# Evaluation the Antimicrobial Activity of Methanolic and Aqueous Extracts of the Aerial Parts of *Chrozophora oblongifolia* Against some Microbes

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**Abstract**

The genus Chrozophora is a large genus belonging to the family Euphorbiaceae with adifference, Species that possess different biological activities such as *C. oblongifolia* that possess antioxidant and antimicrobial activity. Due to the lack of research related to studying the microbial effect of *C.oblongifolia*e this research aims to evaluation the inhibitory of Methanolic and aqueous extracts of the aerial parts of C. oblongifolia against some microbes. The aqueous and alcoholic extracts were prepared in the laboratory of the Pharmacy College University of Aden . The results of this study both alcoholic and aqueous extracts had significant effects at all microgronisms tested indicating by increasing of inhibition diameter zone compare to control. However the ethanolic extract showed higher effects comparing to the aqueous extracts. This effects of plant extracts due to available of many active ingredients in chrozophora plants.

**Key words**: antimicrobial, chrozophora, flucanzole, ciprofolxacin

## 1. Introduction:

Plants are the main and alternative source for treating infectious diseases. In the past years, a clear interest in using medicinal plants has emerged with the development of countries due to the entry of derivatives of these plants into the pharmaceutical industry, which were found to be safe and their use does not lead to the emergence of side effects. Among these plants is *Chrozophora oblongifolia*, where it belongs to the family Euphorbiaceae, which is a family of sporophyte, as mentioned in the research of (Kamel et al.,2016). Genus Chrozophora is a large genus belonging to family Euphorbiaceae with different species which possess different biological activities as C. oblongifolia which possess antioxidant and antimicrobial activity (Abdallah et al .2022). The whole plant is

utilized for wounds to improve healing, other parts such as seeds and leaves are utilized as a laxative, a depurative agent and in treating skin diseases( **Kamel et al, 2018** )

Due to the lack of research on this plant and the lack of research for Chrozophora's plant in warm and coastal areas in Yemen further this study to evaluation the inhibitory of Methanolic and aqueous extracts of the aerial parts of C. oblongifolia against some microbes.

## Materials and Methods

### Preparation of plant material

The aerial parts (fruits, leaves, stems) of *C. oblongifolia* were collected from Jarad r egion- Lhij governorate. The plant was identification by prof. Dr. Abdul Nasser Al-Jifri, Professor of Plant taxonomy, collage of Education, Department of Biology, University of Aden. The collected *C. oblongifolia* plants were washed with tap water and distilled w ater and shade dried, then powdered in an electric grinder.

### Preparation of methanolic and aqueous Extracts:

The extraction process of The powdered plant material carried out was at the College of P harmacy pharmacology lab, University of Aden.

For Preparation of alcohol extract by using 70% methanol as polar solvent.50 g of p lant powder was soaked in 500 ml of 70% methanol and placed in a shaker for 24 hours and suspension was filtered using gauze first to get rid of the large fractions and then by filter paper for several hours(Al-Musayeib et al,.2012) and (Kothawade 2019). The filterat ed were concentrated to dryness in the oven at 45°C for 24 hours then the dryness extract was left at room temperature in pertri dishes and wrapped in alumunium foil and kept in the refrigerator.

For Preparation of aqueous extract 50 g of dried powder plant soaked in 500 ml of d istilled water, then placed in a shaker for 24 hours and preparation was filtered using g auze first to get rid of the large fractions and then by filter paper for several hours (Al-M usayeib *et al*,.2012 and Kothawade *et al* 2019). The filterated were concentrated to dryn ess in the oven at 45°C for 24 hours then the dryness extract was left at room temperatu

re in pertri dishes and wrapped in alumunium foil and kept in the refrigerator.

**Antimicrobial Activity:**

The antimicrobial activity of Methanolic extract and Aqueous extract of *C. oblongifolia* against Gram positive bacteria: *S. aureus*, Gram Negative bacteria: *P. aeruginosa* and *E. coli*, and Fungi (*C. albicans*) Carried out in Supreme Board of Drugs and Medical Appliances at quality control laboratory for drugs and medical appliances in Aden Governorate / Khor Maksar Distric.

**Disc Preperation**

The 6mm (diameter) discs were prepared from whatmann No. 1 filter Paper the discs were sterilized by autoclave at 12°C. After the sterilization the moisture discs were dried on hot air oven at 50°C.Then plant extract discs and control discs were prepared.

**The** antibacterial **and antifungal activity of *C. oblongifolia***

The antibacterial and antifungal activity studies were carried out by disc diffusion technique. The sterile Mueller Hinton agar plates for bacteria and Sabauroud dextrose agar for *C. albicans* plates were prepared. In this study the bacterial test organisms like *Staphylococcus aureus* and *Escherichia coli* were spread over the Mueller Hinton agar by using separate sterile cotton buds. Then the fungal test organism *C. albicans* wAS spread over the Sabauroud dextrose agar plates. After the microbial lawn preparation 50 microns of methanolic and aqueous plant extracts with different concentration were inoculated into one wells in each organism in plates and also control discs were prepared. All bacterial plates were incubated at 37°C for 24 hrs and fungai plates at 25°C for 72hrs. The diameter of the minimum zone of inhibition was measured in mm. For each test, three replicates were performed .

**Statically analysis:**

The data were statistically analyzed using SPSS 16 for windows. Duncan test at probability level 0.05 was used to separate the means when the ANOVA indicated a significant effects of plant extracts.

**RESULTS**

**Antimicrobial Activity of methanolic extraction:**

The results of this study revealed to evaluate antimicrobial activities of methanolic and aqueous extracts of *chrozophira* .The aerial parts extracts using methanol water of *chrozophira* were tested for their antimicrobial efficiency against pathogenic bacteria and fungi (*Staphylococcus aureus, pseudomonas aeruginosa , E.coli*,) and fungi like ( *Candida Albicans* ) at different concentration those 0, 200,400,800 and 1000 mg. The standard drugs used for comparison were ciprofloxacin and Fluconazole against bacteria and fungi respectively . The ethanolic extracted tested for their antibacterial activity, showed moderate to high activity against both gram positive and gram negative bacteria. The higher antimicrobial activity of ethanolic extract against bacteria and fungi were showed a highest inhibition zone at the higher concentration (1000 mg ) except for E.coli that shown not affected by the 1000 mg concentration of methanolic extracts.The results of this study showed significant different among various of methanolic extract on all microbes indicating by diameter of inhibition zoon as show in figure 1 and 2.

The result of Methanolic extract of aerial part *C. oblongifolia against S. aureus* ( figure1 A) Showed that all concentrations had Significant difference between them, no Significant difference between 400mg/ml and 200mg/ml , which produced inhibition zone diameters 16 and 15.7mm respectively , and no Significant difference between 800mg/ml and 600mg/ml , which produced inhibition zone diameters 18.7 and 18mm respectively but the Positive control Ciprofloxacin showed increasing inhibition zone significantly on *S. aureus* compared with all concentrations.While doesn't inhibition zone of the negative control(0mg).The Maximum inhibition zone of high concentrations of Methanolic extract of aerial part *C. oblongifolia* against *S. aureus* was appear at

concentration 1000mg (figure 1 A). with Inhibition zone diameters 20mm.

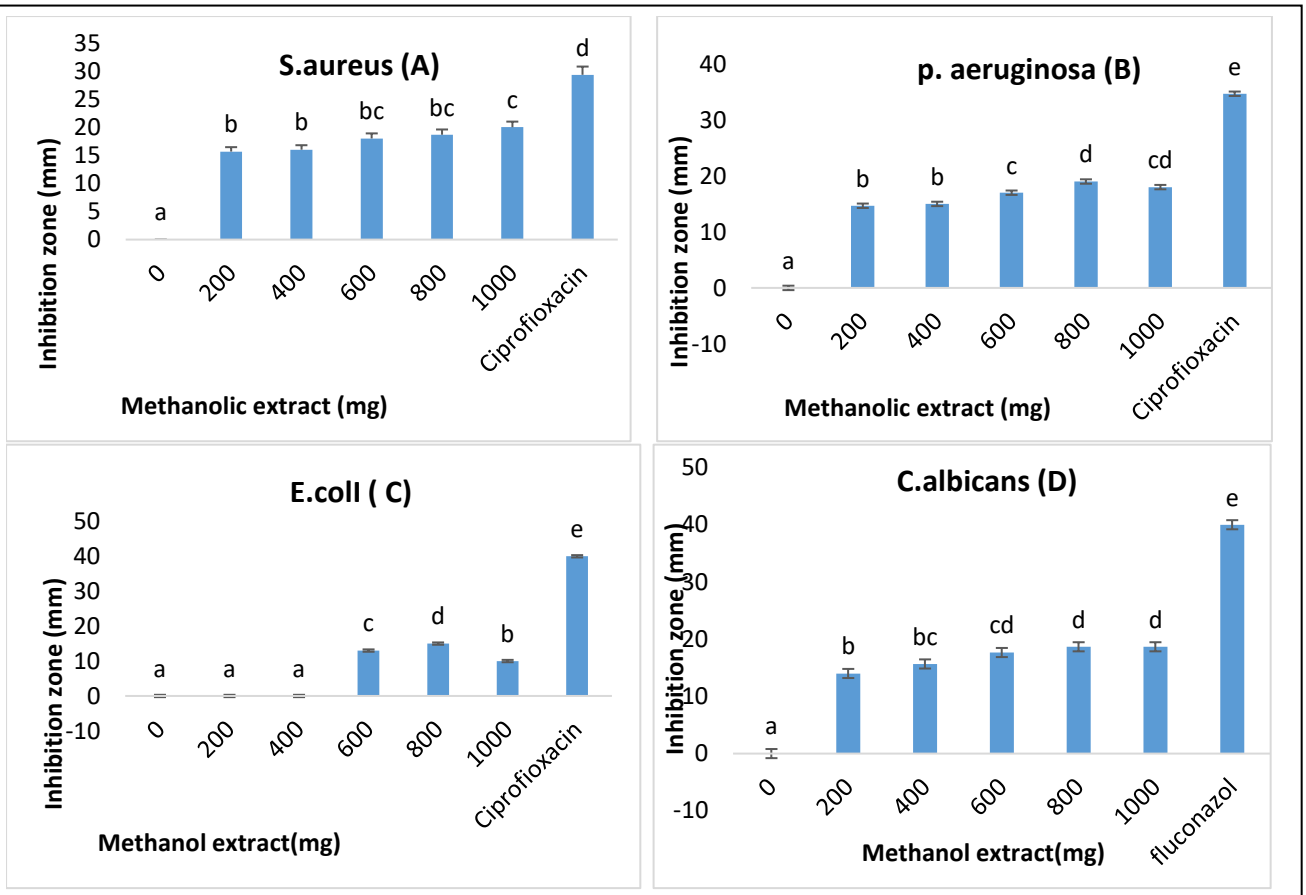


**Figure 1**: Antimicrobial activity of methanolic extract on microbes. *S. aureus* (a), *pseudomonas* (b), *E. coli* (c) and *c. albicans* (d). Bars represent means ± SE. Different letters above the bars indicate significant differences between the different concentration compared to the positive and negative control, according to Duncan test ($\alpha$ = 0.05)

while the antimicrobial activity of methanolic extract aginst *P. aeruginosa* indicated that all concentrations had Significant difference between them and no Significant difference between 400mg/ml and 200mg/ml , which produced inhibition zone diameters 15 and 14.7mm respectively and no Significantly difference between 800mg/ml and 1000mg/ml , which produced inhibition zone diameters 19 and 18mm respectively but the Positive control Ciprofloxacin showed higher antimicrobial activity against *Pseudomonas aeruginosa* compared ta all methanolic concentrations. While doesn't inhibition zone of

control negative (0mg).The Maximum inhibition zone of Methanolic extract of aerial part *Chrozophora oblongifolia* against *Pseudomonas aeruginosa* was produced at concentration 800mg/ml with Inhibition zone diameters 19mm.

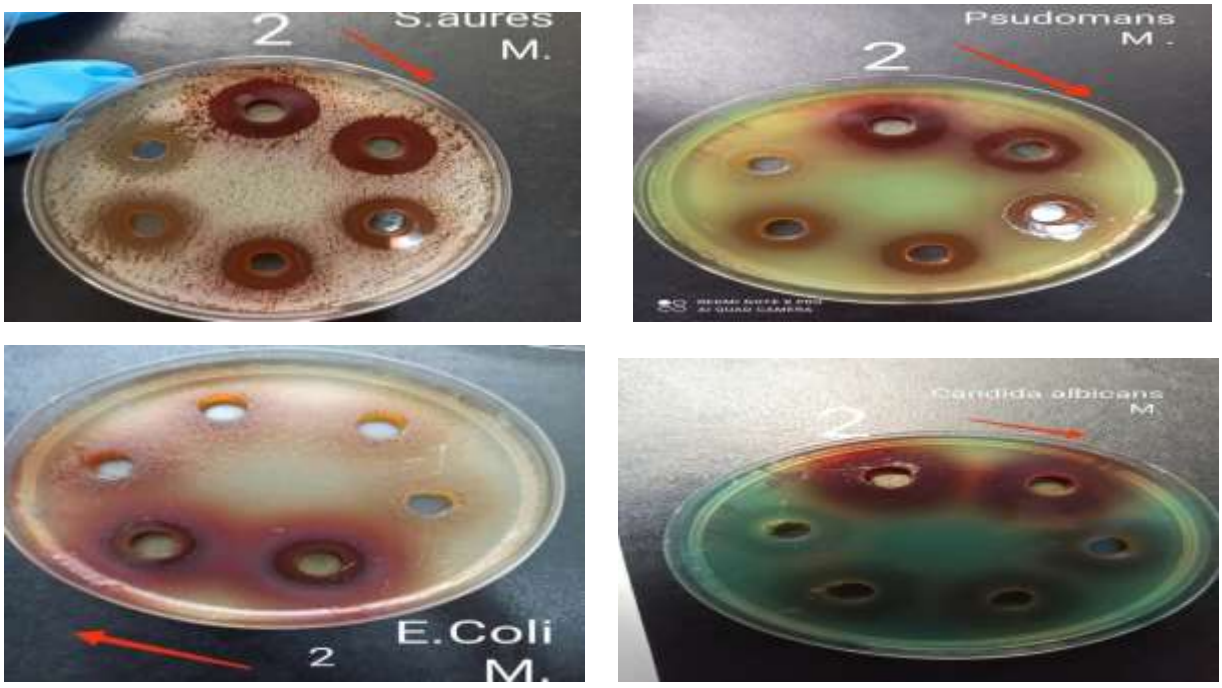


Figure 2: A culture plate showing diameter of inhibition zone for Methanolic extract by well method. Where : *S. aureus P. aeruginosa* , *E. coli* and *C. albicans*

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**Antimicrobial activity of of Aqueous Extract**

The results of this study revealed to evaluate antimicrobial activities of aqueous extracts of *chrozophora* plant as in figure 3 and 4 .The aerial parts extracts using aqueous water of *chrozophira* were tested for their antimicrobial efficiency against pathogenic bacteria and fungi (*Staphylococcus aureus, pseudomonas aeruginosa , E.coli*,) and fungi ( *Candida Albicans* ) at different concentration those 0, 200,400,800 and 1000 mg. The standard drugs used for comparison were ciprofloxacin and Fluconazole against bacteria and fungi respectively . The aqueous extracted tested for their antibacterial activity, showed moderate to high activity against both gram positive and gram negative bacteria. The higher antimicrobial activity of aqueous extract against bacteria and fungi were

showed a highest inhibition zone at the higher concentration (1000 mg ) except for E.coli that shown not affected by the 1000 mg concentration of methanolic extracts.

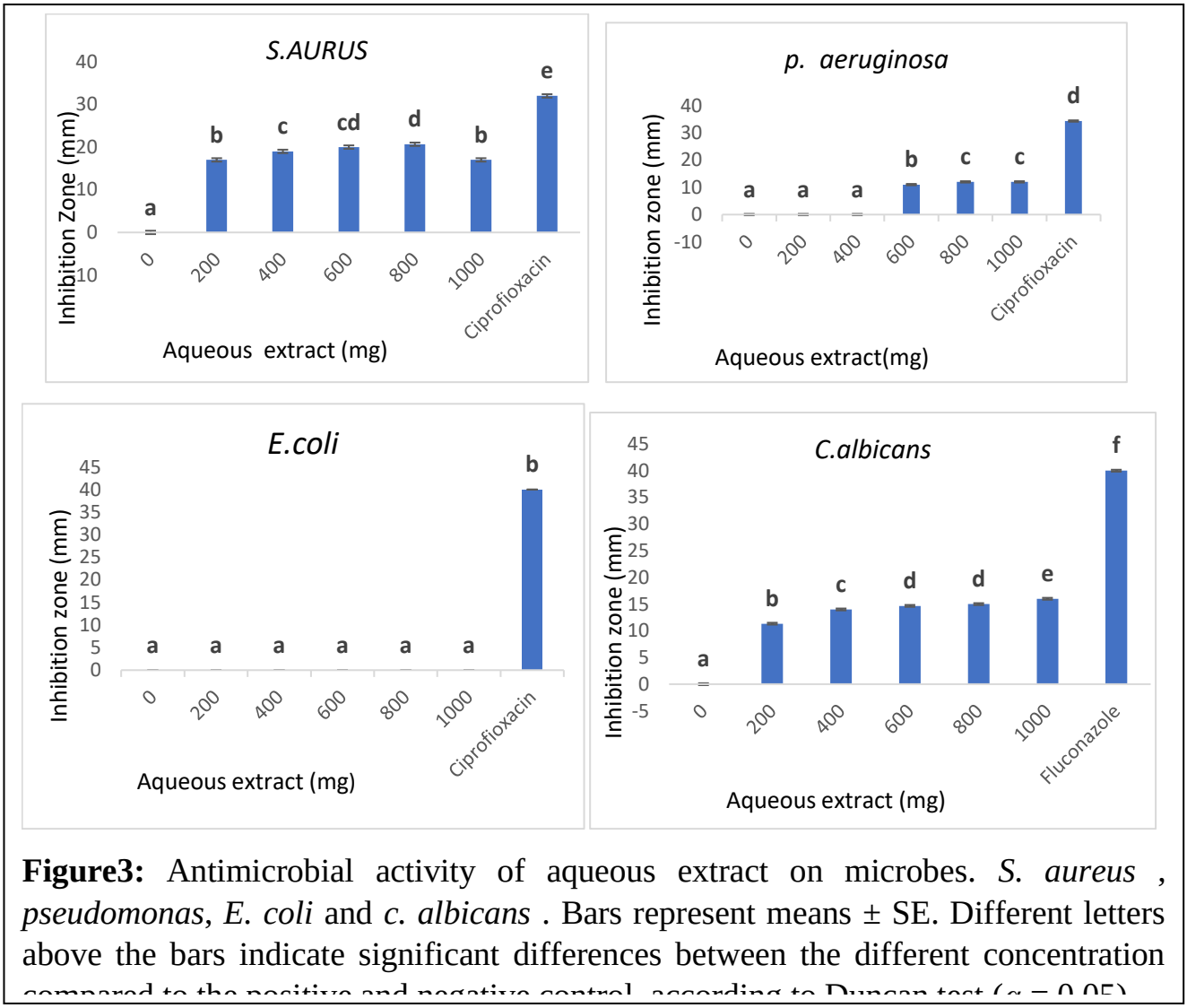


**Figure3:** Antimicrobial activity of aqueous extract on microbes. *S. aureus* , *pseudomonas*, *E. coli* and *c. albicans* . Bars represent means ± SE. Different letters above the bars indicate significant differences between the different concentration compared to the positive and negative control, according to Duncan test ($\alpha = 0.05$)

The result of Inhibition zone for the Aqueous extract of aerial part *C. oblongifolia* at S. Aurus Showed that all concentrations had Significant difference but no Significant difference between 800mg/ml and 600mg/ml , which produced inhibition zone diameters 20.7 and 20mm respectively , and no Significant difference between 1000mg/ml and 200mg/ml , which produced inhibition zone diameters for both was 17mm respectively

but the Positive control Ciprofloxacin showed highest inhibition zone significantly on *Staphylococcus aureus* compared with all concentrations.,While doesn't inhibition zone of control negative (0mg).The Maximum inhibition zone of Aqueous extract against *S. aureus* at concentration 800mg/ml with Inhibition zone diameters (20. 7mm) while at *P. aeruginosa*.The concentration 1000mg/ml and 800mg/ml produced inhibition zone diameters by about 12mm respectively.Most the Inhibition zone of the Aqueous extract of aerial part *C. oblongifolia* against *S. aureus* was the highest Inhibition zone of Microbes. While the Inhibition zone of the Aqueous extract against *P. aeruginosa* was the lowest Inhibition zone of Microbes. finally, Doesn't inhibition zone of the Aqueous extract against *E. coli*.

The Aqueous extract Showed that no inhibition zone on *E. coli* while the Positive control Ciprofloxacin showed higher activity against to *E. coli* compared with all concentrations. For *C. albicans* microbe the result Showed that all concentrations had Significant difference between them but no Significant difference between 800mg/ml and 600mg/ml, which produced inhibition zone diameters 15 and 14.7mm respectively while the Fungi Positive control (Fluconazole ) recorded the higher activity against to *C. albicans* compared to all concentrations ,While doesn't inhibition zone of the negative control (0mg). The Maximum inhibition zone of the Aqueous extract against *C. albicans* was produced at concentration 1000mg/ml with Inhibition zone diameters 16mm respectively.

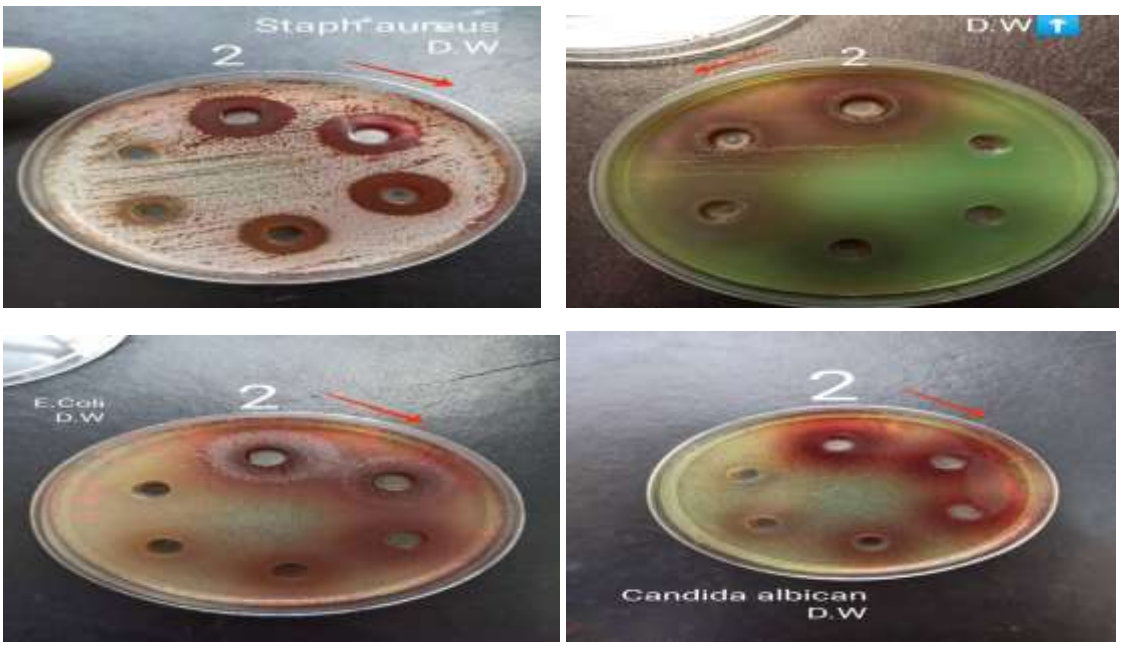


Figure 4: culture plate showing diameter of inhibition zone for Aqueous extract by well method, where :(A) *S. aureus*, ( B) P. *aeruginosa*, (C) *E. coli* and D ( *C. albicans*).

**Compared between antimicrobial Activity of Methanolic and aqueous extracts on microbes:**

The obtained result in Figure 5 , Showed no significant differences between inhibition zone of methanolic and Aqueous extract on *S. aureus*. The maximum inhibition zone was in aqueous extract while the result showed significant differences between inhibition zone of methanolic and Aqueous extract on *P. aeruginosa* with maximum inhibition zone in methanolic extract compared to the aqueous extract.

The obtained result in Figure 5 C Showed significant differences between methanolic and aqueous extract on *E. coli*. The maximum inhibition zone was in methanolic extract while doesn't inhibition zone in aqueous extract. In this study Showed no significant differences between methanolic and aqueous extract on *C. albicans* but the Maximum inhibition zone was in Methanolic extract.

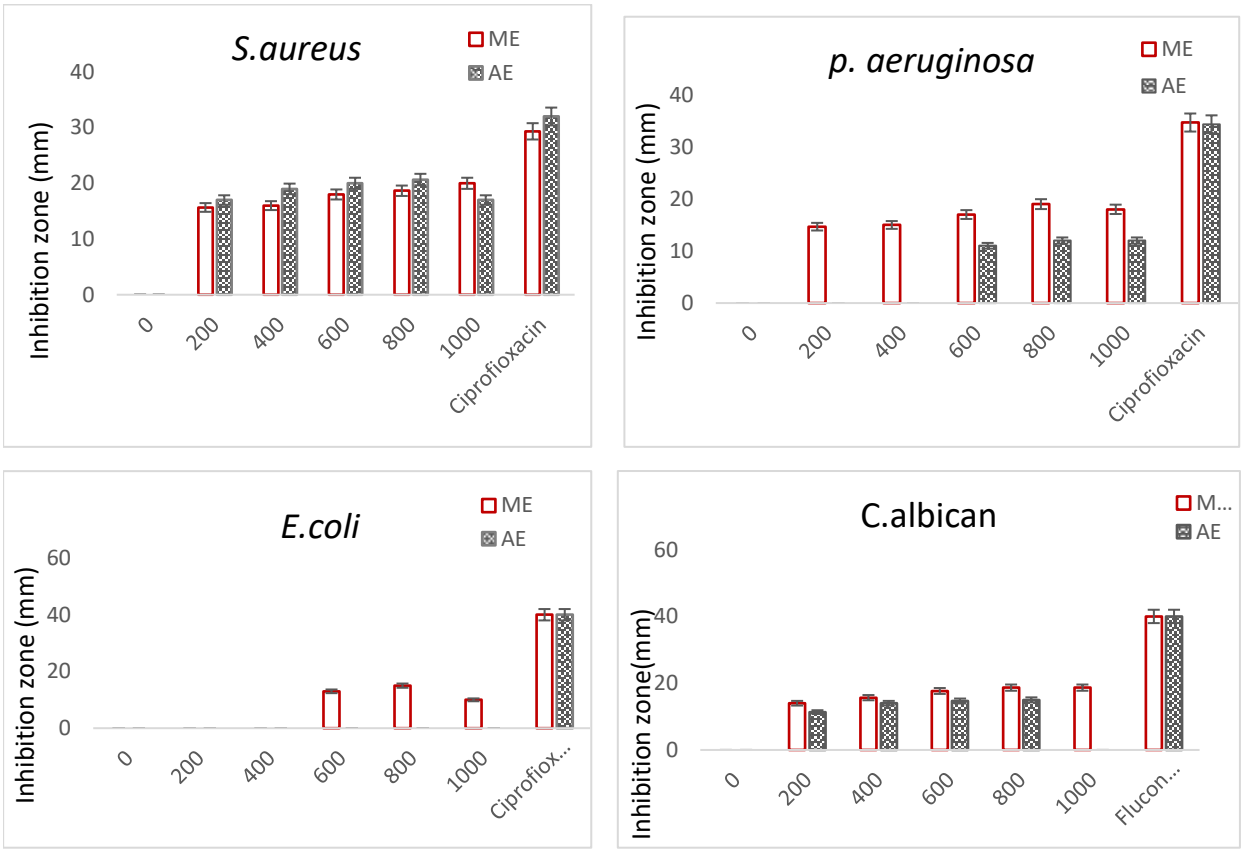


**Figure** 5: Mean Comparing between of methanolic and aqueous extracts S. aureus , pseudomonas, E.coli *and* c. albicans. *Bars represent means ± SE*.

**Discussion**

In this present study the aqueous extract, has shown high zone of inhibition in S.aureus at all concenteration except for 1000mg compared to methanolic extract while at p.auregona the methanolic extract has shown high zone of inhibition and e.coli but the aquoes extract has not shown zone of inhibtion in e.coli . for c.albicans the methanolic extract has shown high zone of inhibition *when compared the zone of inhibition with the*

Standard drugs like ciprofloxacin and fluconazole. The plant extracts have shown almost equal to the standard drug. The above parameter supports the strong scientific basis for the use of these plants in traditional treatment of microbial diseases.

The antimicrobial activity of the extracts and their potency was evaluated by the presence or absence of inhibition zone and zone diameter. The alcoholic extract was found to be a better extract against microbes compared to aquoes extract (Kamel ,et al.2018). Ramzi A. et al.(2011) study the antimicrobial activities of the aerialt parts of Chrozophora against Staphylococcus aureus, Pseudomonas aeruginosa, E.coli and Candida albicans. The antimicrobial analysis it was confirmed that plant extracts showed positive results against bacterial species such as Staphylococcus aureus , Pseudomonas aeruginosa and Escherichia coli and fungi candida. Hence, it can be concluded that the leaf extracts of Chrozophera can effectively act as an antimicrobial agent which have ability to replace most of medium medicines of this era.The antibacterial and fungi activity in this plant due to presence of the compound Carbohydrates and /or glycoside, Sterols and / or triterpenes , Flavonoids , Alkaloids and / or nitrogen bases , Anthraquinone ,Saponins, Flavonoids , Tannins and Terpenoids (Altuntas, et al .2017). On the other hands, Ciprofloxacin exhibited higher activity than both extracts at all concentrations on Bacteria this result agreement with finding Maheshwari et al.,2019. Fluconazole exhibited higher activity than both extracts ( Aqueous and Methanolic ) at all concentrations on C. albicans (Irany et al.,2012)

**Conclusion**

C. oblongifolia is a medicinal plant found in many regions in Yemen. Acc ording to the results obtained from our study, C. oblongifolia Methanolic and Aqueous e xtracts have antimicrobial activity against the tested Microbes.

**Recommendations**

The study recommends further research to study the inhibitory effect of micro bes, as the study showed that this plant contains effective substances.

It also recommends the use of *C. oblongifolia* found in the Yemeni regions and the u se of both an aqueous and an alcoholic extract showing interesting results, and recomme

nd to study leaves, flower and fruits of *C. oblongifolia* on microbes.

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