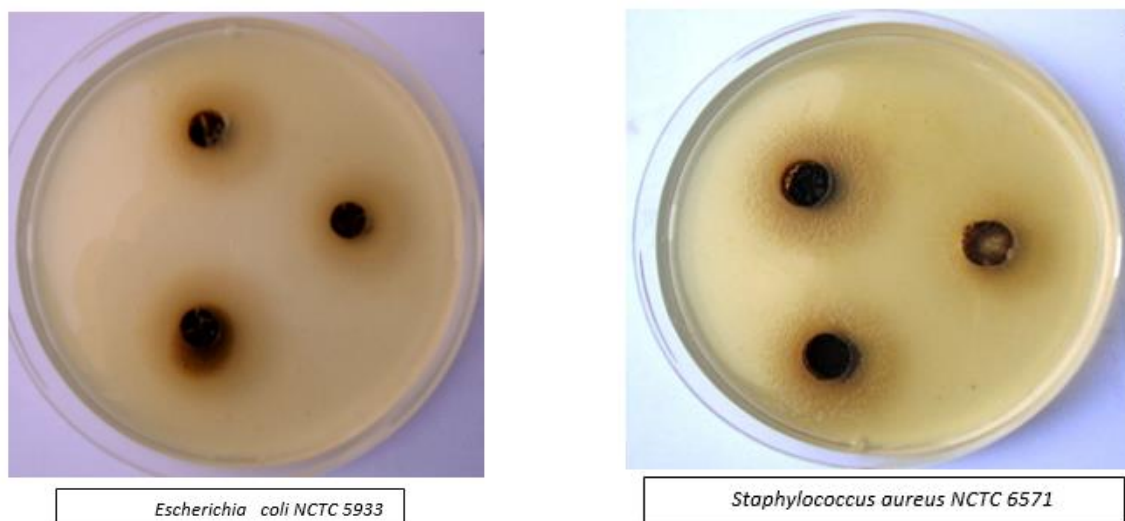


Fig (2) Antimicrobial activity of Alkaloid compound Solanum nigrum against tow species of Fayeza oaid neamahBacteria in different con(500, 250, 125 mg/ ml)

**Results and Discussion**

The results of preliminary qualitative test shown in table (1), where the appearance of isolated compound from alkaloid family . The early search appeared the leaves of Solanum nigrum L. contains a number of active constituents like alkaloid [5].Thin layer chromatography study for isolated compound give only one spot using chloroform :methanol(19.5 :0.5) as a solvent system and we using some reagent as developer for this spot. shown in table(2)and Figure(3), relative of front (R<sub>f</sub>) equal (0.74), it is organic compound have conjugated double bonds .Melting point(m.p), also tested and it was found that the isolated compound has sharp (281-285 °C) which means the isolated compound is pure. The ultraviolet – visible spectrum, figure(2) has shown one peak at max equal to (296nm) due to presence the pairs of electrons (nonbonding type n- The FT-IR spectrum for the isolated compound is shown in figure (1) and table (3), the appearance of a single peak at (3477 cm<sup>-1</sup>) related to the vibration stretching for (-OH ) bond indicated the presence of hydroxide . The band at (3045 cm<sup>-1</sup>) is related to the vibration stretching for (CH<sub>2</sub>=CH<sub>2</sub>) bond of aliphatic group. The band at (1652 cm<sup>-1</sup>) is due to the vibration stretching for

benzene ring in aromatic compound .The band at (1460 cm<sup>-1</sup>) is due to the vibration stretching for (C=C) bond of aliphatic ether.[4],[7].

The antibacterial activity of isolated compound determined by using Agar Diffusion Method [6]. The results, in table(5), show that the isolate compound has good antibacterial activity against gram positive and gram negative bacteria, which are Staphylococcus aureus NCTC 6571 and Staphylococcus aureus and also Escherichia coli NTC5933. The results, this my due to steroidal alkaloid with (OH)group in the structure of the studied increase the activity of the isolated compound to inhibit the bacteria growth, by denaturation of nature cell protein, and increases the permeability of cell membranes [11],[8].

Similar finding were drowned by 2,3 in their experiment which represent a very good mechanism of biological control of microorganisms .In addition the effectiveness of plant was not due to one main active constituent but to the combined action of other chemical compounds involved in it, some example include alkaloids.[12].

Table (1) the qualitative chemical analysis for the isolated compound of Solanum nigrum

Reagent	Dragendroff	Wagner	Mayer 's
Alkaloid compound test	+	+	+
	Formation Orange precipitate	Formation Light brown precipitate	Formation White precipitate

Table(2) the thin layer chromatography, R<sub>f</sub> value for the isolated compound of Solanum nigrum

Solvent system	Developers	Number of spot	R <sub>f</sub> -values	Notes
Chloroform: Methanol 19.5 :0.5 [10]	The eyes	1-spot	0.74	Pure compound
	I2 Vapor	1-spot	0.74	Organic nature
	UV-lamp(366nm)	1-spot	0.74	Conjugated double bond

Table(3)the infared absorption peak and and their related functional group for isolated compound of Solanum nigrum

Frequency rang intensities	Functional Group	Assignment of remark
3477 (Strong)	Alcohol	O-H stretch
3049 (medium)	Aliphatic	CH <sub>2</sub> = CH <sub>2</sub>
1652 (strong)	Benzene ring in aromatic compound	aromatic C =N
1460 (strong)	Aliphatic ether	- C =C in alkene

Table(4) Bacterial strains used in the present study

No	Bacterial strain	Gram(+/-)
1	Staphylococcus aureus NCTC 6571	+
2	Escherichia coli NTC5933	-
3	Staphylococcus aureus*	+

Table (5) the antibacterial activity for the isolated compound of Solanum nigrum

Bacteria strains	Inhibition zone (mm)**
Staphylococcus aureus NCTC 6571	24
Escherichia coli NCTC 5933	26
Staphylococcus aureus *	19

\*clinical strain

\*\* mean of three value each number

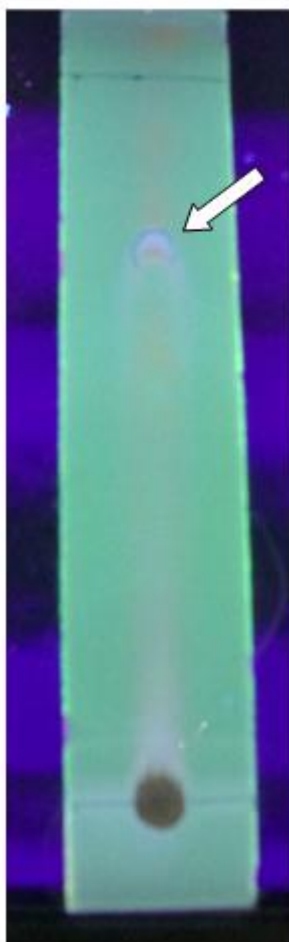


Figure (3) shown The the thin layer chromatography for isolated compound of Solanum nigrum

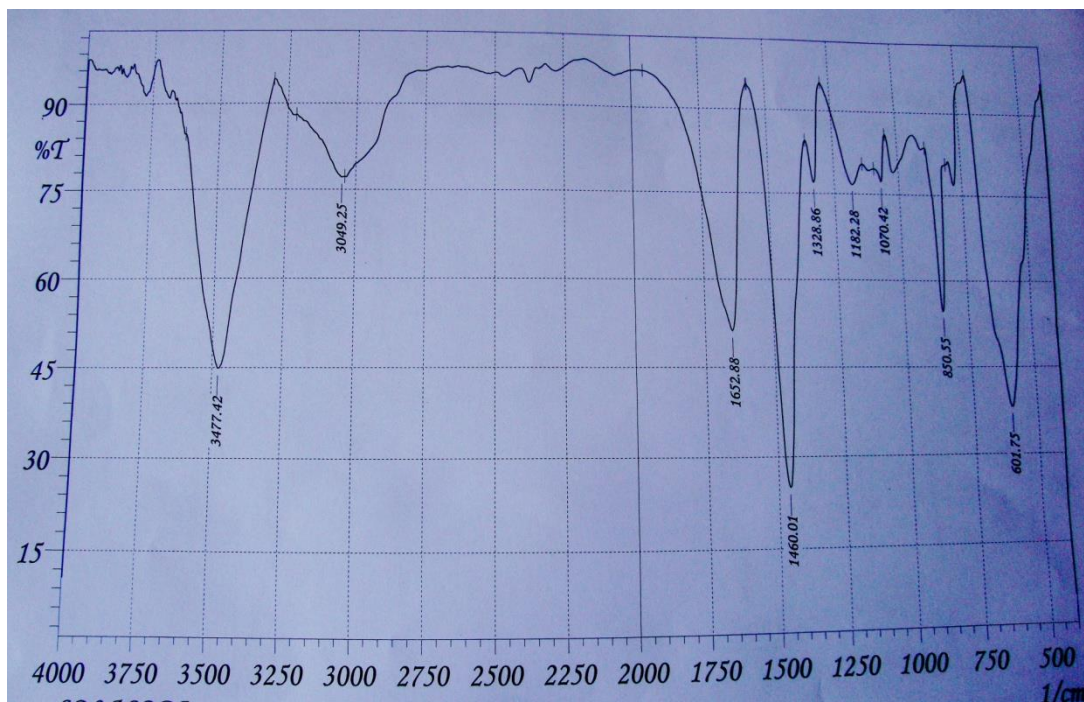


Figure (1) shown infared absorption peak and and their related functional group for isolated compound of Solanum nigrum

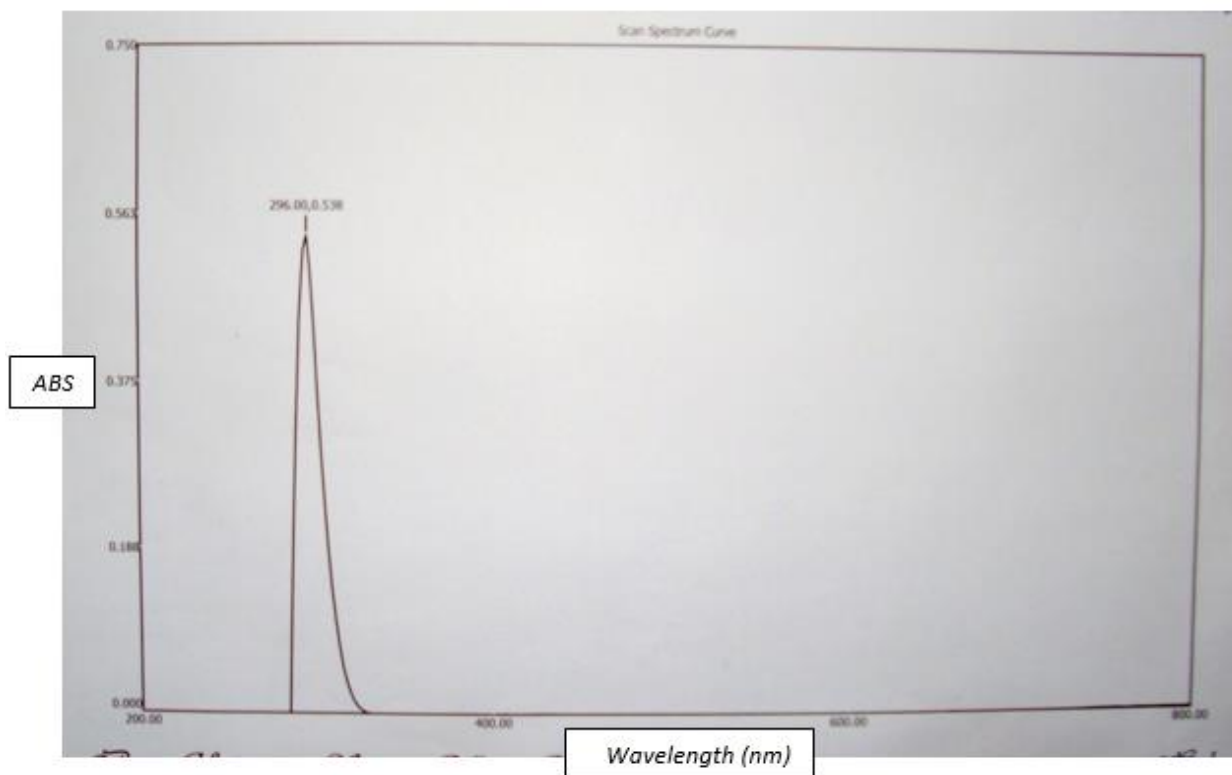


Figure (2) shown The ultraviolet-visible spectrum for isolated compound of Solanum nigrum

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