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ANTIBACTERIAL ACTIVITY OF *CONOCARPUS ERECTUS* LEAVES EXTRACTS ON SOME MICROORGANISMS ISOLATED FROM PATIENTS WITH BURN INFECTION

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Abstract

Medicinal plants are a source for a wide variety of natural active compounds and are used for the treatment of diseases throughout the world. *Conocarpus erectus* L. widely planted all over Iraq and has different secondary metabolites, which has been used in treatment of anemia, cancer, fever and diarrhea. The present study aims to estimate the antibacterial activity of *Conocarpus erectus* leaves extracts on some microorganisms collected from patients with burn infection. The study began with the collection of *Conocarpus erectus* leaves in June 2018 from the trees in university of Baghdad. Maceration method was used to prepare aqueous extract, while Soxhelt apparatus was used to prepare methanolic extract. The results of phytochemical test showed the presence of flavonoids, phenols, alkaloids, glycosides, tannins and saponine in the *Conocarpus erectus* leaves extracts, while alkaloids were not detected in aqueous extract. Agar well diffusion method was employed to determined the antibacterial activity, the best effect was seen on *Staphylococcus aureus* and *Pseudomonas aeruginosa* in concentration 100 mg/ml with inhibition zone (23 and 22 mm) respectively for methanolic extract, and 22 mm on *Staph. aureus* in the same concentration for aqueous extract, and their efficacy in terms of MIC and MBC were ranged from 50 to 75 mg/ml.

Keywords: *Conocarpus erectus*, phytochemical, antibacterial activity, antibiotic sensitivity.

Introduction

Bacterial resistance to antimicrobial drug is one of the most serious threats to global health. Antimicrobial resistance threatens the effective treatment of an ever-increasing range of infections caused by bacteria, parasites, fungi, and virus. The causes of antibiotic resistance are complex and include human behavior at many levels of society; the consequences affect everybody in the world (Laxminarayan *et al,* 2013). In the past few decades, pharmacological companies have been developing new antimicrobial agents but microbial resistance has been increasing due to the ability of bacterial organisms acquiring resistant genes (Gislene *et al.*, 2000). Herbal drugs have been used since ancient times to treat diseases and disorders with their antimicrobial properties making them a potent source of new drugs. The use of herbal medicine has been used to positively prevent and control diseases such as, heart disorders, diabetes and other forms of cancer (Srivastava *et al.,* 2005). Several types of plant extracts or plant-derived molecules have been investigated for their potential as antibacterial sources against several diseases (Madaleno, 2015, AL-Azawi and Salih, 2019).

In the folk medicine, the leaves of *Conocarpus erectus* are eaten and decoctions from bark, leaves and fruits are used against many diseases as catarrh, conjunctivitis, gonorrhea, diarrhea, fever, orchitis and syphilis (Nascimento *et al.,* 2016). Phenolic compounds are the major secondary metabolites of this species, these molecules detected in different *C. erectus* extracts have been described to exhibit antioxidant, antifungal and antiviral activities, as well as act in the activation of the immune system (Chen *et al.,* 2016). Few studies were published until now in relation to possible action mechanism promoted by *C. erectus* extracts in human lymphocytes or in microorganisms collected from human wounds (Santos *et al*., 2018). Thus, main purpose of this research is detection of active ingredients in *Conocarpus erectus* leaves extracts as well as evaluating the antibacterial activity against pathogenic bacteria isolated from patients with burn infection.

Materials and Methods

Plant Collection

Plant leaves were collected from trees in university of Baghdad. Classified as *Conocarpus erectus* L. by the herbarium of the Biology Department, College of Science, University of Baghdad. The leaves were ground using a grinder and stored at -20°C for further analysis.

Preparation of aqueous extract

Water extract was prepared according to N'Guessan *et al*. (2007). Macerated 100 gram of *Conocarpus erectus* leaves in 700 ml of distilled water for 72 hours, after extraction, the mixture was vacuum filtered through Whitman No. 1 paper. The filtrate evaporated to dryness under vacuum at 50°C by a rotary evaporator to eliminate water. The resulting extract stored in amber glass vials at 4 °C until analyzed.

Preparation of methanolic extract

Methanolic extract was prepared according to AACC (1984) by using Soxhelt apparatus. Fifty gram of *Conocarpus erectus* leaves was put in a thimble and 350 ml of 70% methanol was added within 40-60 °C for 6 hours. The solution was filtered through a filter paper Whitman No.1 and evaporated to dryness under vacuum at 40°C by a rotary evaporator to get rid of methanol; the extract was stored in amber glass vials at 4 °C until analyzed.

General chemical detection methods

Methanolic and aqueous extracts were tested for the presence of the phytoconstituents according to the following standard tests (Harborne, 1984; Harborne, 1998; AACC, 1984; Smolensk *et al*., 1972 and Jaffer *et al*., 1988) to detected phenols, Flavonoids, Alkaloids, Tannins, glycosides and saponins.

Microorganism collection

Staphylococcus aureus, *Acinetobacter baumannii* and *Pseudomonas aeruginosa* can be obtained from AL-Yarmouk hospital in Baghdad, collected from patients with burn infection and diagnosed by using the VITEK-2 System. Bacterial cultures were maintained on nutrient agar slops. Subcultures were made monthly and stored at 4 °C until required for use.

Culture preparation

Three – five colonies from the pure culture were suspended in 5-10 ml of sterile nutrient broth. The turbidity of the test suspension was compared with 0.5 McFarland turbidity standards (10^8 CFU / ml) (Sofia *et al*., 2007).

Antibiotic sensitivity test

Antibiotic sensitivity of the bacterial isolates was determined by the standard disc diffusion method (WHO, 2003). Different antibiotics (Oxoid / England) were used in the present work, Amikacin (Ak), 30 µg; Cefotaxime (CTX), 5 µg; Colistin (CT) 10 µg; Doxycycline (DO), 30 µg; Erythromycin (E), 15 µg; Tetracycline (T), 30 µg; Gentamicin (GM), 10 µg; Levofloxacin (LEV), 5 µg; Penicillin (P), 10 µg; Sulphamethoxazole (SXT), 5 µg. The interpretation of antibiotic susceptibility test resistant, (R) intermediate (I), or sensitive (S), according to CSLI, (2018).

Antibacterial assay

The agar well diffusion method was employed for detection the antibacterial activity. 0.2 ml volume of the standard inoculums (10^8CFU/ml) of the test bacterial isolate was spread on Mueller Hinton Agar with a sterile glass rod spreader and allowed to dry. Wells (6 mm diameter) were made in each of these plates using sterile cork borer. 100 µl from each concentration (25, 50 and 100 mg/ml) of the aqueous and methanolic extracts were put in each hole by using micropipette and allowed to diffuse at room temperature for 30 min. The plates were incubated at 37°C for 18-24 hours. The diameter of any resulting zone of inhibition was measured in millimeters (Valgas *et al*., 2007).

Statistical Analysis

The Statistical Analysis Sys-tem program was using to study different parameters. LSD test was used to significant compare between means in this study (SAS, 2012).

Results and Discussion

***Conocarpus erectus* leaves extracts**

The weights of the methanolic and aqueous *Conocarpus erectus* extracts as shown in Table (1).

Table 1 : Weights of dried *Conocarpus erectus* extracts.

Conocarpus erectus extracts	Weight (gm)
Methanolic extract	14.45
Aqueous extract	9.75

Phytochemical tests of *Conocarpus erectus*

Phytochemical screening means the extraction, identification and screening of the medicinally active substances found in plants, some of the bioactive substances that are derived from plants are flavonoids, alkaloids, carotinoids, tannin, antioxidants, and phenolic compounds (Pendyala *et al*., 2017). Different phytochemicals have a wide range of activities that may help in protection against various diseases (Shanmugapriya *et al*., 2011).

Phytochemical characterizations of methanolic and aqueous of *Conocarpus erectus* extracts are presented in (Table 2). The result showed methanolic extract contain flavonoids, phenols, alkaloids, tannins, glycosides and saponine. While the aqueous extract contains all the constituents, which found in methanolic extract, except alkaloids was not detected. The result of phytochemical findings agreed with Dayane *et al*., (2016) who reported the presence of flavonoids, phenols, tannins, glycoside and saponine as active compounds in *Conocarpus erectus* aqueous extracts with absent of alkaloids.

Table 2 : Phytochemical screening of *Conocarpus erectus* extracts.

Phytochemical compound		Aqueous Extract	Methanolic Extract	Result
Flavnonoids		+	+	Yellow colour
Phenols	Ferric Chloride	+	+	bluish green colour
	lead acetate	+	+	reddish brown precipitate
Alkaloids	Wagner's test	–	+	reddish brown precipitate
	Meyer's test	–	+	White precipitate
Tannins		+	+	White gelatin
Glycosides		+	+	Violet ring
Saponine		+	+	thick foam

(+) Positive, (–) Negative

Antibacterial activity of *Conocarpus erectus* leaves extracts

Antibiotics susceptibility

Antibiotics susceptibility of the bacterial isolates was performed on ten antibiotics represented by Amikacin, Cefotaxime, Colistin, Levofloxacin, Gentamicin, Tetracycline, Penicillin, Sulphamethoxazole, Erythromycine and Doxycycline by disc diffusion method.

The antibiogram of the studied isolates revealed that the bacteria *A. baumannii* and *Staph. aurous* were resistant to Doxycycline, Cefotxime and Erythromycin. Moreover, *P. aeruginosa* was resistant to Doxycycline, Penicillin, Gentamicin and Erythromycin. While the isolates were sensitive and intermediate to another antibiotics used in this study as shown in (Table 3). The resistance of local bacterial isolates may be due to the production of beta-lactamase enzymes such as ESBL enzymes which degrade the penicillins and cephalosporins (Salman and Ghaima, 2018).

Appropriate antimicrobial drug use has unquestionable benefit, but physicians and the public frequently use these agents inappropriately hence, it became necessary to perform the antimicrobial susceptibility test as a routine. The aim of antimicrobial susceptibility testing is to determine the lowest concentration of existing or even new antimicrobial agents

which inhibits the visible growth of the bacterium being investigated, under certain test conditions. The disk diffusion, well diffusion, stokes and gradient diffusion methods are manual methods that provide flexibility and possible cost savings. Although available testing methods provide accurate detection of common antimicrobial resistance mechanisms, emerging newer mechanisms of resistance certainly attracts researcher for the development of advanced, reproducible, automated and reliable antimicrobial testing methods (Bagu *et al.,* 2016).

Table 3 : Antibiotic Sensitive test

Micro-organism	Antibiotic									
	DO	CTX	P	CT	SXT	T	LEV	CN	AK	E
Staph. aurous	R	R	S	S	S	I	S	I	S	R
P. aeruginos	R	S	R	I	S	I	S	R	I	R
A. boumanii	R	R	S	S	S	S	S	S	S	R

DO = Doxycycline, CTX = Cefotxime, P = Penicillin, CT = Colistin, SXT = Sulphamethoxazole, T = Tetracycline, LEV = Levofloxacin, CN = Gentamicin, AK = Amikacin, E = Erythromycin, S = Sensitive, R = Resistant, I = Intermediate

Well diffusion Method

Preliminarily, the antibacterial activity of *Conocarpus erectus* leaves extracts was qualitatively evaluated by agar well-diffusion method against gram positive bacteria *Staphylococcus aureus* and gram negative bacteria *Pseudomonas aeruginosa* and *Acinetobacter baumannii.*

For each type of *Conocarpus erectus* extracts, statistical test were performed between different concentrations, for methanolic extract as seen in (Table 4), the best effect was seen on *Staph. aureus* (Figure 1) with the inhibition zone (15.33 ± 0.33, 17.67 ± 0.66 and 23.00 ± 0.57 mm) in concentration (25, 50 and 100 mg/ml) respectively with a significant difference of (P<0.01), while the lowest effect was seen on *P. aeruginosa* (Figure 2) and *A. baumannii* (Figure 3) with inhibition zone (8.67 ± 0.66 , 13.33 ± 0.88 mm) and (9.67 ± 0.88 , 14.33 ± 0.33 mm) respectively in concentrations (25 and 50 mg/ml) with a significant difference (P<0.01). Phenolic compounds exert anti-inflammatory and anti-proliferative (Shahidi and Ambigaipalan, 2015), antimicrobial and antiviral (Abdel-Hameed *et al.,* 2012). These compounds act on the microbial cell altering the cellular permeability, damaging the cytoplasmic membrane and interfering with the energy generation system, finally leading to cell death (Vieitez *et al.,* 2018). The water extract in (Table 5) shows that the concentration 100 mg/ml was the highest effect on *Staph. aureus* with inhibition zone 22.00 ± 0.57 mm (Figure 4), while the inhibition zone of *P. aeruginosa* (Figure 5) and *A. baumannii* (Figure 6) in same concentration was 17.67 ± 0.33 and 17.00 ± 0.57 respectively with a significant difference (P<0.01). This study showed that the methanolic extract of *Conocarpus erectus* leaves have the best effect than the aqueous extract due to the higher concentration of phenolic compounds such as tannins, flavonoids, alkaloids and phenols. Mummed *et al.* (2018) mention the antibacterial properties of the active plants may be due to the presence of different bioactive chemical agents in the extracts (tannins , saponines , flavonoids, glycosides, alkaloids, sterols and phenolics) which are known to act by a different mechanism to exert an antibacterial action.

These results were similar with Bashir *et al.* (2015) which showed the antimicrobial effects of *Conocarpus erectus* methanolic leaves extract exerted antimicrobial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* with inhibition zone 21 and 18 mm respectively. Furthermore, a study by Abdel-Hameed *et al.* (2012) demonstrated the antibacterial potential activity of the *Conocarpus erectus* leaves of the methanolic extract can form inhibition zone of 21.5 mm for *Staph. aureus*. Santos *et al.* (2018) showed that the aqueous extract of the leaves can form inhibition zone of 10 mm for multidrug resistant *Staph. aureus* isolated from cutaneous wounds. Tannins showed a better antibacterial activity which may be related to their ability to inactivate several enzymes, microbial adhesion, and cell envelope transport proteins (Alan and Miller 1996). Flavonoids and saponins have been reported to possess antibacterial activity, which could be attributed to their ability to form complex with extracellular proteins, soluble proteins, and bacterial cell wall (Tazelaar *et al.,* 2009).

Table 4 : Antibacterial activity of methanolic *Conocarpus erectus* leaves extract

Concentration (mg/ml)	Mean ± SE		
	Staph. aureus	*A. baumannii*	*P. aeruginosa*
25	15.33 ± 0.33 c	9.67 ± 0.88 c	8.67 ± 0.66 c
50	17.67 ± 0.66 b	14.33 ± 0.33 b	13.33 ± 0.88 b
100	23.00 ± 0.57 a	19.00 ± 0.57 a	22.00 ± 1.00 a
LSD value	1.883 **	2.208 **	2.978 **
P-value	0.0002	0.0002	0.0001

** (P<0.01). Means having with the different letters in same column differed significantly

Table 5 : Antibacterial activity of aqueous *Conocarpus erectus* leaves extract

Concentration (mg/ml)	Mean ± SE		
	Staph. aureus	*A. baumannii*	*P. aeruginosa*
25	13.33 ± 0.66 c	7.33 ± 0.33 c	9.67 ± 0.33 c
50	17.33 ± 0.33 b	13.33 ± 0.33 b	15.33 ± 0.33 b
100	22.00 ± 0.57 a	17.00 ± 0.57 a	17.67 ± 0.33 a
LSD value	1.883 **	1.489 **	1.153 **
P-value	0.0001	0.0001	0.0001

** (P<0.01). Means having with the different letters in same column differed significantly

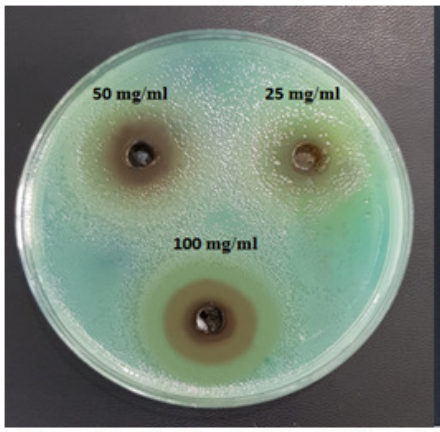

Figure 2: Antibacterial activity of methanolic extract on *p. aeruginosa*

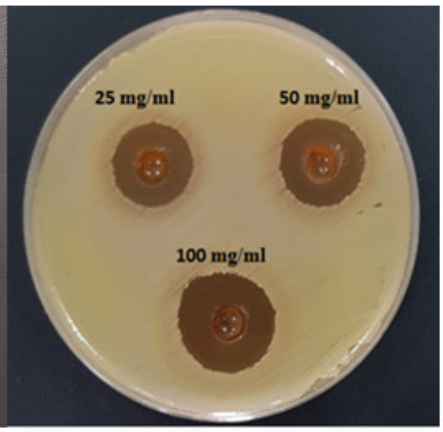

Figure 1: Antibacterial activity of methanolic extract on *staph. aureus*

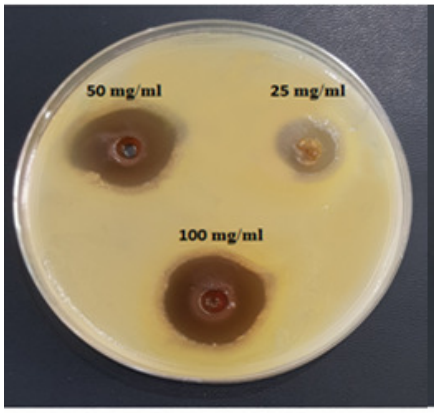

Figure 4: Antibacterial activity of aqueous extract on *staph. aureus*

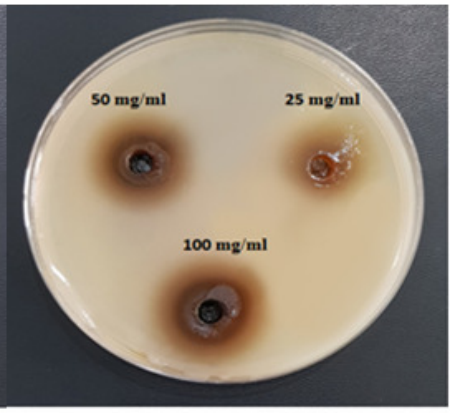

Figure 3: Antibacterial activity of methanolic extract on *A. baumannii*

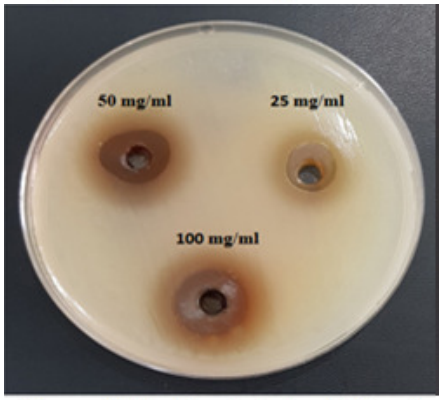

Figure 6: Antibacterial activity of aqueous extract on *A. baumannii*

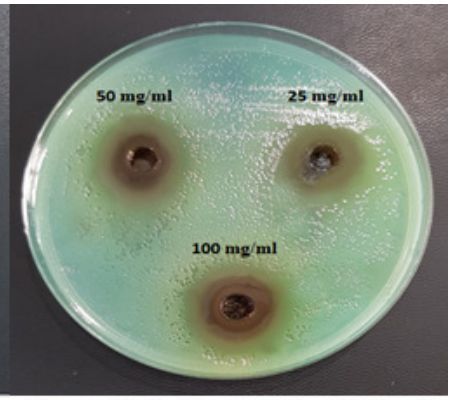

Figure 5: Antibacterial activity of aqueous extract on *p. aeruginosa*

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) method

Broth dilution method was used to determining the MIC and MBC for isolates used in this study. Methanolic extract (Table 6) showed that the MIC and MBC were 25 and 50 mg/ mL for *Staph. aureus* and *P. aeruginosa* (Figure 7 and 8) respectively, while the MIC and MBC for *A. baumannii* found to be 75 and 100 mg/ mL (Figure 9). For aqueous extract (Table 4-8) showed that the MIC and MBC were 50 and 75 mg/ mL for *Staph. aureus* and *P. aeruginosa* (Figure 10 and 11) respectively, while the MIC and MBC for *A. baumannii* found to be 100 and 150 mg/ mL (Figure 12).

Conocarpus erectus contains large amounts of tannins (Abdel-Hameed *et al*., 2012). Tannins are water-soluble polyphenols that are commonly found in higher herbaceous and woody plants (Scalbert 1991). They have been reported to possess both bacteriostatic and bactericidal activities (Akiyama *et al*., 2001). Shohayeb *et al.,* (2013) suggested that tannins of *Conocarpus erectus* extracts are largely responsible for antimicrobial activity of this plant collected in Saudi Arabia, when studied the methanolic extracts fractions of leaves, stem, fruit and flower on *conocarpus Staphylococcus aureus, escherichia coli, salmonella typhimurium, klebsiella pneumoniae* and *Pseudomonas aeruginosa.* In this study, bacterial isolates showed resistant to commonly used antibiotics. The *Conocarpus erectus* extracts showed a promising antibacterial activity against the resistant bacterial strains. The presence of phenolic compounds in the leaves extracts may responsible for the antimicrobial activity.

Table 6: MIC and MBC of *conocarpus erectus* extracts

Microorganisms	*Conocarpus erectus* extracts	MIC mg/mL	MBC mg/mL
Staph. aureus	methanolic extract	25	50
	aqueous extract	50	75
A. baumannii	methanolic extract	75	100
	aqueous extract	100	150
P. aeruginosa	methanolic extract	25	50
	aqueous extract	50	75

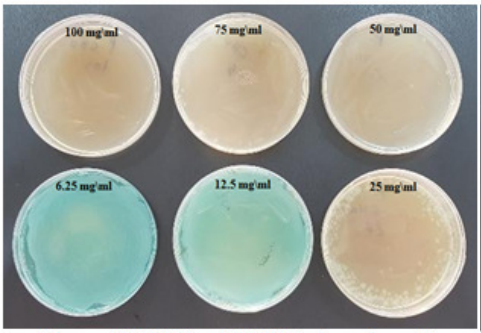

Figure 8: MIC and MBC of methanolic extract on *P. aeruginosa*

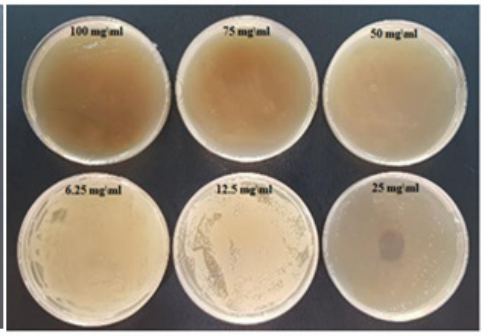

Figure 7: MIC and MBC of methanolic extract on *staph. aureus*

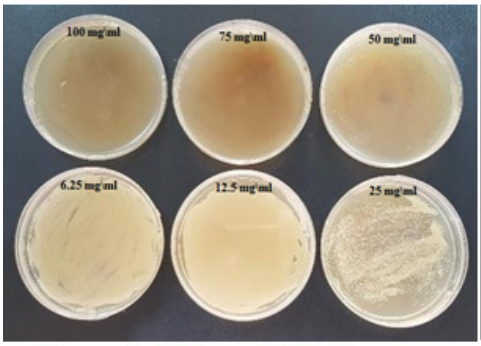

Figure 10: MIC and MBC of aqueous extract on *staph. aureus*

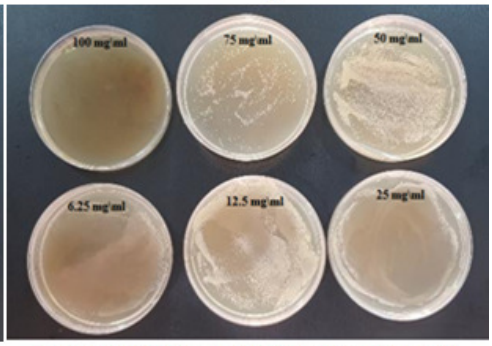

Figure 9: MIC and MBC of methanolic extract on *A. baumannii*

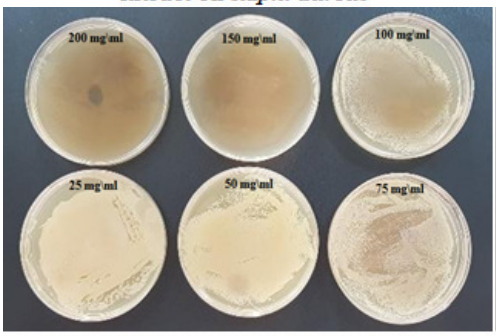

Figure 12: MIC and MBC of aqueous extract on *A. baumannii*

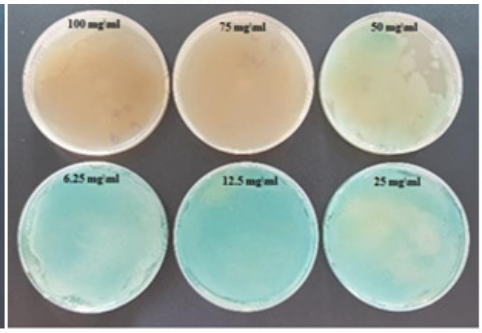

Figure 11: MIC and MBC of aqueous extract on *p. aeruginosa*

Conclusion

Conocarpus erectus leaves extract have rich phytochemical contents responsible for its medicinal activities that offer beneficial effects as a good source to combat numerous diseases. *Conocarpus erectus L.* extract displays a high antibacterial agent against gram positive bacteria *Staph. aureus and* gram negative bacteria *p. arginosa,* and *A. baumannii* despite the bacterial isolates which showed increased resistance to commonly used antibiotics.

Conflict of Interest

The authors declared that present study was performed in absence of any conflict of interest.

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Author Contributions

AHA and SAY contributed to the design of the experiments and performed the experimental work. All authors carried out laboratory tests. AHA wrote the manuscript, all authors revised and approval the final version.

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