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Isolation of alkaloids content from the Iraq local medical plant *Catharanthus roseus* (L.) leaves

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Abstract

The following alkaloids were isolated from *Catharanthus roseus L.* leaves from the yellow crystal as white powder at (38°C). The chemical and physical properties were studied by thin layer chromatography (TLC), IR-spectrum (IR), Ultraviolet - visible spectrum (UV) and melting point (mp). Standard strains of bacteria: *Staphylococcus aureus NCTC 6571, Escherichia coli NCTC 5933* and the clinical multidrug resistance (MDR) *S. aureus, E. coli* and *P. aeruginosa* were tested with the alkaloids. The *Catharanthus ruseus (L.*) alkaloids inhibited both type of bacteria strains of gram positive strains *S. aureus NCTC 6571,*S. aureus* and **E. coli* as gram negative strains when concentrations increase (125, 250, 500 mg/ml) with *C. ruseus L*. alkaloids being the most active in (500 mg/ml) concentrations against gram positive *S. aureus* bacteria than gram negative bacteria *E. coli*, while the clinical resistance **P. aeruginosa* showed resistance against *C. ruseus L*. alkaloids in all concentrations (500, 125, 250 mg/ml). The minimum inhibitory concentration (MIC) (µg/ml) of the alkaloidal compound were also determined. Finally, a test was also carried out to examine the cytotoxicity assay methods towards human red blood cells have no cyto-toxicity in all concentration. The good antimicrobial potency of the alkaloidal compound of *C. ruseus L*. indicates the treatment of (MDR) as an alternative to the costly antibiotics.

Keywords: *Catharanthus roseus* L. leaves, thin layer chromatography (TLC), alkaloid compound, antibacterial activity.

INTRODUCTION

Infectious diseases account for high proportion of health problems in the developing countries (Sashi et al., 2003). The continuous use of the same antibiotic per disease gave the appearance of resistance bacteria to antibiotic. In particular, emergence of resistance to antibiotics has hampered the pace by which newer antibiotics are being

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Figure 1. *Catharanthus roseus* (L.) plant.

introduced into the public domain (Russell, 2002). Despite ever increasing advancement in the field of medicine and molecular diagnosis it is estimated that 80% of the world population is still dependent on the plant derived pharmaceuticals. As published, plant natural products or its derivatives accounts for available in the market (Newman et al., 2003). In recent years, many drugs have been isolated from natural source as the modern medicine system treats the symptoms and suppresses the disease but does little to ascertaine the real cause. Medicinal plants are rich source of antibacterial drugs substances (Jaleel et al., 2007). *Catharanthus roseus*, from the family Apocynaceae (Figure 1), is used as plant medicine. Catharanthus roseus alkaloids have anticancer activity (Jaleel et al., 2007). The crude extracts of different parts of *C. roseus* are used in clinic as a antibacterial agent, it is published that more than 130 of different alkaloids are found in *C. roseus* (Muhammad et al., 2009). The aim of this study was isolation of alkaloids content from the Iraq local medical plant *Catharanthus roseus* (L.) leaves and to study the physiochemical properties and the antibacterial activity of these alkaloids.

MATERIALS AND METHODS

Plant

C. roseus were cultivated and collected at the flowering stage from arboretum garden at Missan in south of Iraq. In this study, the leafs of *C. roseus* were used for testing their antibacterial activity. The plant materials were dried in shade at room temperature (25°C).

Preparation of plant extracts

Material

1. Leaves of *C. roseus* were collected, dried, broken and kept at (4°C).
2. Standard bacteria strains; *S. aureus* (NCTC 5671) and *E. coli* (NCTC 5933).
3. The medical sensitivity for some clinical bacterial isolates, that were isolated from some patients burns in Myssan Public Hospital were tested. These isolates involve *S. aureus, E. coli* and *P. aeroginosea*.
4. Ready culture media; culture media (Muller Hinton Agar) was prepared according to information of the manufacturing company.

Isolation of crude alkaloid compound from dry leaves of Catharanthus roseus (L.)

100 mg of finely powdered material and 40 ml of 95% ethanol were refluxed in 100 ml flask for 30 min. The extract was then filtered and then the residue washed twice with 5 ml of ethanol. The washed residues are added to the original filtrate and transfered into a 50 ml standard flask then added ethanol 95% and adjusted to the mark. 5 ml of this solution was pipette into a test tube and ethanol completely removed by evaporation on a water bath, then we treated the residue with 3 ml of 1 N NaOH and then we added acetic acid and the contents transferred to 25 ml standard plates (2 x 9 cm) in a pre-saturated chamber of the mixture of (chloroform: methanol) (0.5: 9.5). The glass plates were dried and the spot which appeared were developed with UV-lamp at (336 to 200 nm), iodine vapor. Melting point electro-thermal is used for the determination of melting point of the isolated compounds (Teresa and Ivan, 2003).

Spectroscopy

1. 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.
2. 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 spectrophoto-meter UK.

Antibacterial activity

Agar diffusion method (John et al., 1996) was used to determine the antibacterial activity of the isolated compound (30.000 µg/ml) against types of reference strains of gram positive and gram negative bacteria (*S. aureus* NCTC 5671) and (*E. coli* NCTC 5933), **S. aureus*, **E. coli* and **P. aeruginosa* as clinical Mullidrug resistance (MDR), clinical strain which are tested using plate of Muller-Hinton agar. The antibacterial activity was defined as the clear zone of growth inhibition (Sashi et al., 2003).

The preparation of alkaloid compound

500 mg/ml (0.5 mg of alkaloid compound soluble into 1 ml DMSO) as stock solution was put in a 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, then we finely recrytallized the dry powdered by methanol of 80% and allowed it to dry in room temperature.

Determmination of MIC by agar plate dilution method

According to the methods of National Committee for Clinical Laboratory Standards (NCCLS) (2002), agar plate dilution test was used to determine the minimum inhibitory concentration (MIC) of an antimicrobial agent.

Cytotoxicity assay

According to the methods of Xian-guo and Ursula (1994), human red blood cells were used for toxicity test.

Identification

1. Preliminary qualitative test: The chemical family of the isolated compound was implemented using several test such as:

a) Dragendroff test (Harborne, 1984).
b) Wagner reagent (Harborne, 1984).
c) Mayer's test (Harborne, 1984).

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 45 min on glass plates (2 × 9 cm) in a pre-saturated chamber of the mixture of (chloroform: methanol) (0.5: 9.5). The glass plates were dried and the spot which appeared were developed with UV-lamp at (336 to 200 nm) iodine vapor (Figure 2).

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 the spectrum recorded with the Spectroscan 80D UV-vis spectrophoto-meter UK.

5. The preparation of alkaloid compound: 500 mg/ml (0.5 mg of alkaloid compound soluble into 1 ml DMSO) was used as stock solution.

RESULTS AND DISCUSSION

Qualitative test which describes the appearance of alkaloid is listed in the Table 1 and was isolated from *C. roseus* (L.). The result was similar to that published previously on the leaves of *C. roseus* (L.) alkaloid content (Muhammed et al., 2009). In this study, we did not find saponin, glycoside, amino acid, flavonoid and carbohydrate

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Table 1. The qualitative chemical analysis for the isolated compound of Catharanthus roseus (L.).

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

Table 2. Thin layer chromatography , R*f* value for the isolated compound of *Catharanthus roseus* (L.).

Solvent system	Developers	Number of spot	R*f* values	Notes
Methanol : NH4OH 9.5 : 0.5	The eye	1	0.83	Pure compound
	I2 Vapor	1	0.83	Organic nature
	UV- Iamp (366 nm)	1	0.83	Conjugated double bond

on *C. roseus* . The thin layer chromatography shows (Table 2) the appearance of one spot using different solvent system and different types of (TLC) plates and diffrenent reagent as spot developer. The Rf value of the peak was 0.83.

Melting point (m.p)

The melting point of the isolated alkaloids shows a sharp melting point peak at 165 to 168°C. These results indicate that the isolated compound is pure. The FT-IR spectra were recorded in KBr on a SHIMADZU 8400S Japan spectrophotometer for the isolated compound as shown in Figure 3 and Table 4. The appearance of a single broad peak at 3411 cm^{-1}, related to the vibration stretching for (-OH) bond which indicated the presence of alcohol group. The band at 2923 to 2856 cm^{-1} related to the vibration stretching for (C – H) bond of aliphatic CH2 and CH3 group (1731 cm^{-1}) is due to the vibration stretching for (C = O) bond of carbonyl group (1441, 1392 cm^{-1}) related to the vibration stretching for (CH3–) bond of aliphatic CH3 group (CH antisymmertic and symmetric). The band at (1060 cm^{-1}) is related to the vibration stretching for (C – O – C) bond of aliphatic ether. The band at 871 cm^{-1} is due to the vibration stretching (-CH) banding of Tri –substitution. 594 cm^{-1} is related to vibration stretching for (C – O – C) bond of ethers bond (Table 4). The result of IR spectrum appeared, the isolated compound is an aliphatic compound containing carbonyl and ether group (Jaleel et al., 2007).

The ultraviolet–visible spectrum (Figure 4), has shown one peak at ƛ max equal to 300 nm due to the presence of pairs of electrons (non-bonding type $\pi \rightarrow \pi^*$) on the oxygen atom (Donald et al., 2009; Silverstein et al., 1991). Test for the sensitivity of some clinical bacterial isolates under study life towards some antibiotics was used (John et al., 1996). Isolation bacteria isolated from some patients burns under study were resistant multiple (MDR) as shown in Figure 5 Table 3. Drug susceptibility testing of some bacterial isolates isolated from burns and surgical operations under study for antibiotic showed all of isolation clinical bacteria *S. aureus* and isolation strain clinical *P. aeruginosa* resistance to 100% of the direction of six types of antibiotics used as shown in the Figure 5, while it showed isolation clinical strain isolate of *E. coli* resistance to both antibiotics cephalothine and metronidalzole and rifampin as shown in Figure 5 Table3, thus all of these isolates are multi-resistance to antibiotics multi drug resistance because they showed resistance to more than one antibiotic (Majeed, 1992; Enright, 2003).

The antibacterial activity of isolated compound was determined by using agar well diffusing methods. The results, in Table 5, Chart 1, show that the isolated compound has good antibacterial activity against gram positive and gram negative bacteria which is evaluated for their ability to inhibit the growth against both a standard bacteria *S. aureus NCTC6571* and *E. coli NCTC* 5933, and the multidrug resistant bacterial isolates *S. aureus* and *E.coli* studied showed an inhibition activity at 500, 250 and 125 mg*/*ml in the valuable level (0.01), and the results in Table 6 shows that the MIC value of the isolated compound were 1 µg*/*ml against gram positive and gram negative bacteria while that of the MIC value of the isolated compound were 1.5 µg*/*ml against clinical (MDR) *S. aureus* and *E. coli*, this may be due to the presence of (OH) group in the structure of the studies which increased the activity of the isolated compound to inhibit the bacteria growth by changing the nature of cell protein (denaturationa), thus increasing the permeability

Table 3. The antibiotic test in this study.

Antibiotic	Symbol	Concentration (µg)
Cephalothen	KF	30
Erythromycin	EA	15
Metronidozle	MET	5
Nalidixic acid	NA	30
Riphampin	RA	5
Streptomycin	S	10
Tetracycline	TE	30

Table 4. The infrared absorption peak and their related functional group for the isolated compound of *Catharanthus roseus* L. leaves.

Frequency rang intensities (cm^{-1})	Group or class	Assignment of remark
3411 (strong)	Alcoholic or phenol	O – H Stretch
2923, 2856 (medium)	Aliphatic CH2 and CH3	C – H Stretch
1731 (strong)	Carbonyl group	Stretch C = O
1441, 1392 (medium)	CH antisymmertic and symmertic	CH3- Stretch
1153 (strong)	Aliphatic ether	Stretch C – O – C
1060 (strong)	Aliphatic ether	Stretch C – O – C
871 (very strong)	Tri - substitution	banding - CH
594 (medium strong)	Ether band	banding C – O – C

Table 5. The bacterial activity for the isolated compound of *Catharanthus roseus L.*

Bacteria strains	Inhibition zone (mm)			
	0.065 µg/ml	125 µg/ml	250 µg/ml	500 µg/ml
Staphylococcus aureus (NCTC5671)	13	19	23	26
Escherichia coli (NCTC5933)	10	19	20	23
*Staphylococcus aureus**	0	13	19	23
*Escherichia coli**	0	10	19	20
*Pseudomonas aerugenosa**	0	0	0	0

*Clinical isolate **three value each number ***diameter of well (8 mm).

Table 6. The minimum inhibition concentration (MIC) for the isolated compound of Catharanthus roseus(L.) leaves.

Bacteria strains	**MIC (µg/ml)**
Staphylococcus aureus (NCTC5671)	1
Escherichia coli (NCTC5933)	1
*Staphylococcus aureus**	1.5
*Escherichia coli**	1.5

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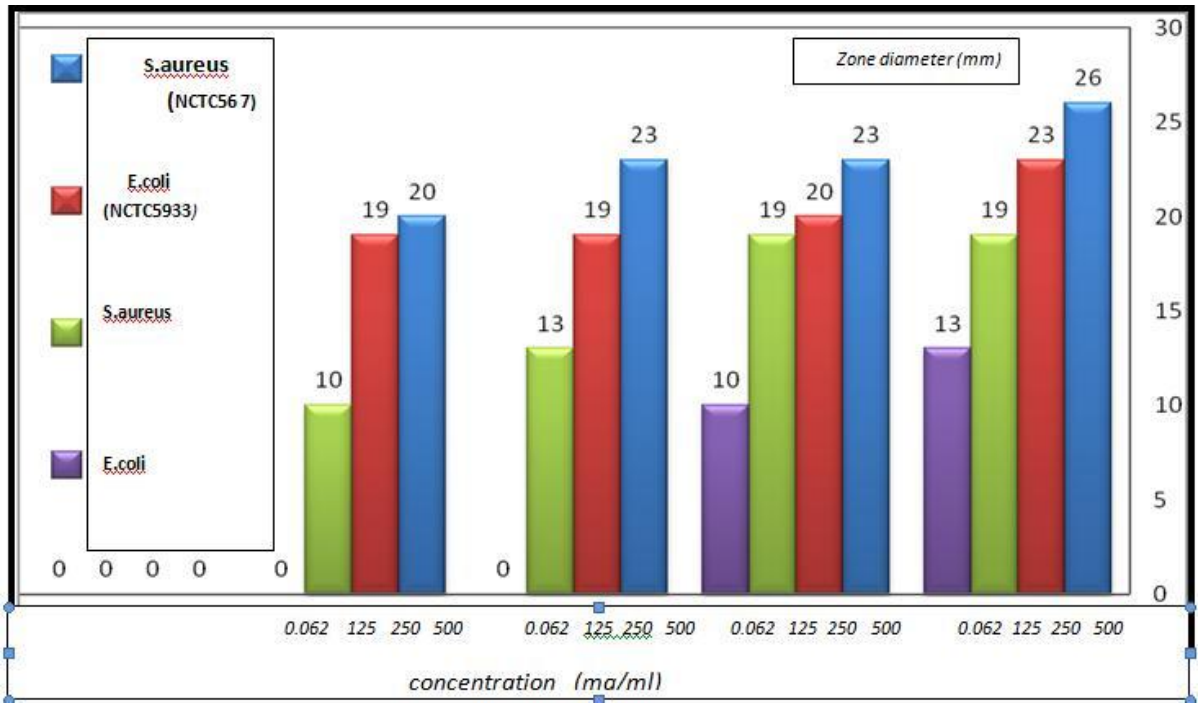

Chart 1. Showing zone of inhibition diameter against different bacteria strains of different concentrations of whole alkaloid compound isolated of Catharanthus roseus(L.) leaves

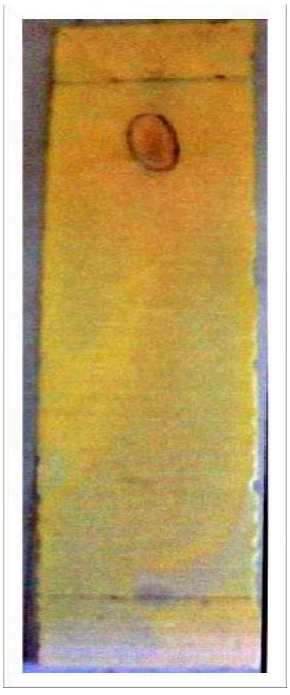

Figure 2. Thin layer chromatography for isolated compound of *Catharanthus roseus L.*

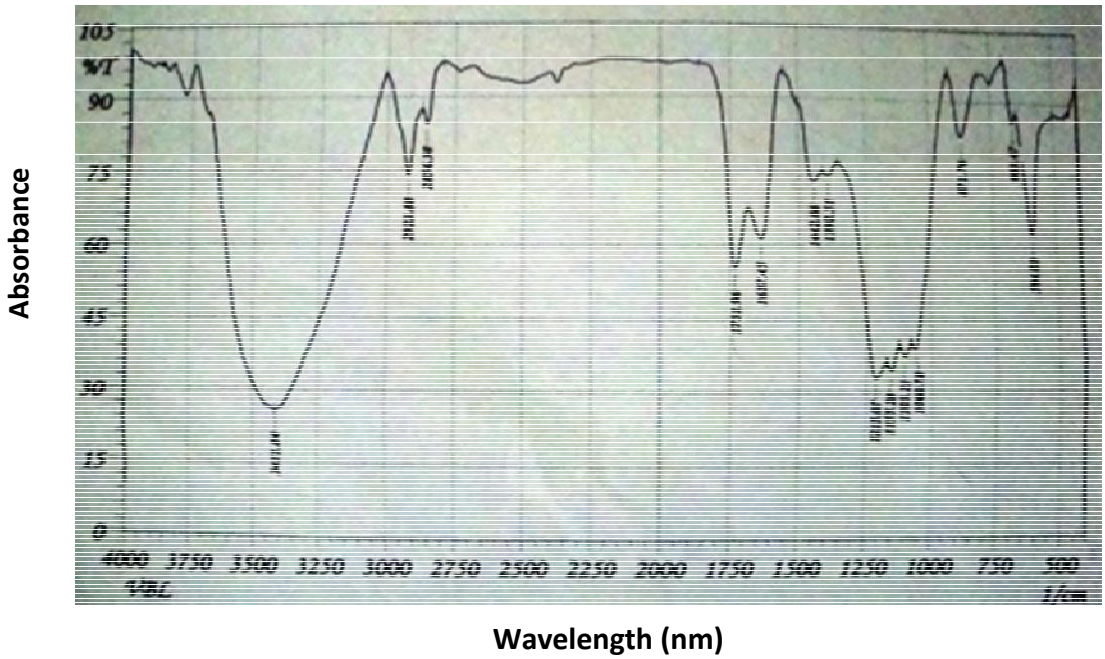

Figure 3. Infared absorption peak and and their related functional group for isolated compound of *Catharanthus roseus (L.).*

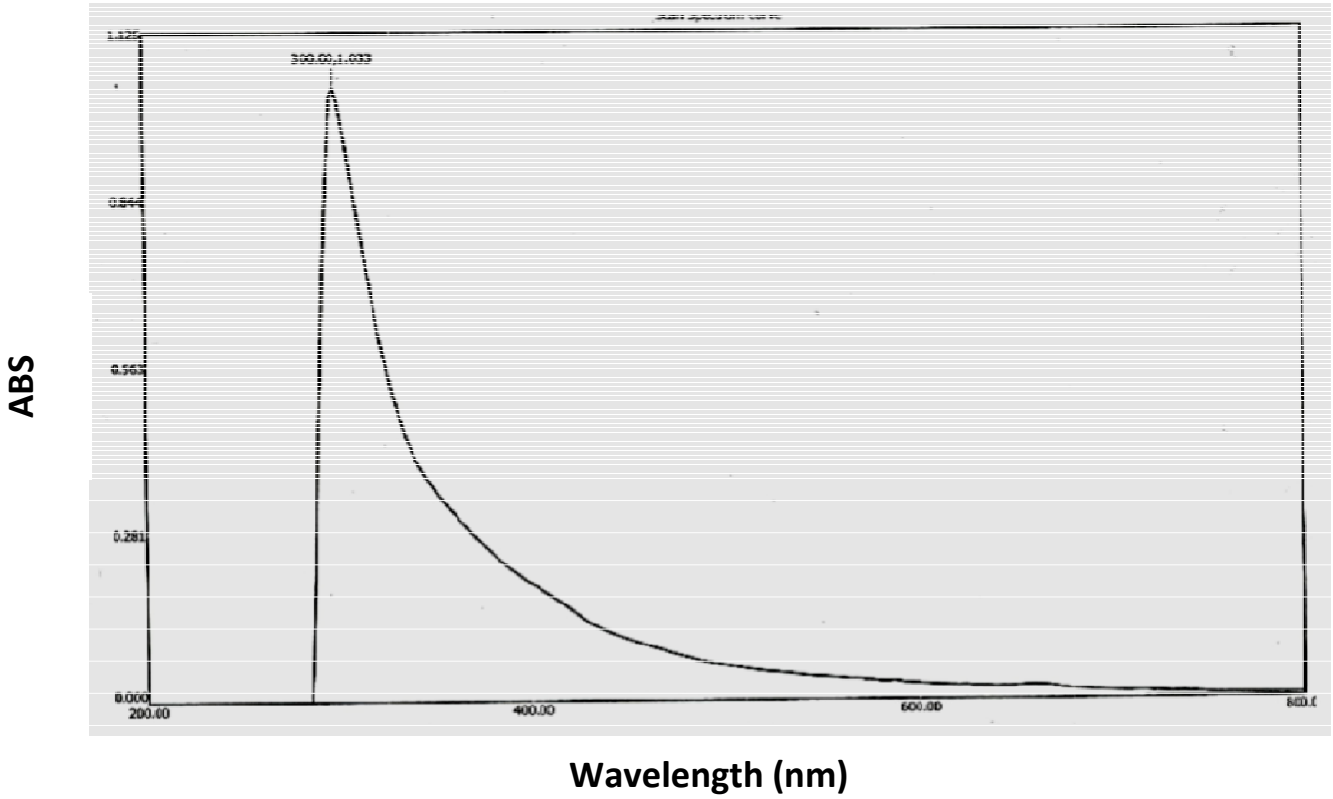

Figure 4. The ultraviolet-visible spectrum for isolated compound of *Catharanthus roseus (L.).*

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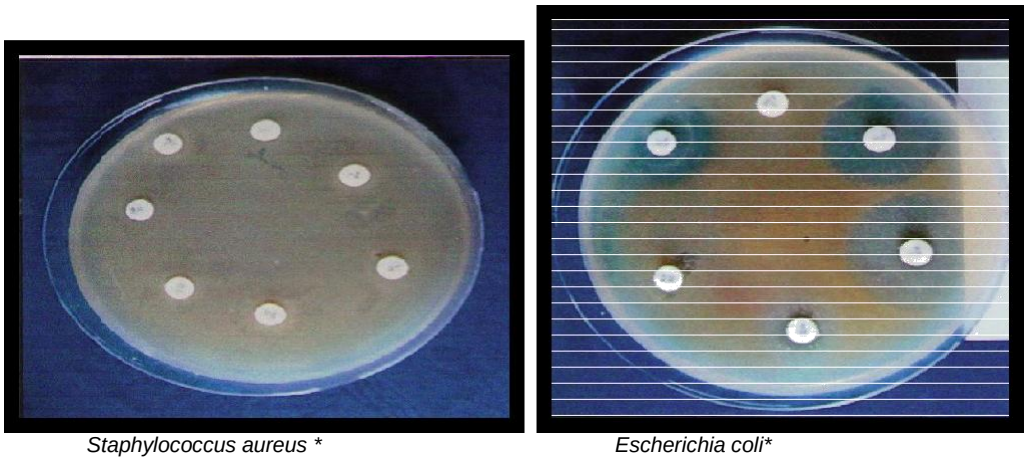
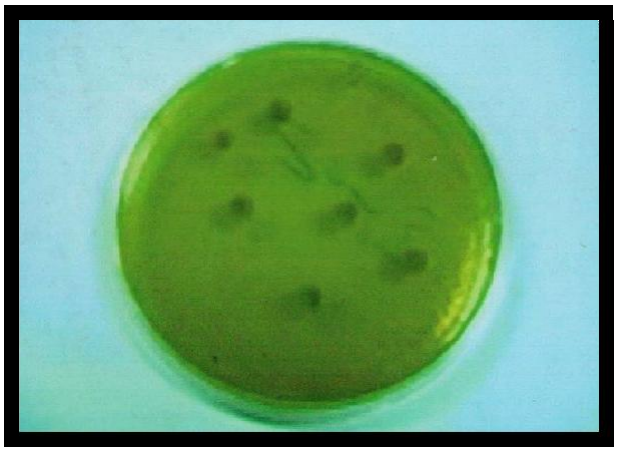
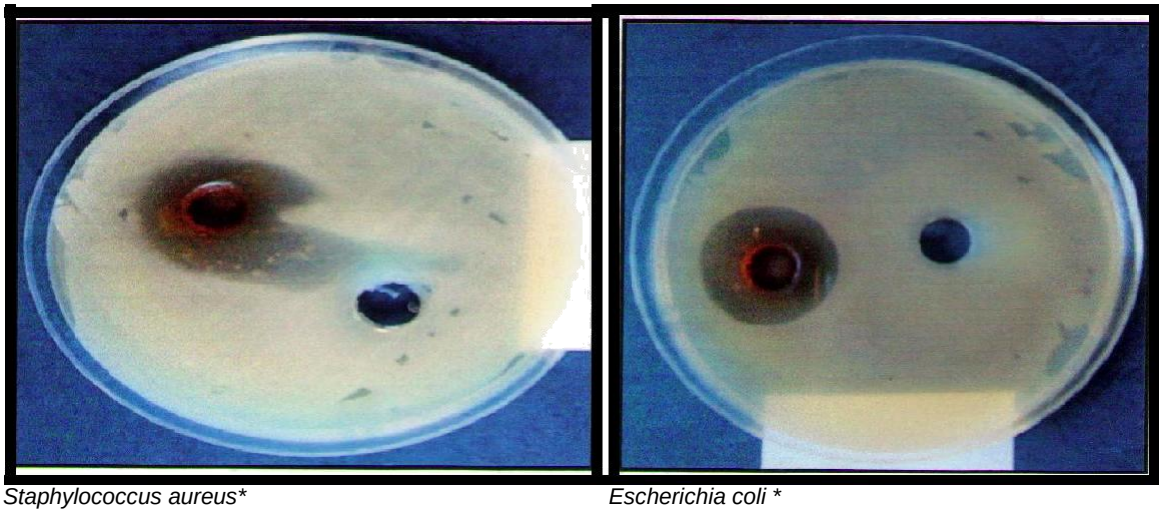


*Staphylococcus aureus ** *Escherichia coli**



*Pasuedomonas aeruginosa **

Figure 5. Isolated multi drug resistant (MDR) (*clinical isolate).



*Staphylococcus aureus** *Escherichia coli **

Figure 6. Antibacterial activity of alkaloid compound isolated from Catharanthus roseus leaves against MDR **Staphylococcus aureus and *Eschericha coli.*

of cell membranes (Feeny, 1998), either by increasing the permeability of the membrane cell bacteria. The cell membrane causes loss or leakage of the contents of a cell of bacteria to the outside or through a direct link membrane of cell bacteria, causing the demise of polar membrane of bacteria, which leads to the death of a cell bacteria gradually (Carpenter and Chambers, 2004; Straus and Hancock, 2006).

Finally, a test was also carried out to examine the cytotoxicity assay by using Xian-gou and Ursula (1994) methods towards human red blood cells in which the alkaloid compound of *C. roseus* (L.) were found that they are not having cytotoxicity on 1 to 500 µg*/*ml. Results of this study suggested that the alkaloidal compound may be useful either alone or when combined with antimicrobial agents to treat MDR bacterial infections.

Conflict of interest

The authors declare that they have no conflict of interest.

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