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# ANTIBACTERIAL EFFECTS OF AL - AQUEOUS AND AL-COHOLIC EXTRACT OF COLUTEA CILICICA L . PLANT

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**Abstract:**

In this study, samples were collected from the plant Colutea cilicica L. , which is a member of the family Papilionoideae (legume family), and it is the only species of the genus Colutea L spread in Iraq.

Aqueous and alcoholic extract of the plant were prepared for antibacterial efficacy study. All of extracts were tested for their antibacterial activity against ( 8 ) pathogenic bacterial three of them Gram positive bacteria ( Staphylococcus albus , Staphylococcus aureus and Streptococcus pyogenes ) and five were negative for Gram stain ( Escherichia coli , Klebsilla pneumonia , Pseudomonas aeruginosa , Serratia marcescens and Sallmonella typhi ) by perpendicular streak method on Muller - Hinton agar. The extracts have antibacterial activity against pathogenic bacteria. The study proved the effectiveness of the extract against bacteria . The diameter of inhibition zone of the alcoholic extract reached (30) mm against Gram positive bacteria and reached (7.27) mm against Gram negative bacteria. While the inhibition diameter of the aqueous extract reached (29) mm against Gram-positive bacteria(5.30 mm) against Gram-negative bacteria. The results showed that the minimum inhibitors concentration (MICs) of alcoholic extract against bacteria was ( 0.625) µg / ml for all gram negative and gram positive bacteria except against E. coli was ( 1.25) µg / mL and the minimum bactericidal concentration ( MBCs) ranged from ( 0.312-0.625 ) µg / ml against Gram-positive and negative bacteria. While minimum concentration inhibitors (MICs) of aqueous extract were ( 0.625) µg / ml against Gram negative and Gram positive bacteria , (MBCs) were ( 0.312 ) µg / mL against Gram positive and negative bacteria .

**Key words:** Antibacterial effects; (MICs); Colutea cilicica L; ( MBCs); Aqueous extract; alcoholic extract

**Introduction :**

Leguminosae is the third largest angiosperm family in terms of number of species after the Asteraceae and Orchidaceae family ( LPWG, 2017 ) . It is second in economic importance after Poaceae .

Leguminosae is classified into six subfamily, based on the family genetic structure. , which is Duparquetioideae , Papilionoideae , Caesalpinioideae , Cercidoideae , Detarioideae , Dialioideae , ( LPWG, 2017 ) .

The genus Colutea is a genus of Papilionoidea subfamily ( Leguminosa family ). common as bladder senna , and includes about 30 species. Found in southern Europe, southern and eastern Africa, to southern and central Asia . It is native to the Mediterranean and It grows as shrubs, small trees with inflated fruits from 2 to 5 m tall , Mirzaei et al. (2015) . the leaves are pinnate and light green . The flowers are yellow to orange arranged in clusters .

The genus Colutea has been classified into several flora, within the Galegeae tribe , in Iraq it was classified by Townsend (1974) within the Coluteae (Benth.) Hutch tribe . The genus is represented by one species in Iraq, which is C. cilicica Boiss. Its spread was limited to the north of the country, AL-Rawi and Chakravarty (1964) .

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Colutea cilicica is a medicinal plant, and some studies have been conducted to show its medicinal effect. The study of Suntar et.al ,(2011) showed that the ethanolic extract of Colutea cilicica had an antibacterial and inhibitory effect . Eser et al. ( 2017 ) also explained in general , that the secondary metabolites present in the plant are responsible for the biological activity of the plant, such as an anti-cancer or anti-oxidant . Cell stimulation was observed after using D-pinitol (0-80) µM for 24, 48 and 72 hours, which was isolated from Colutea cilicica leaves . It was determined that D-pinitol inhibits the protein expression of Cox-2 in K562 cells, confirming the effective role of this substance in significantly reducing inflammation and may be an anti-inflammatory agent for the treatment of K562 cells.

## Material and methods

### 1: Primary Screening Test..

This antibacterial activity of extracts were tested by perpendicular streak plate method (Dhanasekaran and Selvamani., 2010 **;** Dhananjeyan et al., 2010). This method was prepared by putting extracts through made cross lines from these isolates in the middle of Muller Hinton agar media . After this , columns from the pathogenic test bacteria were streaked at right angles on each side of extracts at straight line colony (Zhomghui and Wei., 2000 ; Suthindhiran and Kannabiran., 2009 ). The media then incubated at 37°C for 24 h. and the results were recorded as positive through inhibition growth of pathogenic test bacteria (Nanjwade et al., 2010).

### 2: Secondary Screening Test of Actinomycetes.

The positive results were obtained from the primary screening protocol , batch culture fermentation as well as agar well diffusion assay were used to determining the antimicrobial activities of the extracts by use the Muller Hinton agar ( Pallavi et al., 2013).

### 3: Minimum Inhibitory Concentrations and Minimum Bactericidal Concentrations (MICs & MBCs) Determination:

The minimum inhibitory concentrations (MICs) of the extracts were determined by a serial dilution technique. MICs were defined as the lowest concentration of an antimicrobial that inhibits growth of a microorganism after their incubation for overnight. To determine MIC:

**1.** An appropriate amount (0.1mg antimicrobial plus 10 ml respective solvent) of antimicrobial extract was dissolved in respective solvent to prepare an antimicrobial solution containing 10 µg/ml.

**2.** Two fold dilutions of the antibiotic solution in Mueller Hinton broth were prepared and describe below:

**(a)** Ten sterile tubes were placed in a rack and were labeled each 1 through 8 and first one labeled as antibiotic control) and last one was labeled as G.C (growth control).

**(b)** 1 ml of Mueller Hinton broth was added in each test tube.

**(c)** 1 ml of antimicrobial solution was added to test tube No 1 and A.C.

**(d)** With a sterile micropipette and tips, after adequate mixture 1 ml was transferred from tube No. 1 to tube No. 2.

**(e)** After a through mixing, 1 ml was transferred with a separate micro pipette from tube No 2 to tube No 3.

**(f)** This procedure was repeated through the next-to-next up to the tube No. 8. Except tube No G.C. (using fresh pipette for each dilution). From tube no 8 1 ml was removed and discarded. The last tube (tube G.C) received no antimicrobial agent and was served as a growth control. First A.C labeled test tube was served as antibiotic control.

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**3.** Each tube was inoculated (including the growth control except antibiotic control) with 1 ml of the culture of respective organism. The final concentration of antimicrobial agent in this test tube was half of the initial dilution series because of the addition of an equal concentration of inoculums in Mueller Hinton broth.

**4.** The tubes were incubated at 37ºC for 24 hours.

**5.** The tubes were examined for growth and were determined the MIC of tested antibiotics, which is bacteriostatic for the test organism. The tubes were examined for visible growth (cloudy) and was recorded growth as (+) and no growth as (-). **6**. For determination of minimum bactericidal concentrations (MBC), the concentration which was bactericidal, was then found by sub cultured the contents of selective tubes into a series of Mueller Hinton broth, which did not contain any antibiotic and started from last two non-visible tube to the first two visible tube (direction tube No. 1 to tube No. 8). Then was inoculated into Mueller Hinton agar containing Petri plate by 0.1 sterile micropipette and separate 0.1 ml sterile tips in drop method.

**7.** The plates were incubated at 37ºC for 24 hours. (Reiner, 1982) .

**Results and Discussion:**

The results showed that the aqueous and alcoholic extracts of **colutea cilicica** had anti-bacterial

| No. | extract of colutea cilicica L plants | Diameters (mm) ± SE | | |
|---|---|---|---|---|
| | | S. aureus | S. albus. | S. pyogenes |
| 1 | alcoholic extract | 30±0.01 | 19.5± 0.2 | 6.3±0.7 |
| 2 | aqueous extract | 18.5±0.03 | 18.3±0.4 | 29±0.4 |

**.**effects as shown in the tables (1 & 2)

**Table(1) : Antibacterial activity of colutea cilicica extract on pathogenic Gram positive bacteria**

**Table (2): Antibacterial activity of colutea cilicica extract on pathogenic Gram negative bacteria .**

| NO. | extract of colutea cilicica L plants | Diameter(mm) | | | | |
|---|---|---|---|---|---|---|
| | | E. coli | Pj. Aeruginosa | K. Pneumoniae | S. typhia | .A. hydrophila |
| 1 | alcoholic extract | 4±0.3 | 7±0.06 | 0 | 0 | 3±0.1 |
| 2 | aqueous extract | 1±0.8 | 4±0.5 | 2±0.4 | 5±03 | 3±0.3 |

The reason for different sensitivity between Gram positive and Gram negative bacteria could be described to the morphological differences between these microorganisms, Gram negative bacteria having an outer polysaccharide membrane carrying the structural lipopolysaccharide components. This makes the cell wall impermeable to lipophilic solutes, The Gram positive should more susceptible

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having only an outer peptidoglycan layer which is not an effective permeability barrier (Scherrer &Gerhardt, 1971).

Rana and Salam (2014) are summarize the antibacterial screening , if the microbial pathogens were susceptible to the antimicrobial extract ; it would not allow their growth as presented in Plate in figure (1).

**A/ alcoholic extract** **B/ aqueous extract**

**Figure (1) ; Antibacterial activity of extract on pathogenic bacteria** .

Alcoholic extract and aqueous extract were was used to determine its MICs and MBCs against Gram positive and Gram negative pathogenic bacteria. The concentrations were used ranged 0.039, 0.078, 0.156, 0.312, 0.625, 1.25, 2.5 and 5 µg/ml, and the Mueller Hinton broth was used as a culture medium broth.

The data presented in Tables (3&4) summarize minimum inhibition concentrations (MIC) and minimum bactericidal concentrations (MBC). There are various factors affecting the activity so The MIC and MBC are not a constant for a given agent, because it is affected by the nature of the test organism used, the inoculum size , and the composition of the culture medium , the incubation time , and aeration ( Pandey et al. 2004) . Our results were agreed with those Mukai et al. (2006) , Xie et al. (2007), Ababutain et al. (2012) and Gebreyohannes et al. (2013) which were intended for the activities of the bioactive metabolites against both gram positive and negative bacteria .

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Table (3): Minimum inhibition concentrations and minimum bactericidal concentrations alcoholic extract against bacterial pathogens.

| Pathogenic Bacteria | | 5 | 2.5 | 1.25 | 0.625 | 0.312 | 0.156 | 0.078 | 0.039 | A.C | G.C |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **S. aureus** | | - | - | - | --MIC | MBC | + | + | + | - | + |
| **S. albus** | | - | - | - | MBC --MIC | - MBC | + | + | + | - | + |
| **S. pyogenes** | | - | - | - | - MIC- | - MBC | + | + | + | - | + |
| **E. coli** | | - | - | - | -- MIC -MIC | MBC+ | + | + | + | - | + |
| **P. aeruginosa** | | - | - | - | - MIC- | -MBC | + | + | + | - | + |
| **K. Pneumoniae** | | - | - | - | - MIC- | -- MBC | + | + | + | - | + |
| **S. typhia** | | - | - | - | - MIC- | -- MBC | + | + | + | - | + |
| **A. hydrophila** | | - | - | - | - MIC- | -MBC | + | + | + | - | + |

A.C = Antibiotic Control G.C = Growth Control

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Table (4): Minimum inhibition concentrations and minimum bactericidal concentrations aqueous

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | G.C | + | + | + | + | + | + | + | + | A.C = Antibiotic |

extract against bacterial pathogens

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| Pathogenic Bacteria | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 5 | 2.5 | 1.25 | 0.625 | 0.312 | 0.156 | 0.078 | 0.039 | A.C | | |
| **S. aureus** | - | - | - | - MIC -MBC MIC | -MBC | + | + | + | - | | |
| **S. albus** | - | - | - | -MBC | -MBC | + | + | + | - | | |
| **S. pyogenes** | - | - | - | - MIC- | -MIC -MBC | + | + | + | - | | |
| **E. coli** | - | - | - MIC- | -MBC | + | + | + | + | - | | |
| **P. aeruginosa** | - | - | - | - MIC- | -MIC | + | + | + | - | | |
| **K. Pneumoniae** | - | - | - | - MIC- | -MIC -MBC | + | + | + | - | | |
| **S. typhia** | - | - | - | - MIC- | -MIC -MBC | + | + | + | - | | |
| **A. hydrophila** | - | - | - | - MIC- | -MIC -MBC | + | + | + | - | | |

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