

# Study effect of plant extraction for *Cuscuta europaea* (Dodder) against two species of bacteria *Staphylococcus aureus* and *Escherichia coli*

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**Objective:** The goal of this study is focused on the effect of crude extract of *Cuscuta europaea* plant and compared with Amoxicillin drug, and then we showed these effects on *Staphylococcus aureus* and *Escherichia coli*. The analysis shows that the side effect of drug is high on human. We try to find the alternative antimicrobial to treat the bacterial infection.

This study focuses on two treatments to inhibit the growth of two species of bacteria that are Amoxicillin as drug and *Cuscuta eurooaea* as extract until show the activity antimicrobial of these amount on bacteria.

**Methods:** The current study included *Cuscuta europaea* as extract and Amoxicillin as drug and two species of the bacteria *Staphylococcus aureus* and *Escherichia coli*. This study has been investigated to show the antimicrobial activity of plant extract and drug on the bacteria.

**Results:** During this study, the results reveal that the activity of antimicrobial of extract plant was higher than Amoxicillin on *Staphylococcus aureus* but the both Amoxicillin and extract did not have any effect on *Escherichia coli* in each concentration. And we showed the best result recorded in concentration 20 mg/ml of extract plant when compared with amoxicillin drug.

**Conclusion:** There are no significant differences between the concentration of plant extract of bacteria, and we showed the plant extract have a high effect on gram positive bacteria but do not have any effect on gram negative bacteria.

**Keywords** bacteria, amoxicillin, dodder, antimicrobial activity

## Introduction

Pathogenic bacteria have been always considered as a major cause of morbidity and mortality in human. Even though pharmaceutical companies have produced a number of new antibacterial drugs in the last year, the global emergence of multi-drug resistant bacteria is increasingly limiting the effectiveness of current drug and significantly causing treatment failure; Due to the increase of resistance of antibiotics, there is a pressing need to develop new and innovative antimicrobial agent. The potential source of new agent plants that have long been investigated contain many bioactive compounds that can be of interest in therapeutic because of their low toxicity.

### Amoxicillin

This is a penicillin antibiotic. It is used to treat certain kinds of bacterial infection. It will not work for colds, flu, or other viral infection. This drug also comes in other forms including oral capsules, oral suspension and oral table used for bacterial infection such as some respiratory infection, infection of the urine, ear infection and dental abscesses. It works by killing the bacteria causing the infection, and sometimes used to get rid of *Helicobacter pylori*. This is the bacterium believed to cause stomach ulcers and works by inhibiting the synthesis of the bacterial cell wall. This means that it stops any cross linkage that would normally occur between the linear peptidoglycan polymer chains that compose that large component of the cell wall.<sup>1</sup>

### Singular bacterium

This microscopic single-cell (unicellular) life form that exists practically everywhere on the earth, and is simpler than the cells of animals, fungi, and plants. It is of about three million species of bacteria believed to exist, only about 4000 are known and are divided into general groups according to their shape.<sup>2</sup>

### *Staphylococcus spp*

This is a genus of Gram positive bacteria. Under the microscope, they appear round (cocci) and form in grape-like cluster.<sup>2</sup> The staphylococcus genus includes at least 40 species. Nine have two subspecies, one has four subspecies.<sup>3</sup> Most are harmless and reside normally on the skin and mucous membranes of humans and other organisms, and found worldwide. They are a small component of soil microbial flora,<sup>4</sup> and are the most common cause of localized suppurating infection; pathogenic species include: *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*. *S. aureus* strain carries on the skin, nose and pharynx as harmless commensal bacteria. Strains of staphylococcus are known to enter through the breaks in the skin to cause localized infection or spread via blood to cause more generalized infection like that of the blood (sepsis), bone (osteomyelitis), brain (meningitis), lungs (pneumonia) etc., individuals with a compromised immune system are particularly vulnerable, etc.<sup>5</sup>

### *Escherichia coli*

This is a Gram-negative, facultative anaerobic, rod-shaped bacterium of the genus *Escherichia coli* that is commonly found in the lower intestine of warm-blooded organisms endotherms,<sup>6</sup> Most *E. coli* strains are harmless, but some serotypes can cause serious food poisoning in their hosts, and are occasionally responsible for product recalls due to food contamination.<sup>7,8</sup> The harmless strains are part of the normal flora of the Gut, and can benefit their hosts by producing vitamin K<sub>2</sub>,<sup>9</sup> and preventing colonization of the intestine with pathogenic bacteria.<sup>10,11</sup> *E. coli* is expelled into the environment with fecal matter. The bacterium grows massively in the fresh fecal matter under aerobic condition for 3 days.<sup>12</sup>

### Medical plant

*Cuscuta europaea* Roxb belongs to the family Convolvulaceae (Fig. 2). It is leafless green yellowish and thread-like twinning herb. It has a weak root under the ground but only grown as a

parasitic twin on other plants, hence, it is known as Akaswel (sky twinner) or Amarbel (immortal twin); in English it is known as Dodder. The plant is bitter acrid and hence useful in aphrodisiac alternative and the bilious disorder (Fig. 1). Therefore, seeds are used as carminative purgative, and the juice of the plant is used as anthelmintics to purify the blood, however, fruit decoction is used in cough and fever and the stem is useful in constipation, flatulence, liver complaints.<sup>13</sup>

## Plant Extraction

Plant remains the most important source of natural drug, more than 30% of prescription drugs are natural products, more than 60% of anticancer and anti-infective drugs are natural product.<sup>13</sup>

### Properties of good solvent in plant extraction

- Low toxicity
- Ease of evaporation at low heat
- Promotion of rapid physiologic absorption of the extract
- Preservative action
- Inability to cause the extract to complex or dissociate

## Material and Methods

### Preparation of plant extract

We sterilized the blender to destroy the infectants. A small amount of plant extract was added (40 g) in conical flask and then 140 ml of ethanol alcohol was added to the plant extract. The mixture was incubated in a shaker incubator for 24 hours to dissolve the plant in alcohol and the mixture was poured in many test tubes and put the tubes in a centrifuge to separate sediment from solution and removed the sediment. The separated plant extract was poured in glass petri dishes and incubated until it gets vapor. The extracted plant pours in glass petri dishes and incubate until vapor the alcohol and accumulate the extracted plant.<sup>16</sup>

The media was prepared according to the protocol given by the company and sterilized the media in an autoclave for 15 minutes in 121°C and 1 psi.

### Dilutions preparation with *E. coli* and *S. aureus*

We used four test tubes for each bacteria (e.g. *S. aureus*). 10 ml of nutrient broth was poured in first tube and 9 ml in remain

tubes. From suspension bacteria, we took 1 ml and put to first tube and mix. We took 1 ml from the first tube and put in the second tube and we chose the second dilution.

The concentration of drugs and extract of the plant were prepared as five different concentrations to and put 10 ml distilled water to each concentration (5 mg/ml, 10 mg/ml, 15 mg/ml, 20 mg/ml, 25 mg/l). The medium was poured in a petri dish and bacteria was streaked on medium (30 petri dish to *E. coli* and 30 petri dishes to *S. aureus*, 3 petri dishes to each concentration).

We made holes on media. Three holes in each petri dish and poured 50 µm from concentrations in the holes. The plates were incubated for 24 hours under 35–37°C. Later, the results were recorded and calculated. We calculated zone of inhibition by ruler to drugs and to the plant extract.

### Preparation of MIC test

our put (250 mg ) from extracted plant to nutrient broth and mix and prepare pure nutrient broth and we sterilize the media in autoclave in 121C for 15 minute after pouring the media in wells, and incubated the wells in incubator in 37°C for 24 hours and the results were recorded.

NOTE:- Used of ethanol because found easier to penetrate the cellular membrane to extract the intracellular ingredient (polyphenols) from the plant material.

Scientific classification	
Kingdom:	Plantae
(unranked):	Angiosperms
(unranked):	Eudicots
(unranked):	Asterids
Order:	Solanales
Family:	Convolvulaceae
Genus:	<i>Cuscuta</i>
Species:	<i>C. europaea</i>
Binomial name	
<i>Cuscuta europaea</i>	
L.	

Fig. 2 Plant classification.



Fig. 1 *Cuscuta europaea* (Dodder)<sup>13</sup> (Left) General morphology of Dodder, (Right) Tendrils of Dodder when coiled on stem of plant.

### Statistical Analysis

In statistical analysis, ANOVA table was included, and we entered the data to excel program. The data include the average of inhibition zone and the number of isolate of bacteria and other data and we used an ANOVA table to know the difference between the numbers.<sup>14</sup>

### Results and Discussion

The results show that the effect of plant extract on *Staphylococcus aureus* as the best than Amoxicillin drugs, and did not show any significant difference between the concentration of plant extract for bacteria. Also the best result appeared in the concentration of plant extract is 20 mg/ml that is  $25 \pm 1.93$ , and follow 5, 10, 15, 25 mg/ml are  $24.33 \pm 3.2$ ,  $24.33 \pm 1.9$ ,  $22 \pm 2$  and  $20.5 \pm 1.05$ , respectively, and we showed the effect of plant extract and drug on *E. coli* bacteria also (Figs. 3, 4, 5, 6, 7, 8). The

results appeared effective on plant extract on gram positive bacteria and did not show an effect on gram negative bacteria, due to the increasing prevalence of antibiotic-resistant pathogens in the hospital and homes. The deliberate search is in progress for alternative treatment to combat the further spread of antibiotic resistant-pathogen. This result is shown in Table 1.

In some study, the effect of extract plant on gram negative bacteria show more antimicrobial activity than gram positive bacteria, under many concentrations, such as 50, 100, 150, 200 mg/ml. In *Staphylococcus aureus*, the best inhibition zone appear in 200 mg/ml (9.5 mm) and the smallest inhibition zone in 50 mg/ml (7.1 mm), in *E. coli*, the best inhibition zone appears in 200 mg/ml (9.7 mm) and the smallest inhibition zone in 50 mg/ml (8.2 mm),<sup>15</sup> therefore, Researchers have been shown the effect of extract plant activity various from one area to another. The effect of extract plant in *E. coli* less than other species that found in the research,<sup>16</sup> however, in the research, the methanol leaf extract of *Momordica charantia* showing

Table 1. Inhibition zone (mm) of plant extract on *Staphylococcus aureus* and *Escherichia coli*

No. of Bacteria	Inhibition diameter (mm)					LSD <sub>0.01</sub> concentration	
	Anti con Amoxicillin (mg/ml)	Concentration of plant extract (mg/ml)					
		5	10	15	20	25	
<i>Staphylococcus aureus</i>	17.33 ± 0.802 A	24.33 ± 3.2 A	24.33 ± 1.9 A	22.0 ± 2.0 A	25.0 ± 1.93 A	20.5 ± 1.05 A	7.715
<i>Escherichia coli</i>	-	-	-	-	-	-	

\*The number refer to mean of inhibition diameter (mm) ± standard error. \*Homologous horizontally capital letters refer to no significant differences (P < 0.01) between the concentration of plant extract for bacteria.

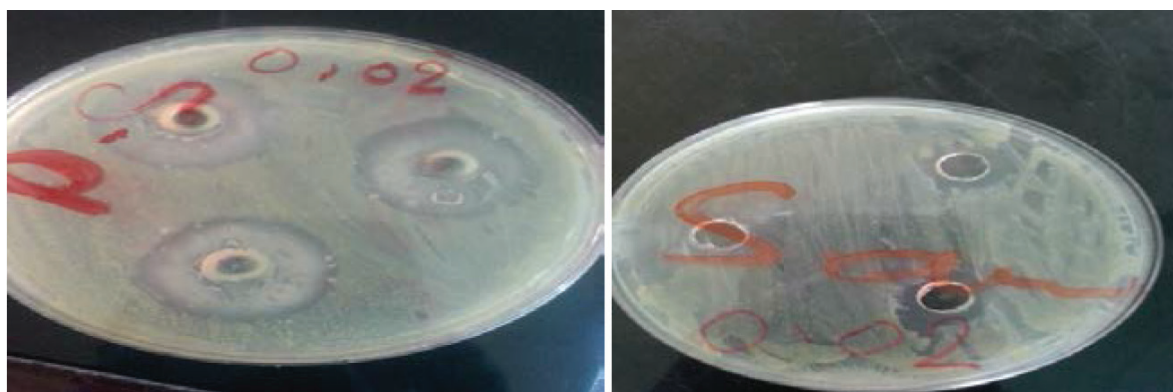


Fig. 3 *S. aureus* Plant conc. 20 mg/ml Control drug conc. 20 mg/ml.

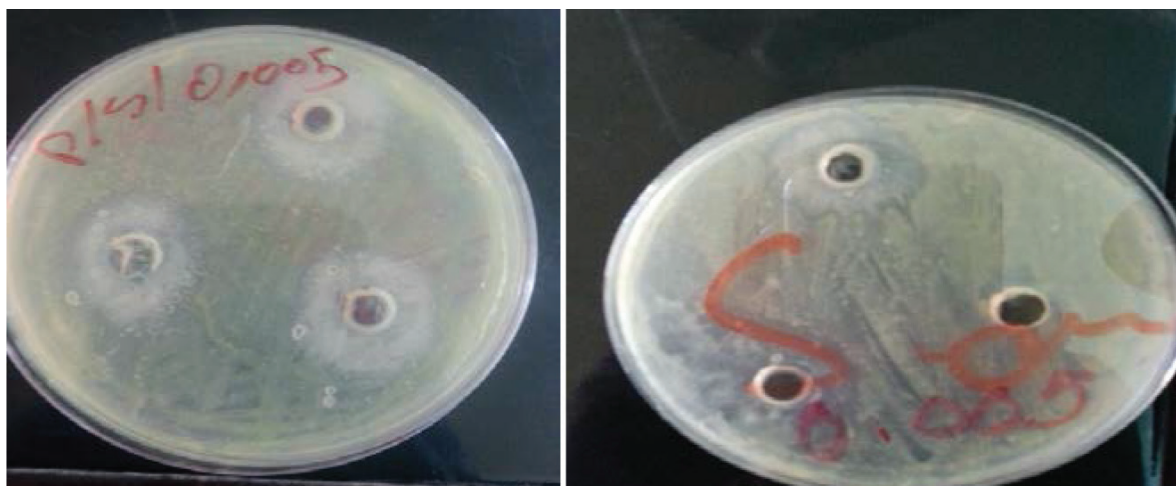


Fig. 4 *S. aureus* Drug conc. 5 mg/ml Plant conc. 5 mg/ml.



Fig. 5 *S. aureus* Drug conc. 10 mg/ml, Plant conc. 10 mg/ml.



Fig. 6 *S. aureus* Drug conc. 15 mg/ml, Plant conc. 15 mg/ml.



Fig. 7 *S. aureus* Drug conc. 25 mg/ml, Plant conc. 25 mg/ml.

significant activity against *S. aureus* (40 mm) and *E. coli* (35 mm), leaf extract of *Ocimum exhiple* high activity against *E. coli* (34 mm), the methanol leaf extract of *Acacia* show significant activity against *E. coli* and *S. aureus* around (15 mm), dark extract of a *Acacianilotica* exhibit high activity against *S. aureus* (15 mm) and *Sidacordifolia* leaf extract possess maximum activity against *S. aureus* (18 mm) and Roots extract of extract

plant showed high inhibitory action against *S. aureus* and least activity in *E. coli*.<sup>17</sup> as well as, It was noted worthy that the lowest concentration of the leaf extract of (50 mg/ml) *P. niruri* was found to be very effective in inhibiting the growth of all the selected strains of *S. aureus* (3 strains), whereas, *P. niruri* has no inhibitory effect on the *E. coli* even at 400 mg/ml, the fruit extracts of *T. bellerica* inhibited the growth of the *S. aureus* at



Fig. 8 *E. coli* Plant conc. 5 mg/ml, Drug conc. 5 mg/ml.

(50 mg/ml) and exhibited growth inhibition of *E. coli* at (200 mg/ml) only.<sup>18</sup> Some study, the researcher is using many extracted plant of which *C. arvensis*; he was obtaining on result, when he used ethanol leave the inhibition zone is obtaining (8 mm) to *E. coli* and *S. aureus* (0 mm), and used aqueous leave; the inhibition zone of *E. coli* (3 mm) and *S. aureus* (0 mm) and when he used ethanol seed; the inhibition zone of *E. coli* (2 mm) and *S. aureus* (2 mm), also when he used aqueous seed; the inhibition zone of *E. coli* (1 mm) and *S. aureus* (2 mm).<sup>19</sup>

## Conclusion

The *Cuscuta europaea* extract plant have high antimicrobial activity on *Staphylococcus aureus*, the crude extract consider

best than drug, The effect of extract on gram positive bacteria best from gram negative bacteria, The best concentration of extract on *S. aureus* is 20 mg/ml.

## Recommendation

The plant extract can be tested against all microorganisms (bacteria, fungi, etc.)

The active compound can be isolated and purified from the extract plant by applying various methodologies.

## Conflict of Interest

None. ■

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