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ANTIBACTERIAL OF *CASSIA ACUTIFOLIA* EXTRACT AGAINST SOME BACTERIA AND ANTI-BIOFILM FORMATION ACTIVITIES AGAINST *PSEUDOMONAS AERUGINOSA*

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ABSTRACT

The present study was carried out to determine the potential antibacterial effect of *Cassia Acutifolia* ethanolic extract against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas Aeruginosa* using the agar diffusion technique. All microorganisms showed the minimum inhibitory concentrations were investigated to characterize the antimicrobial activities of this extract. The result showed that the least MIC value of alcoholic extract of *Cassia Acutifolia* was 1.25mg/ml against *S. aureus* and 20 mg/ml against*E. Coli* and *P. aeruginosa* whereas the highest MBC value of alcoholic extract of *Cassia Acutifolia* was 50mg/ml against*E. Coli* and *P. aeruginosa* and 5mg/ml for *S. aureus.*

The biofilm inhibitory concentration of the *Cassia Acutifolia* extract was 1.034-0.418mg/ml against *P. aeruginosa* in different plant concentration started with 50 mg/ml to 5mg/ml.

KEYWORDS: Cassia Acutifolia**,** Antibacterial Activity, Antibiofilm Activity

INTRODUCTION

The incidence of severe infections in humans caused by pathogenic microorganisms has increased globally and is a key cause of morbidity and mortality in developing countries (1).Development of agriculture further contributed to this, since these infections could only be sustained in large and dense human populations (2).The discovery of antibiotics during the twentieth century, coupled with significant advances in antimicrobial drug development, improved human health through improved treatment of infections (3,4) and due to the indiscriminate uses of commercial antimicrobial drugs leading to multiple drug resistant microorganisms have developed (5). However, the past record of rapid, widespread emergence of resistance to newly introduced antimicrobial agents indicates that even new families of antimicrobial agents will have a short life expectancy (6). For this reason, researchers are increasingly turning their attention to herbal products, looking for new leads to develop better drugs against MDR microbe strains (7).

Bacteria in nature found in communities attached to solid surfaces and is consist of biological polymers include exopolysaccharide (EPS), protein, and DNA. The function of the biofilm is to adhere to biotic surfaces, the epithelia of multi cellular organisms, and interfaces such as that between air and water. Surface attaché of bacteria is an important step and is necessary for the bacteria to arrange themselves in their environment (8-12).

This study is aimed at investigating the antimicrobial and anti biofilm activities of *Cassia Acutifolia* extract against some pathogens.

MATERIALS AND METHODS

Preparation of Plant Extracts

The dried plant sample (50 g) was chopped and placed in a conical flask, then soaked with 350ml of 98% (v/v) ethanol for 10 days infreez. The mixtures were filtered through what man No. 4 filter paper and the filtrates were evaporated at 50 C. The extracts were stored at4C until use.

Selection of Test Organisms

Gram-positive bacteria, *Staphylococcus aureus*, Gram-negative bacteria, *Pseudomonas aeruginosa*, *Escherichia coli* were medical isolates obtained from culture, libraries of the zoonotic diseases unit Laboratory of Veterinary Medicine College and were checked for purity then pure culture were grown in nutrient broth and preserved at 4°C.

Preparation of Inoculums

The inoculums of Bacterial strains were prepared by growing cells in Mueller Hinton Broth (Himedia) for 24 h at 37 ^{o} C then diluted with sterile MHB to supply10^{8} cfu/ ml cell counts (0.5 McFarland turbidity standards).

Assay for Antibacterial Activity

Agar well diffusion method Agar well-diffusion method was followed to determine the antimicrobial activity for plant extract. One milliliter of extract was used against the test microorganisms. Approximately, 100 μL of fresh culture (approximately 10^{8} CFU/mL) was uniformly spread into Mueller Hinton agar (MHA) plates by sterile cotton swab. Then, allowed to drytheinoculated plates at room temperature for 20 min. After that, five wells of 6 mm in diameter were made in the agar using a sterilized stainless steel borer, then The *Cassia Acutifolia* extract was separately redissolved in sterile distilled water at concentrations of (40, 20, 10 and 5mg/ml) after that 100μL of each extract diluted was poured in the wells. Steriledistaled water was used a control. Plates were incubated at 37∘C for 24h. Antibacterial activity was proofed by the presence of a clear inhibition zone around each well. The diameter of this zone was measured and recorded (13).

Minimum Inhibihitory Concentration (MIC)

The minimum inhibitory concentration is known as the lowest concentration able to inhibit any visible bacterial growth on the culture tube. MIC was determined from concerning on the culture tubes after incubation. The tube dilution method and agar dilution methods were the most commonly employed methods and Serial dilutions are made of the plant extract in bacterial growth media. After that the test organism was added to the dilutions of the herbal extract, then incubated and scored for growth, Preparation of Inoculums Test for antibacterial activity the antibacterial assay was carried out by tube dilution method in order to determine the antibacterial activity of extract tested against the pathogenic bacteria. The bacterial suspensions were adjusted with sterile saline to a concentration of 1.0×10^{8} CFU/ml. The minimum inhibitory concentrations (MIC) were performed by a serial dilution technique using seven tubes for each tested microorganisms. The plant extracts was taken (20 mg/ml) and serial dilution of the extract with Muller Hinton broth for bacterial culture then incubated the tube for 24 hours at 37^{o}then the MIC assay determined by visualize the bacterial growth, 0.5 ml (0.04mg/ml) of *p*-iodonitrotetrazolium violet (trazolium salt) ((INT) was added to each tube and incubate all tubes at room temperature for 6 hrs. The tubes were examined for color change and the MIC was indicated by the first clear tube that not changed to red color when compared with the control tubes or none inhibited concentrations.

Determination of Minimum Bactericidal Concentration (MBC)

Determination of the MBC was carried out by serial sub-cultivation of 2 µl into Muller Hinton agar plates and incubation for 24 hours. The lowest concentration with no visible growth was defined as the MBC, indicating 99.5% killing of the original inoculums.

Biofilm Inhibition Assay

Biofilm inhibition carried out in seven glass tube take on modified method of biofilm inhibition spectro photometric assay (14).1ml of cell suspension of P. aeruginosathus prepared was added into each glass tube and different concentration of plant extract as 20, 10, 5, 2.5, 1.25, 0.625 and 0.312mg /ml was added and incubated at37º C for 3 days. After the incubation, the liquid suspension was removed and 1ml of 1% w/v aqueous solution of crystal violet was added. Following staining at room temperature for 30 minutes the dye was removed and the wells were washed thoroughly, 95% ethanol was added and incubated for 15 minutes. The reaction mixture was read spectrophotometrically at 570nm. Inhibition mediated reduction of biofilm formation was calculated by the following formula

% of inhibition = OD in control –OD in treatment × 100 / OD in control

RESULTS

Antimicrobial Activities of the Extracts

The present research work investigated the antimicrobial activity of crude extract plant (*Cassia Acutifolia)* was evaluated according to their zone of inhibition against three pathogens (*S. Aerus E. coli, P. aeruginosa*) and the results (zone of inhibition). The results revealed that the extract is potent antimicrobials against all the microorganisms studied. Among the different concentrations extract studied 50 and 20 mg/ml showed a high degree of inhibition followed by 10 mg/ml concentrated extract.

Ethanolic *Cassia Acutifolia* extract at 50mg/ml show maximum inhibition zone diameter obtained in *S. Aureus, E. coli* and *P. aeruginosa* in with diameter 23.5±0.42 mm ,19.5±0.52 mm, and 17.3±0.32 respectively (Table 1; Figure 1 (A-C) and Figure 2).

Table 1: Antimicrobial Activity (Zone of Inhibition, mm) of Plant Extract *Cassia Acutifolia* against Clinical Pathogens

Bacteria	Zone of Inhibition of *Cassia Acutifolia*			
	50 mg/ml	20 mg/ml	10mg/ml	5 mg/ml
S aureus	23.5±0.42	20.5±0.35	14±0.25	-
E. coli	19.5±0.52	16±0.35	10±0.15	-
P. aeruginosa	17.3±0.32	15±0.35	9±0.35	-

Values are mean of triplicate readings (mean ± S.D).

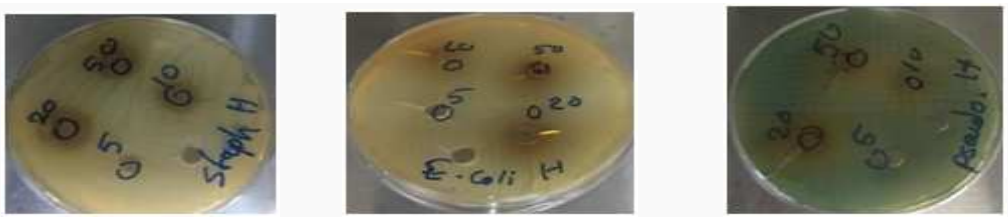


Figure A: S aureus **Figure B: E. coli** **Figure C: P. Aeruginosa**

Figure 1: Antimicrobial Activity of Cassia Acutifolia Extract Against

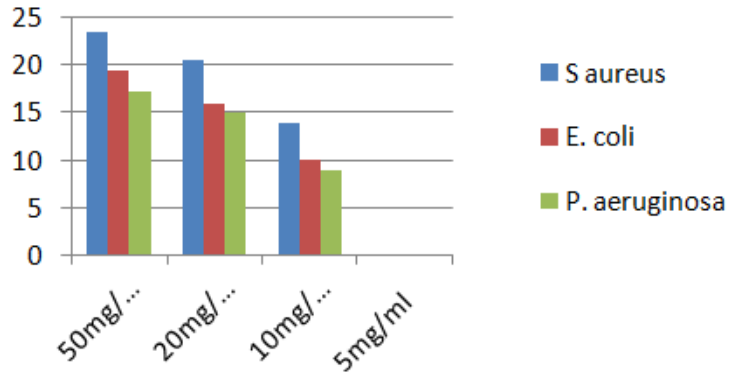


Figure 2: Antimicrobial Activity of *Cassia Acutifolia* Extract Against

Determination of MIC, MBC Values

The antimicrobial activity of the extract and their potency were quantitatively assessed by determining the MIC and MBC, respectively, as given in Table 2.

The MIC for each tested bacterial strain had been shown in Figure 3. From the data in Figure 3, it was clarify that all of the tested bacterial isolates showed the minimum value of MIC for 20 mg/ml extract except *S aureus* The minimum MIC value of *S aureus* (1.25 mg/ml) was observed visualize using0.5 ml(0.04mg/ml) of *p*-iodonitrotetrazolium violet(trazolium salt)((INT) after that the tubes were examined for color change and the MIC was indicated by the first clear tube that not changed to red color when compared with the control tubes or none inhibited concentrations.

Table 2: MIC and MBC Values for Crude Extract of *Cassia Acutifolia* against Three Microorganisms

Microorganisms	Antimicrobial Activity of *Cassia Acutifolia*	
	MIC mg/ml	MBC mg/ml
E. coli	20	50
S aureus	1.25	5
P. aeruginosa	20	50

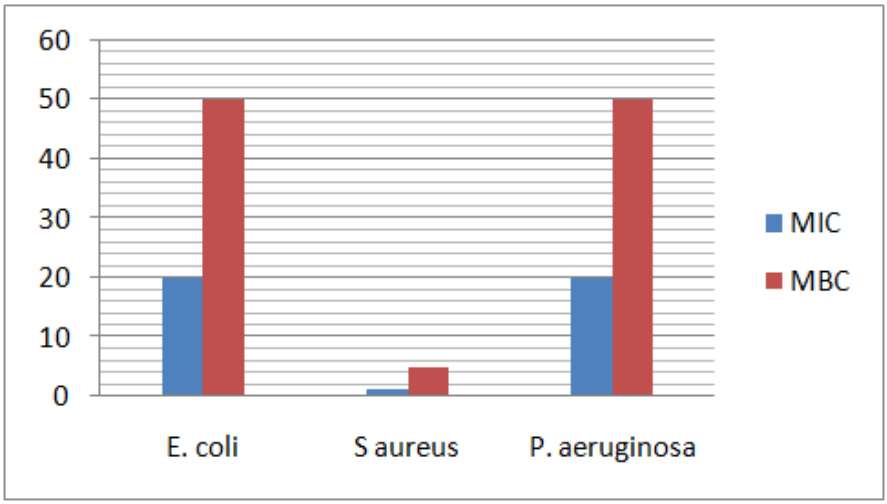


Figure 3: MIC and MBC Values for Crude Extract of *CASSIA Acutifolia* against Three Microorganisms

The MBC was carried out by sub culturing the test dilution that used in MIC on to a fresh solid medium and incubated further for 24 h. The concentration of plant extract that completely killed the Bacteria was taken as MBC. Furthermore MBC value that is almost two fold higher than there corresponding MIC , extract of showed least MBC value 5mg/ml against *S. aureus* while ethanol extract 50 mg/ml against *P. aeruginosa* and *E. coli* (Table 2)(figure 4.A,B,C) respectively.

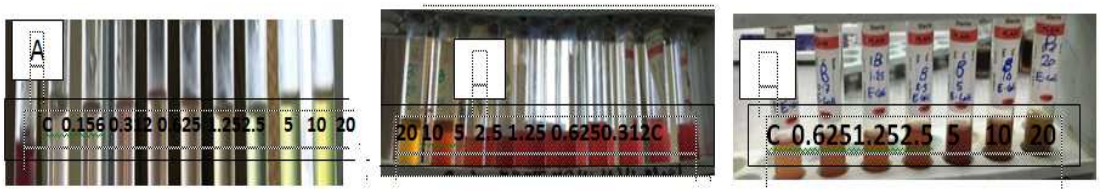
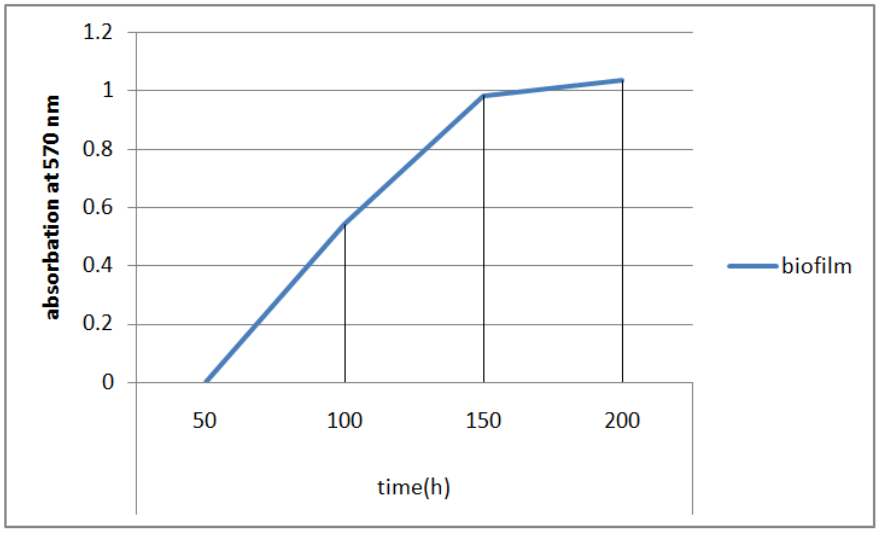


Figure 4: The Minimum Inhibition Concentration of C. Acutifola against A.S Aureus B.P. Aeruginosa and C .E. coli

Biofilm Assay

Biofilm inhibition studies carried out using plant extract and at all the tested concentration have successfully inhibited biofilm formation of *p. aeruginosa* as dose dependent manner.

The results clearly indicate the enhanced anti biofilm effect of herbal extract in glass tube assay. Extract of *Cassia Acutifolia* with 10, 20 and 50mg/ml recorded0.418, 0.541, 0.982 and 1.034% of biofilm inhibition as shown in figure 5.



Inhibition of biofilm formation on glass tube surfaces for *P. aeruginosa* by *Cassia Acutifolia* were additionally visualized by crystal violet assay which is illustrated in Figure 6.

DISCUSSIONS

Antibacterial activity was recorded for ethanolic extracts of *Cassia Acutifolia* which proves its ethno pharmacological use against some infectious diseases. The herbal we have studied display edantibacterial activity against Gram Positive bacteria with smaller effect against Gram Negative bacteria. Ethanolic extract of *Cassia Acutifolia* exhibited maximum antibacterial activity against *S.aureus* in different concentrated extract producing a maximum zone of inhibition. This antibacterial activity against gram positive bacteria is related with the presence of flavonoids (15, 16). Plant extract may consist of certain component which inhibit the cell wall synthesis of bacteria and may be active against all three tested organisms.

Minimum Inhibitory Concentration (MIC) is defined as the highest dilution or less concentration of the extracts that inhibit growth of organisms, it can measurement the activity of an antimicrobial agent against an organism. Determination of the MIC is important in diagnostic laboratories because it helps in confirming resistance of microorganism to an antimicrobial agent and it monitors the activity of new antimicrobial agents. Minimum bactericidal effects were exhibited with various degrees in the ethanol extracts. These effects were also observed on 3/3 tested microorganisms likewise the known antibacterial mechanisms of medicinal plants against microorganisms were inhibit cell wall synthesis (17, 18), aggregate in cell membranes causing energy depletion (19) , or overlap the permeability of cell membrane which lead to increasing of permeability and loss of cellular shapes, membrane disturbance and alter the structure and function of key cellular frames, resulting in mutation and cell damage then death (20).

CONCLUSIONS

The plant extract tested inhibited biofilm as dose dependent manner. Antibiofilm effect of plant extract against biofilm of P. aeruginosa bacteria has been reported by workers [21, 22, and 23].

The anti biofilm activity could be related to the higher proportion flavonoids in the plant.

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