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Studying the efficacy of Eucalyptus Leaves Extract on the
growth of some pathogenic species of bacteria

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Summary

This study was carried out to study the in vitro antimicrobial activity of water and mixture of (methanol and ethanol) alcoholic extract of Eucalyptus Leaves on selected bacterial isolates. The bacteria used in this study were Escherichia coli and Staphylococcus aureus. Eucalyptus Leaves were obtained from the general garden of Erbil city. Disk and well diffusion methods were used for the sensitivity assessment.

Two solvents used to extract Eucalyptus Leaves and the water extract shows significantly the best inhibitory effect on E.coli bacteria more than Staphylococcus aureus in which it seems that it works better on gram negative bacteria (E.coli) comparing to the gram positive bacteria (Staphylococcus aureus).

The statistical analysis of the data conducted by measuring the significancy of the results using t test analysis at level of 1%

Introduction

The aim of our study is to detect the effects of Eucalyptus leaves extract on the activity of bacteria and to know which concentration has more effects.

The use of higher plants and preparations from them to treat infections is an age-old practice. Interests in plants with antimicrobial properties have come to use again because of emergence of resistance strains against antimicrobials such as penicillin (*Cavallito and Bailey, 1944*). Using of plant extracts as defensive or therapeutic tools has begun in many studies and used directly or indirectly in the preparation of pharmaceuticals.

Anticancer, anti-inflammatory, antioxidant, antifungal and antiviral effects have been attributed to the leaf extracts of this plant. In Some studies, it has been reported that phytochemicals such as essential oils, sterols, alkaloids, glycosides, flavonoids, tannins and phenols are effective substances present in Eucalyptus. In recent years with regard to the emergence of multidrugresistant pathogenic bacteria, searching new antibacterial substances from natural sources such as plants has gained more attention (*Seyyednejad, 2014*).

Eucalyptus is a plant that is used in traditional medicine for the treatment as antimicrobial drugs. The antimicrobial activities of extracts of the leaves were evaluated against some common bacteria. The dried powdered leaves were extracted using aqueous and organic solvents (methanol, acetone and water). The antimicrobial activity of the concentrated extracts was evaluated by determination of the diameter of zone of inhibition against both gram negative and gram positive bacteria using the paper disc diffusion method. The least activity was shown by aqueous extract against *E. coli* (6.5 mm) and *Bacillus subtilis* (10 mm) while the maximum was recognized by the alcoholic extract, with a recorded zone diameter for *E. coli* (14 mm) and *Bacillus subtilis* (17 mm). The results of this analysis support the traditional use of Eucalyptus leaves as an antibacterial agent (*Jahan et al. 2011*). The results of Sidkey

and Omran (2017) confirmed the effect of *E. camaldulensis* extracts on retarding the growth of bacteria. The essential oil could control resistant pathogenic bacteria. The greatest effect of essential oil was reported against *Klebsiella pneumoniae* with an inhibition zone diameter of 35 mm and MIC and MBC of 500 and 1500 ppm, respectively (Asiaei et al. 2018).

The dried powder, aqueous and alcoholic extracts of *Eucalyptus* leaves contained resins, tannins, glycosides, saponins, phenols and flavonoids.

Materials and methods

1- Extraction:-

Plant Extract Preparation: - Plant leaves were dried in the shade at room temperature for ten days and then powdered using electronic blender. The first concentration is 0% (control) by using only D.W and the first concentration (C1) of water extract (10%) prepared by mixing 20 gm of finely powdered of plant leaves with 200 mL D.W and the second concentration (C2) is 20% prepared by mixing 40 gm of finely powdered of plant leaves with 200 ml D.W. The first concentration (C1) of alcoholic extract is prepared by mixing 20 gm of finely powdered of plant leaves with mixture of 100 mL of ethanol and 100ml of methanol (10%). The second concentration (C2) is prepared by mixing 40 gm of finely powdered of plant leaves with mixture of 100 mL of ethanol and 100ml of methanol (20%) (Seyyednejad et al.2014).

2- Antibacterial activity test

The antibacterial activity test of the Eucalyptus Leaves Extract against the isolates of bacteria was carried out by the Agar- diffusion method.

a- Preparation of culture media:

MHA is used for antibiotic susceptibility test which prepared as following:

- 1-dissolve 0.76 gm of the medium in 200ml of distilled water
- 2-Heat with frequent agitation and boil for one minute to complete dissolve the medium.
- 3-Autoclave at 121°C for 15 minutes, then cool to room temperature.
- 4-Pour cooled Mueller Hinton Agar in to sterile petri dishes on a level
- 5-Allow cooling to room temperature.
- 6- Store the plates in refrigerator.

b- Isolation of the pathogenic bacteria from the patients:

The bacteria (*E. coli* as a gram negative and *Staphylococcus aureus* as a gram positive) isolated from various specimen (urine, sputum, stool, blood and pus) identified properly by standard microbiological procedures. The isolated bacteria then transferred to the microbiology department and stored in a refrigerator until required.

c- Antibacterial test:

1- Well diffusion method:

By using Agar well diffusion method (*Prabhakar et al, 2012*)

The agar plate surface is inoculated by spreading a volume of the microbial inoculum over the entire agar surface. Then, a hole with a diameter 6 mm is punched with a sterile cork borer and a volume (20–100 μ L) of the extract solution is introduced into the well. Then, agar plates are incubated under suitable conditions depending upon the test microorganism. The antimicrobial agent diffuses in the agar medium and inhibits the growth of the tested bacteria.

2- Disc diffusion method:

Antibacterial activity of leaf extracts were tested using disc diffusion method. The 6-mm diameter disc soaked in the extract placed on the inoculated agar plates. The inoculate of the test organisms were prepared by transferring a loopful of culture into 15 mL of sterile Nutrient Broth (NB Hi media) and incubated at 37°C for overnight. After the inoculate dried, the extract soaked discs were placed on the agar gently pressed down to ensure contact. The inoculated plates were incubated at 37°C overnight. The results were expressed as the diameter of inhibition zone around the paper disk Hoque et al. (2007)

Results and discussion:

Two experiments conducted for this study but the first one is failed because we don't get growth for the bacteria so we can't decide whether there is effect of the extract on the inhibition of the bacteria growth or not.

To overcome this problem we conduct the second experiment by activation the bacteria then cultured on the media by two methods the first one by disk method and the second by well diffusion method to identify which method is better for application.

Generally the zone of inhibition indicate the effect of the extract on the growth of the bacteria by measuring the diameter of the zone and by increasing the diameter of the zone it mean that the Eucalyptus Leaves Extract effected the growth of the bacteria and oppositely by decreasing the diameter it mean that the bacteria has resistance to the Eucalyptus Leaves Extract.

Table (1) Effect of Eucalyptus Leaves Extract on the growth of Escherichia coli by well diffusion method

bacteria	Solvent	treatment	concentration	Inhibition Zone(mm)
<i>Escherichia coli</i>	control	C	0%	6 a
	Water extract	C1	10%	14cd
		C2	20%	16d
	alcohol extract	C1	10%	11b
		C2	20%	13c

Note: Means with the same letters showed no significant differences at level 1%

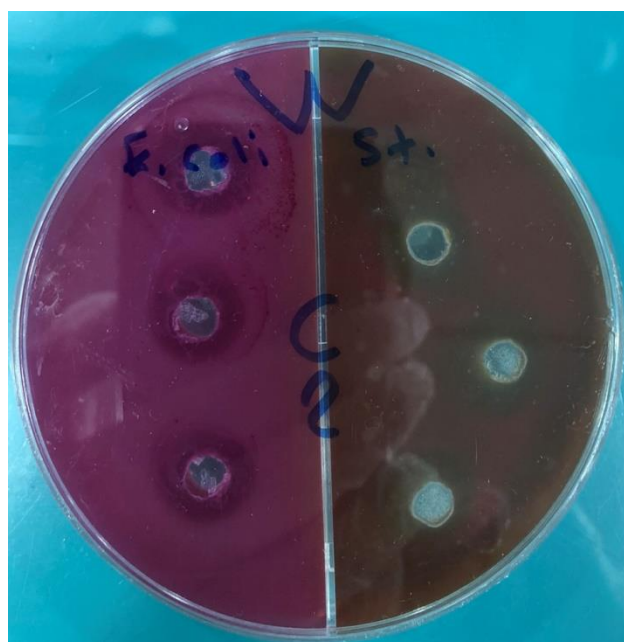


Fig. (1): Effect of water extract of Eucalyptus Leaves on the growth of Escherichia coli and Staphylococcus aureus

The results in Table (1) and figure (1) showed significant differences between the treatments where the higher value 16 mm was recorded for treatment of water extract at higher concentration while the lowest affecting value is recorded for the lower concentration of alcohol extract. So the effect of solvent appeared clearly that water extract has more effect on the inhibition of Escherichia coli bacteria at the higher concentrations than alcohol extracts of Eucalyptus Leaves. These results agreed with results of Raho and Benali (2012).

Table (2) Effect of Eucalyptus Leaves Extract on the growth of Escherichia coli by disk method

bacteria	Solvent	treatment	concentration	Inhibition Zone(mm)
<i>Escherichia coli</i>	control	C	0%	6a
	Water extract	C1	10%	11bc
		C2	20%	15d
	Mixture of alcohol extract	C1	10%	10b
		C2	20%	12c

Note: Means with the same letters showed no significant differences at level 1%

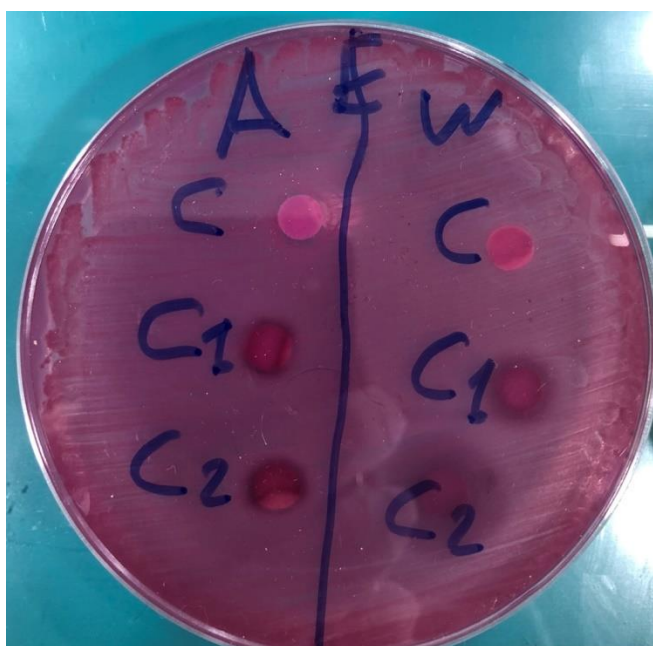


Fig. (2): Effect of water and alcohol extract of Eucalyptus Leaves on the growth of Escherichia coli

The table (2) and fig (2) showed that the higher concentration of water extract has more effect than the alcohol extract by disk method.

Table (3)Effect of Eucalyptus Leaves Extract on the growth of Staphylococcus aureus by well diffusion method:

bacteria	Solvent	treatment	concentration	Inhibition Zone(mm)
Staphylococcus aureus	control	C	0%	6a
	Water extract	C1	10%	10b
		C2	20%	15c
	Mixture of alcohol extract	C1	10%	10b
		C2	20%	13c

Note: Means with the same letters showed no significant differences at level 1%



Fig. (3): Effect of water and alcohol extract of Eucalyptus Leaves on the growth of Staphylococcus aureus by well diffusion method

It is clear from table (3) and fig(3) that the higher significant effect resulted from the application of the higher concentration of water extract of Eucalyptus Leaves on the inhibition of Staphylococcus bacteria comparing to the other concentrations which there are no significant differences between them which agree with results of Seyyednejad (2014).

Table (4) Effect of Eucalyptus Leaves Extract on the growth of Staphylococcus aureus by disk method:

bacteria	Solvent	treatment	concentration	Inhibition Zone(mm)
Staphylococcus aureus	control	C	0%	6a
	Water extract	C1	10%	10b
		C2	20%	12c
	Mixture of alcohol extract	C1	10%	8ab
		C2	20%	10b

Note: Means with the same letters showed no significant differences at level 1%



Fig. (4): Effect of water and alcohol extract of Eucalyptus Leaves on the growth of Staphylococcus aureus by disk method

From table (4) and fig(4) we observe that there is a little effect of the extracts on the inhibition of bacteria growth by disk method where the higher concentration of the water extract (20%) has more effect on growth of the bacteria comparing with low concentration of alcohol extract (10%) and the control.

Table (5) Effect of method of extract application on growth of the two bacteria

Bacteria	Disk method	Well method
E.coli	48	54
Staph. aureus	40	50

Table (5) showed that the application of Eucalyptus Leaves extract by well diffusion method have more effect than application by disk method.

It is clear that Eucalyptus Leaves Extract possess anti-bacterial activity against both gram negative and gram positive bacteria.

Finally we find from table 1 and 2 that the inhibitory effect of Eucalyptus Leaves extracts against Escherichia coli is more effective than the Staph.aureus. This has been attributed to the anti-bacterial activity of Eucalyptus Leaves extract which is more effective against gram negative bacteria than gram positive. This could be also in regard with the nature of permeability of E.coli, which means 20% of membrane of E. coli is made of lipid while that of S.aureus is only made of 2% lipid(Abiy and Berhe 2016),

Conclusion and Recommendations

So we can conclude and say that using water as solvent for Eucalyptus Leaves is more suitable for inhibition of Escherichia coli and staphylococcus than the mixture of methanol and ethanol alcohol.

It is clear that Eucalyptus Leaves extracts possess anti-bacterial activity against both gram negative and gram positive bacteria.

Furthermore, it can be seen that the inhibitory effect of Eucalyptus Leaves extract against E.coli is more effective than Staph.aureus.

On the light of the previous results, the following points can be recommended:

- 1- Conducting further studies regarding the effect of Eucalyptus Leaves extract on other bacteria.
- 2- Extraction and measuring the quantity of the active material in Eucalyptus Leaves extract and then studying their effects.
- 3- Studying the mechanism of action for the compounds present in Eucalyptus Leaves extract and in which mechanism can inhibits the bacteria growth which it is unclear till now.

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