# Antimicrobial effect of Carboxy Methyl Cellulose (CMC) containing aqueous and ethanolic *Eucalyptus camaldulensis* L. leaves extract against *Streptococcus pyogenes*, *Pseudomonas aeruginosa* and *Staphylococcus epidermidis*

**Behrooz Alizadeh Behbahani[1], Farideh Tabatabaei Yazdi[1], Ali Mortazavi[1], Mohammad Mahdi Gholian[*,2], Fatemeh Zendeboodi[3], Alireza Vasiee[1]**

[1]Department of Food Science and Technology, Ferdowsi University of Mashhad, Mashhad, Iran
[2]Young researchers and elite Club, Aligoudarz Brunch, Islamic Azad University, Aligoudarz, Iran
[3]Department of Food Processing, Research Institute of Food Science and Technology, Mashhad, Iran

* Corresponding Author: Email address: m.mahdi.gholian@gmail.com (M. M.Gholian)

## ABSTRACT

Oil from the eucalyptus tree (*Eucalyptus camaldulensis* L.) is used today in many over the counter cough and cold products, to relieve congestion. Eucalyptus oil is also used in creams and ointments to relieve muscle and joint pain, and in some mouthwashes. In this study *Eucalyptus camaldulensis* leaves extracted with water and ethanol 96°and the antimicrobial effects of extracts were evaluated by "using the method of Collins" and "disk agar diffusion method". Antimicrobial properties of Carboxy Methyl Cellulose (CMC) films containing 20, 40, 60, and 80 mg/ml concentration of the extract studied against on *Streptococcus pyogenes* PTCC 1447, *Pseudomonas aeruginosa* PTCC 1310 and *Staphylococcus epidermidis* PTCC 1435. The results showed that aqueous and alcoholic extract were quite effective in 2000 µg/ml concentration on *Streptococcus pyogenes* and *Staphylococcus epidermidis* and have inhibition effect, while both extracts have no certain antimicrobial effect on *Pseudomonas aeruginosa.* Minimum Inhibitory Concentration (MIC) of ethanolic extract of *Eucalyptus camaldulensis* leaves were performed for each microorganism. Minimal Bactericidal Concentration (MBC) for bacteria was performed using the dilution method. The edible films containing mangrove extract presented more effective impact on the growth of *Streptococcus pyogenes* than *Pseudomonas aeruginosa* ($p<0.05$). The result indicates extracts of *Eucalyptus camaldulensis* leaves have the greatest effect on gram-positive bacterium *Streptococcus pyogenes*. As a result, aqueous and ethanloic extracts of *Eucalyptus camaldulensis* leaves, have been strong antimicrobial activity against many food pathogen bacteria.

**Keywords:** *Eucalyptus camaldulensis*, Carboxy Methyl Cellulose, Extract, Antimicrobial effects.

## INTRODUCTION

The emergence of resistance to conventional antimicrobials is a serious problem that physicians face. This necessitates constant development of newer agents, which can inhibit the growth of resistant organisms. Use of medicinal plants has been known for centuries, and therapeutic efficacy of several herbal species has been widely described [1]. Eucalyptus is native to Australia, and the genus Eucalyptus contains about 600 species. Of all the species, *Eucalyptus camaldulensis* is the most widely cultivated in subtropical and Mediterranean regions. As eucalyptus is a fast-growing tree ,and is a suitable ingredient for paper manufacture, there has been extensive overseas forest plantation of eucalyptus trees . Leaves are a byproduct of tree cutting, and the use of the excess leaves for biomass resources is considered to be an important research subject [2]. *Eucalyptus camaldulensis* is one of these plants which have antimicrobial affects of its extract has long been used to treat influenza and colds in most parts of the world.

The Myrtaceae family represents an important source of essential oils with diverse biological activities including bacteriostatic, fungistatic and anti-inflammatory effects. Various Myrtaceae species possess strong antimicrobial potential and their volatile oils are used as antimicrobial and antifungal agents in creams, soaps and toothpastes. Within the family, the Eucalyptus genus has been cultivated and exploited on a large scale for many years. Several species of eucalyptus are used in folk medicine as an antiseptic and

against infections of the upper respiratory tract, such as cold, influenza and sinus congestion [3].

In its native Australia, the *Eucalyptus camaldulensis* tree is the main food for koalas. It's been used in the past as an antiseptic to kill germs and the oil was used in traditional Aboriginal medicines to heal wounds and fungal infections. Teas made of eucalyptus leaves were also used to reduce fevers. Eucalyptus was soon used in other traditional medicine systems, including Chinese, Indian (Ayurvedic), and Greek and European. Therefore according to the problem of bacterial resistance to antibiotics, it is essential to find antimicrobial compounds with minimal side effects. Edible films have received consideration attention in recent years because of their advantage over synthetic films.

The advantage of edible films over other traditional synthetic films is that can be consumed with the packaged products. The films can function as carriers for antimicrobial and antioxidant agents Antimicrobial edible films may supply an effective way to control food-borne pathogens and spoilage microorganisms to thus enhance food safety and reduce product spoilage. The use of edible films as antimicrobial carriers represents an interesting approach for the external incorporation of plant extract onto food system surfaces.

Eucalyptus is one of the most popular medicinal plants. This plant is a rich source of poly phenols and Terpenoids and the base composition (70 to 80 mg/ml) of the leaves are the Eucalyptol or Cineole [4]. Southwell [5] was evaluated the important survival and damaging effects of cineole on tea oil tree and evaluate the antimicrobial activity in order to determine MIC, agar dilution method was used. In this experiment 8-cineole concentration were used on growing of gram-positive, gram-negative and yeast from food pathogen microorganisms. This essential oil showed a wide spectrum of antimicrobial, antifungal, anticandidal, antibacterial, expectorant and cough stimulant activity. Due to its disinfectant action, the essential oil is used externally, applied to cuts and skin infections but it has deleterious effect on the body in high doses [6, 7, 8, 9].

The aim of this study was evaluating of the antimicrobial effects of aqueous and ethanolic Eucalyptus leaves extracts on some of the important food pathogens and spoilage in foods and food products. If the results of the paper have a confirmation about antimicrobial effect of the extract, we can increase the storage time of fruits and vegetables by spraying Eucalyptus extract in the space industrial food storage refrigeration or treat the fruit's cover by Eucalyptus extract.

## MATERIALS AND METHODS

### *Plant material*

*Eucalyptus camaldulensis* fresh leaves collected from Behbahan (Khuzestan Province) and the species were identifyed in the herbarium of Ferdowsi University of Mashhad. The leaves were dried in shadow in appropriate condition and then milled in to fine particle for extraction.

### *Preparation of film solutions*

Films were prepared according to Wang. CMC, were solubilised with distilled water. Glycerol was added as plasticizer to each solution at a constant glycerol: powder ratio of 1:2 (w/w). Glycerol solutions (glycerol and distilled water) were preheated at the designated heating temperature for 5 minutes. All solutions were stirred continuously on a magnetic stirrer hotplate, until powders were completely dissolved. Solutions were placed in 60 and 80°C water bath, held for 30 minutes and subsequently cooled to 40°C.

### *Extract preparation*

Maceration method was used to prepare extracts. The amount 50 gram of Eucalyptus leaves powder was Added to 250 ml ethanol 96 degree or distilled water. The alcoholic extract mixture was preserved at laboratory temperature for 24 hours and was stirred every few hours with a glass rod. The aqueous mixture was boiled for 20 minutes with low flame until the cream colored liquid was obtained. The collecting supernatant was centrifuged by 3000 rpm for 10 min. The resulting extract (supernatant) volume has reached to the original with ethanol or distilled water, and then samples were stored into the dark container at refrigerator temperature after filtering by 0.45 µ Whatman filter paper [10].

### *Determination dry weight of extracts*

At first, the weight of a tube was measured, and then 1ml of aqueous and ethanolic extracts was poured in it. The contents of the tube were dried at room temperature. After drying the extract, the tubes were weighed again. Weight differences are equivalent weight of 1ml

aqueous or alcohol extract. Average of three replicates, was calculated as the dry weight of the extract [11].

***Source of microorganisms***

The used bacterial strains were *Streptococcus pyogenes* PTCC 1447, *Pseudomonas aeruginosa* PTCC 1310 and *Staphylococcus epidermidis* PTCC 1435. Fresh medium was prepared to evaluate the antimicrobial effect.

***Preparation of Microbial suspension***

Microbial suspensions preparation requires 24-hour culture from each microorganism. So, 24 hours before experiments, microorganisms were inoculated from storage medium to nutrient agar medium slope. After 24 hours, the cultures were washed by Ringer solution and microbial suspensions were prepared. Then some of the bacterial suspension was poured in sterile tubes containing Ringer solution and its turbidity, was measured by spectrophotometer at 530 nm wavelength. It was diluted by Ringer solution until the solution turbidity equalizes with 0.5 McFarland standard solutions. Suspension should have contains $1.5\times 10^8$ CFU/ml [12, 13].

***Antimicrobial activity***

Adding extracts to the culture medium "according to the method of Collins [14]" and "disk agar diffusion method" were done and to evaluated the antimicrobial effects of aqueous and alcoholic *Eucalyptus camaldulensis* leaves extracts. Then 0.2 gram of aqueous and ethanol extract, were added to 5 ml of sterile distilled water. Then it was stirred with vortex system to assist being steady. Then 1 ml of this solution was added to sterile plates. The final concentration of the extract was 2000 µg/ml [15]. In the next step, Mueller Hinton agar (Merck-Germany) medium were sterile and used for bacteria, were added to the plates, and placed at room temperature, so the medium was prepared. One loop of each standard strain culture media was cultured inoculums on these medium. The plates were incubated for 48 hours at 37 ° C. The culture with extract and without bacteria was used as control [15]. The disk agar diffusion method, at first a loop of each standard strain culture media was cultured on the plates, and then paper discs (from Whatman filter with 6 mm diameter), and 6 mm diameter cut from the films were placed on Mueller Hinton (Merck) plates were saturated with 100 µl of the test compound allowed to dry and was introduced on the upper layer of the seeded agar plate with 20, 40, 60 and 80 mg/ml extract concentrations, were prepared in distilled water and was treated with Eucalyptus extract and placed in culture medium by Sterile loop. Then it was fixed on the media with a light little pressure. After incubation time, the diameter of free zone was measured exactly by using a ruler in millimeters. All experiments were performed with 3 replicates [16].

***Determination of Minimum Inhibitory Concentration (MIC)***

MIC was determined according to agar dilution method [17]. Various concentrations (20, 40, 80, 160, 320, 640, 1280, and 2560 mg/ml) of extract was prepared in 10 cm experimental tubes containing Mueller Hinton broth for bacteria. Each tube contains 9 ml of Mueller Hinton for bacteria were sterilized by autoclaving. On cooling, 1 ml of each extract (watery & ethanoli) concentration were added to each tube, to make the final concentrations of 2, 4, 8, 16, 32, 64, 128, 256 mg/ml. The mixture of Mueller Hinton and extract was poured into plates aseptically in a laminar flow cabinet. On solidification of the agar medium, 2 µl of adjusted spore suspension were added to each plate by micropipette and incubated 35 °C for bacteria [18]. The Mueller Hinton without any herbal extract served as control. The MIC was regarded as the lowest concentration of the extract that did not show any visible growth after 12 days of incubation (compared with control)

***Determination of Minimum bactericidal concentration (MBC)***

The in vitro based on MBC was determined for each extracts (watery & ethanoli) as previously described [19] with slight modifications. The MBC were determined by incorporating various concentrations of extracts (2-256 mg/ml) in muller Hilton broth for bacteria.The tubes which showed no visible growth after 2 days incubation were subculture on extract free Mueller Hinton plates and incubated at 35°C for 2days [18]. The MBC was regarded as the lowest concentration of the extract that prevented the growth of any bacteria colony on the solid medium.

***Statistical analysis***

All the assays were carried out in triplicates. The experimental results were expressed as mean ± standard deviation. The data were analyzed using one way analysis of variance (ANOVA) using SPSS version 17.

## RESULTS

The results of the antimicrobial effects of aqueous and alcoholic extracts, by "using the method of Collins [14]" were shown in Table 1. The results showed 2000 µg/ml concentration of both aqueous and alcoholic extracts, were quite effective on *Streptococcus pyogenes* and *Staphylococcus epidermidis* and prevented their growth over the medium. However, 2000 µg/ ml concentration aqueous and alcoholic extracts, have no significant antibacterial effect on *Pseudomonas aeruginosa* and not able to prevent the growth of bacteria on culture. Negative sign (-) in the table shows the growth of bacteria on culture, and the lack of antimicrobial activity of aqueous and alcoholic Eucalyptus extracts. The results of the antimicrobial effects of aqueous and alcoholic Eucalyptus extracts, by "the agar diffusion method" are presented in Table 2.

**Table 1**. Antimicrobial effects of 2000µg/ml ethanolic and aqueous *Eucalyptus camaldulensis* leaves extract concentrations, on *Streptococcus pyogenes*, *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* (using the method of Collins [14])

| **Microorganism** | **Antimicrobial effects of *E. camaldulensis* leaves extract** |
|---|---|
| Aqueous *P. aeruginosa* | - |
| Aqueous *S. pyogenes* | + |
| Aqueous *S. epidermidis* | + |
| Ethanolic *P. aeruginosa* | - |
| Ethanolic *S. pyogenes* | + |
| Ethanolic *S. epidermidis* | + |

- (-) in Table showed the growth of bacteria on culture and the lack of antibacterial activity of aqueous Eucalyptus extract
- (+) in Table showed no bacterial growth on culture and antibacterial activity of aqueous Eucalyptus extract

**Table 2**. Average diameter (mm) of microbial free zone area of ethanolic and aqueous *Eucalyptus camaldulensis* leaves extract on *P. aeruginosa, S. pyogenes* and *S. epidermidis* (disk agar diffusion method).

| | **concentration (mg/ml)** | | | |
|---|---|---|---|---|
| **Microorganism** | **20** | **40** | **60** | **80** |
| Aqueous *P. aeruginosa* | - | - | 6.10±0.57 | 6.70 ±0.57 |
| Aqueous *S. pyogenes* | 13.30±0.57 | 15.50±0.57 | 17.00 ±0.28 | 20.50±0.28 |
| Aqueous *S. epidermidis* | 12.2±0.57 | 13.6±0.57 | 15.20±0.57 | 17.90±0.28 |
| Ethanolic *P. aeruginosa* | - | - | 7.70 ±0.57 | 9.40±0.28 |
| Ethanolic *S. pyogenes* | 16.10±0.76 | 18.90±0.57 | 22.20 ±0.57 | 25.90±0.57 |
| Ethanolic *S. epidermidis* | 14.30±0.28 | 16.20±0.57 | 18.40 ±0.76 | 20.30±0.57 |

[a]Values are means ± standard deviations, n=3.
(-) in Table showed no inhibitory effects was shown

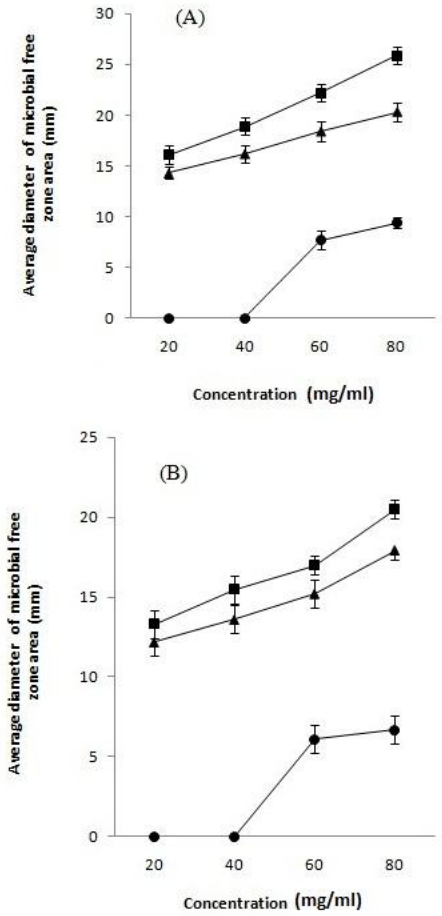


**Figure 1.** Antimicrobial activity of ethanolic (A) and aqueous (B) at different concentration of *Eucalyptus camaldulensis* leaves extract on *Pseudomonas aeruginosa*, *Streptococcus pyogenes* and *Staphylococcus epidermidis*. ●, *Pseudomonas aeruginosa*; ■, *Streptococcus pyogenes*; ▲, *Staphylococcus epidermidis* (Measurements were carried out in triplicate).

The results of the edible films antimicrobial effects of aqueous and alcoholic Eucalyptus extracts, by "the agar diffusion method" are presented in Tables 3.

**Table 3**. Average diameter (mm) of microbial free zone area of edible films containing ethanolic and aqueous *Eucalyptus camaldulensis* leaves extract on *P. aeruginosa*, *S. pyogenes* and *S. epidermidis* (disk agar diffusion method).

| | **concentration (mg/ml)** | | | |
|---|---|---|---|---|
| **Microorganism** | **20** | **40** | **60** | **80** |
| Aqueous *P. aeruginosa* | - | - | - | - |
| Aqueous *S. pyogenes* | 10.20±0.28 | 12.70±0.57 | 14.00 ±0.28 | 17.20±0.57 |
| Aqueous *S. epidermidis* | 8.40±0.57 | 10.70±0.76 | 11.70±0.57 | 14.10±0.28 |
| Ethanolic *P. aeruginosa* | - | - | 6.30 ±0.57 | 7.60±0.28 |
| Ethanolic *S. pyogenes* | 12.40±0.76 | 14.40±0.57 | 17.30±0.57 | 19.90±0.76 |
| Ethanolic *S. epidermidis* | 9.90±0.28 | 11.10±0.57 | 12.90 ±0.76 | 15.90±0.57 |

[a]Values are means ± standard deviations, n=3.
(-) in Table showed no inhibitory effects was shown

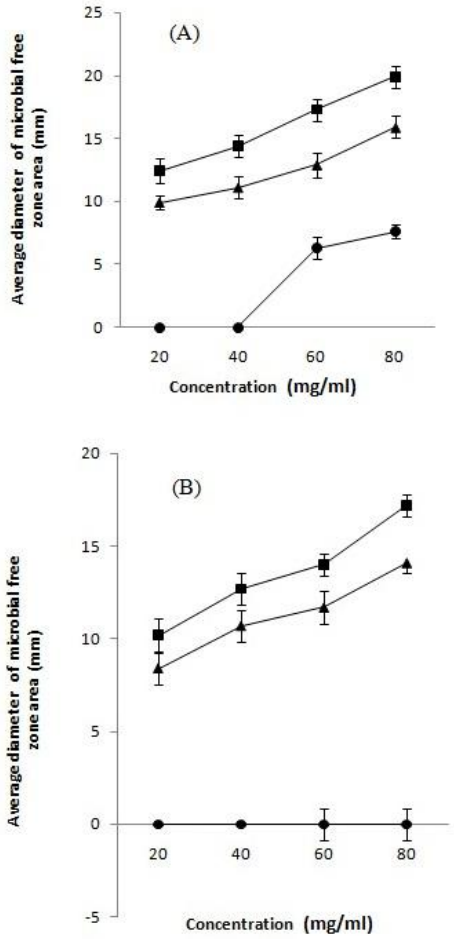


**Figure 2.** Antimicrobial activity of ethanolic (A) and aqueous (B) at different concentration of *Eucalyptus camaldulensis* leaves extract on *Streptococcus pyogenes*, *Pseudomonas aeruginosa* and *Staphylococcus epidermidis*. ●, *Pseudomonas aeruginosa*; ■, *Streptococcus pyogenes*; ▲, *Staphylococcus epidermidis* (Measurements were carried out in triplicate).

**Table 4**. Minimum Inhibitory Concentration (MIC) of ethanolic and aqueous *Eucalyptus camaldulensis* leaves extract on *Streptococcus pyogenes*, *Pseudomonas aeruginosa* and *Staphylococcus epidermidis*

| | **concentration (mg/ml)** | | | | | | |
|---|---|---|---|---|---|---|---|
| **Microorganism** | **64** | **32** | **16** | **8** | **4** | **2** | **control** |
| Aqueous *P. aeruginosa* | + | - | - | - | - | - | - |
| Aqueous *S. pyogenes* | + | + | + | + | - | - | - |
| Aqueous *S. epidermidis* | + | + | - | - | - | - | - |
| Ethanolic *P. aeruginosa* | + | + | - | - | | - | - |
| Ethanolic *S. pyogenes* | + | + | + | + | + | - | - |
| Ethanolic *S. epidermidis* | + | + | + | + | - | - | - |

+: Positive inhibition
- : Negative inhibition

MIC results of the ethanolic extract of *Eucalyptus camaldulensis* leaves are given in Table 4 and Figure 1. The results shows that MIC of ethanolic extract of *Eucalyptus camaldulensis* leaves for *Streptococcus pyogenes* was 4 mg/ml, for *P. aeruginosa* was 32 mg/ml and for the mold *S. epidermidis* was 8 mg/ml. The results indicate that ethanolic extract of *Eucalyptus camaldulensis* leaves mostly had been effective on *S. pyogenes* and has the least impact on *P. aeruginosa*.

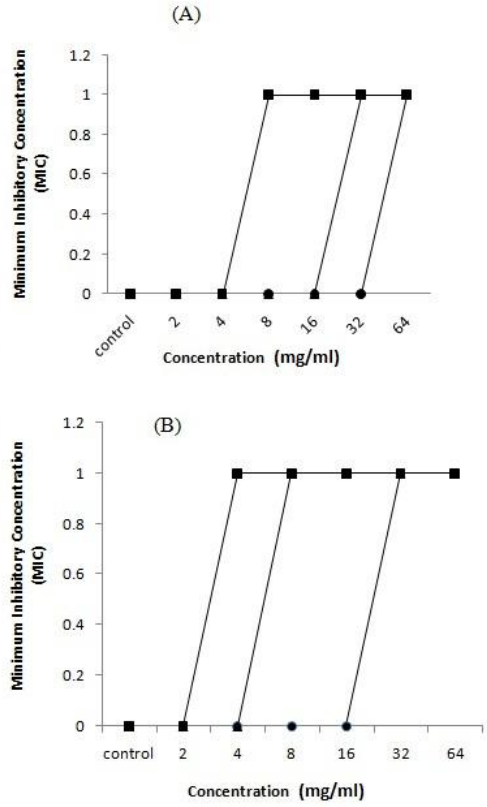


**Figure 3.** Minimum Inhibitory Concentration (MIC) of ethanolic (A) and aqueous (B) at different concentration of *Eucalyptus camaldulensis* leaves extract *Streptococcus pyogenes*, *Pseudomonas aeruginosa* and *Staphylococcus epidermidis*. ●, *Pseudomonas aeruginosa*; ■, *Streptococcus pyogenes*; ▲, *Staphylococcus epidermidis* (Measurements were carried out in triplicate).

MIC results of the aqueous extract of *Eucalyptus camaldulensis* leaves are given in Table 4 and Figure 2. The result show that MIC of aqueous extract of *Eucalyptus camaldulensis* leaves for *S. pyogenes* was 8 mg/ml, for *P. aeruginosa* was 64 mg/ml and for the mold *S. epidermidis* was 32 mg/ml. The results indicate that aqueous extract of *Eucalyptus camaldulensis* leaves had been effective on *S. pyogenes* and has the least impact on *P. aeruginosa*.

Minimum Bacterial Concentration (MBC) results of the aqueous extract of *Eucalyptus camaldulensis* leaves are given in Table 5. The results shows that MBC of aqueous extract of *Eucalyptus camaldulensis* leaves for *S. pyogenes* was 32 mg/ml, for *P. aeruginosa* was 256 mg/ml and *S.epidermidis* was 128 mg/ml. Minimum Bacterial Concentration (MBC) results of the ethanolic extract of *Eucalyptus camaldulensis* leaves are given in Table 5. The results shows that MBC of ethanolic extract of *Eucalyptus camaldulensis* leaves for *S. pyogenes* was 8 mg/ml, for *P. aeruginosa* was 128 mg/ml and *S.epidermidis* was 32 mg/ml.

**Table 5**.Minimum Bacterial Concentration (MBC) of ethanolic and aqueous *Eucalyptus camaldulensis* leaves extract on *Streptococcus pyogenes*, *Pseudomonas aeruginosa* and *Staphylococcus epidermidis*

| | **concentration (mg/ml)** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Microorganism** | **256** | **128** | **64** | **32** | **16** | **8** | **4** | **2** | **control** |
| Aqueous *P. aeruginosa* | + | - | - | - | - | - | - | - | - |
| Aqueous *S. pyogenes* | + | + | + | + | - | - | - | - | - |
| Aqueous *S. epidermidis* | + | + | - | - | - | - | - | - | - |
| Ethanolic *P. aeruginosa* | + | + | - | - | - | - | - | - | - |
| Ethanolic *S. pyogenes* | + | + | + | + | + | + | - | - | - |
| Ethanolic *S. epidermidis* | + | + | + | + | - | - | - | - | - |

+: Positive inhibition
- : Negative inhibition

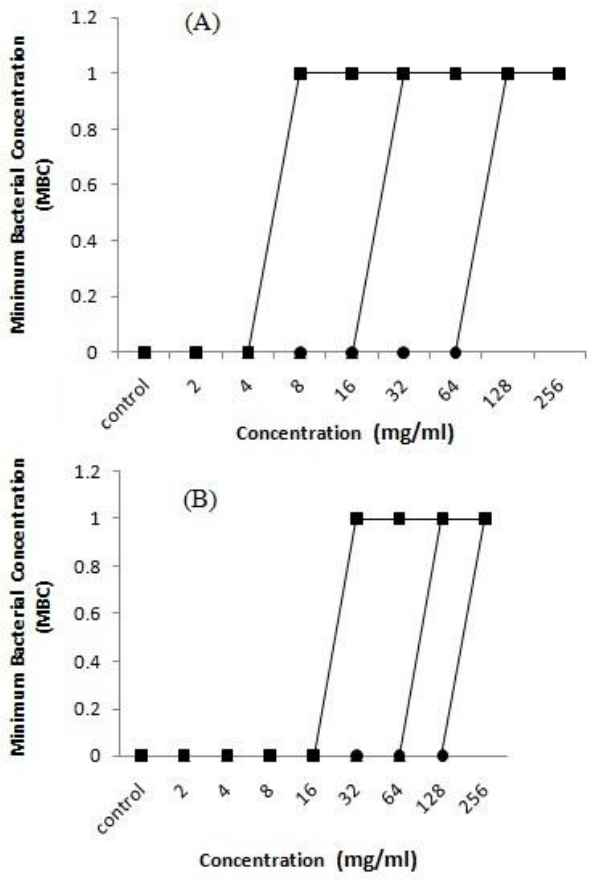


**Figure 4.** Minimum Bacterial Concentration (MBC) of ethanolic (A) and aqueous (B) at different concentration of *Eucalyptus camaldulensis* leaves extract on ●, *Pseudomonas aeruginosa*; ■, *Streptococcus pyogenes*; ▲, *Staphylococcus epidermidis* (Measurements were carried out in triplicate).

## DISCUSSION

Based on the results, aqueous and ethanolic leaf extract of *Eucalyptus camaldulensis* in this study have significant antimicrobial activity on the studied microorganisms. Antimicrobial effect of the extracts was different, depending on the type of microorganisms, thus, the gram-positive bacterium *Streptococcus pyogenes*, was higher sensitivity compared to gram-negative bacteria *Pseudomonas aeruginosa* (Figure 1, 2), and showed inhibitory effects at lower concentrations of *Eucalyptus camaldulensis* leaves extracts.

gram-positive bacteria are more sensitive than gram-negative bacteria to Eucalyptus camaldulensis leaves extract, Due to differences in cell structure of gram-negative and gram-positive bacteria, because gram-positive bacteria have more mucopeptide in their cell wall composition while gram-negative bacteria have only a thin layer of mucopeptide and most of their cell structure is lipoprotein and lipo-polysaccharides. Thus, gram-negative bacteria are more resistant [20,21]. These points were consistent with the results obtained in this study. The results showed all aqueous and alcoholic extract concentrations have inhibition effect on growth of *Streptococcus pyogenes* and *Staphylococcus epidermidis* However, aqueous and alcoholic extract of *Eucalyptus* leaves were able to inhibitory of growth the *Pseudomonas aeruginosa* only in 60 and 80 mg/ml concentrations and no antimicrobial activity was observed at 20 and 40 concentrations. Oyedeji [22] examined antimicrobial properties five species *Eucalyptus* essential oils. At 5 mg/ml concentration of five species *Eucalyptus* leaves volatile oil, have been significant antimicrobial properties, against gram-positive bacteria, gram-negative bacteria and molds. Also, alcoholic extract compared to the aqueous extract was more effective and has a greater deterrent. The reason of these phenomena may be extracting more effective materials extracted by ethanol from *Eucalyptus*. These results are consistent with the findings of a study by Mahasneh [23] on Qataris mangrove species and it was found the aqueous mangrove extract, did not have a significant antimicrobial effect, and the butanol extract, is able to inhibit *Pseudomonas aeruginosa*. Sattari [11] showed 3.2 µg/ml concentration of alcoholic *Eucalyptus* extract and 17.5 µg / ml concentration of aqueous *Eucalyptus* extract, can be good to prevent the growth of *Pseudomonas aeruginosa* standard isolates. Tian [24] investigated the antibacterial effects of "Galla chinesis" (a medicinal plant native to China) reported that the juice extracted by the solvent ethyl acetate, ethanol and water are the highest antibacterial effect. In this study the plant extracts were showed that gram-positive bacteria (*Bacillus cereus*, *Staphylococcus aureus*, *Bacillus Subtlis*) were more sensitive than gram negative bacteria (*Escherichia coli*, *shigella dysenteriae*). This result was consistent with the findings of this study [24]). In another study researchers studied the antibacterial effects *Triticum sativum* Lam, and experiments were done to study the antibacterial effect of aqueous, ethanol, ethyl ether and hexane extract of *Wheat* on some microorganisms, and they found the antimicrobial properties of the plant.

The results showed that aqueous extract had no antimicrobial effect, although, organic extracts have the better effects on gram-positive bacteria in compare to gram-negative. This result is consistent with finding of this study (Table 1, Figures 1 and 2).

MIC concentrations of ethanolic extract of *Eucalyptus camaldulensis* leaves for *Streptococcus pyogenes* was 4 mg/ml, for *Pseudomonas aeruginosa* was 32 mg / ml, and for *Staphylococcus epidermidis* 8 mg/ml while the MIC concentrations of aqueous extract of *Eucalyptus camaldulensis* leaves for *Streptococcus pyogenes* was 8 mg/ml, for *Pseudomonas aeruginosa* was 64 mg/ml and for *Staphylococcus epidermidis* 32 mg/ml. Several mechanisms are discussed to explain the antimicrobial effect. Kotzekidou [25[ found that the antimicrobial compounds in the plant extract, have interaction with the phospholipids' two layers membrane, and affect the permeability of the bacterial cell membrane, and released the intracellular components.

In many studies, the mechanism of the cell wall is considered. They have reported that cell wall and cell membrane affected and changed their permeability cause Release of intracellular contents, which can be associated with impaired membrane function, such as electron transfer, enzyme activity or nutrient uptake. MBC of ethanolic extract of *Eucalyptus camaldulensis* on *Streptococcus pyogenes* was 8 mg/ml, for *Pseudomonas aeruginosa* was 128 mg/ml and for *Staphylococcus epidermidis* 32 mg/ml. While MBC aqueous extract of *Eucalyptus*

*camaldulensis* on *Streptococcus pyogenes* was 32 mg/ml, for *Pseudomonas aeruginosa* was 256 mg/ml and for *Staphylococcus epidermidis* 126 mg/ml. Burt (26) reported that a hydrophilic outer membrane, composed of lipids and LPS, with the property of selective permeability, is an important factor in the resistance of gram-negative bacteria to antimicrobial compounds.

Delaquis [27] were evaluated the antimicrobial activity of essential oils and fractions of dill, parsley, coriander and *Eucalyptus* tested alone or in mixed. Essential oils of this plant was analyzed by fractional distillation and evaluated by gas chromatography and spectroscopy. Then *Salmonella typhimurium*, *Listeria monocytogenes*, *Pseudomonas fragi*, *Serratia*, *Enterobacter agglomerans*, *Yersinia enterocolitica*, *Bacillus cereus*, *Saccharomyces cerevisiae* and *Streptococcus* were tested. The minimum inhibitory concentration (MIC) was determined for each of fractions against gram-positive bacteria, gram-negative and yeast. Results showed mixture fraction, increasing, -synergistic or antagonistic effects against microorganisms. Eucalyptol or alpha-cineole are highest materials identified in the *Eucalyptus* leaves and have more diverse biological effects, including antimicrobial agent. 12.5 μm/ ml and 6.25 μm/ ml MIC has been reported for *Streptococcus mutans* and *Streptococcus sobrinus* respectively [28]. The results showed of the effect antimicrobial edible films containing of extracts aqueous and alcoholic Eucalyptus at all concentrations have inhibition effect on growth of Streptococcus pyogenes and *Staphylococcus epidermidis* However, alcoholic extract of *Eucalyptus* leaves were able to inhibitory of growth the *Pseudomonas aeruginosa* only in 60 and 80 mg/ml concentrations and no antimicrobial activity was observed at 20 and 40 concentrations, the advantages of using an edible film with extract plant for food products are that it may be easy to use and it may be able to enhance quality and extend the shelf life while reducing packaging waste. Moreover, in this regard, extract plant is a valuable component for processing biodegradable packaging which can extend shelf-life and inhibit pathogens and spoilage. Therefore, using *Eucalyptus camaldulensis* as a natural antimicrobial compounds "*in vitro"* requires further research on mechanism of the pharmacy plant on the microorganisms. The results indicate that alcoholic extract of *Eucalyptus camaldulensis* leaves has a greater impact on all strains in compare to aqueous extract, probably because of more efficient extraction by ethanol.

In conclusion the article suggests that *Eucalyptus camaldulensis* leaf extract "*in vitro*", have considerable antimicrobial ability over the studied strains. In addition, further studies need to be performed *"in vivo"*, to identify the effective dose of the extract on the microorganisms, and finally introduce the extract as a natural and novel antimicrobial compound.

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