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Determination of antibacterial activity of Green Coffee Arabica bean extract on Multidrug resistance Pseudomonas aeruginosa (ATCC 27853)

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Abstract: The emergence of drug resistant pathogens becomes a crucial problem for infectious diseases worldwide. Among these bacteria, P.aeruginosa is one of which highly resists to many currently used drugs and becomes a major concern in public health. Our study aims to assess an in vitro antibacterial activity of green coffee Arabica bean aqueous extract against the standard P.aeruginosa (ATCC 27853). The standard bacteria, P.aeruginosa (ATCC 27853) were prepared by sub-culturing a loopful of each strain in nutrient agar at 37°C for 24 hr. The turbidity of bacterial suspension was adjusted to 0.5 McFarland's standard giving a bacterial load of about 1 × 108 CFU/ ml . Antimicrobial susceptibility was done by Kirby Bauer technique and Zone of inhibition was measured to the nearest millimeter using scale. Aqueous extracts of coffee were obtained by a coffee brewing procedure and the antibacterial activity of green coffee Arabica bean extract was determined by disc diffusion method. Green coffee Arabic bean aqueous extract showed the strongest antimicrobial activity against Multidrug-resistant P.aeruginosa (ATCC 27853) with minimum inhibitory concentrations of 0.2 mg/ml and MBC of 0.25 mg/ml.

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I. INTRODUCTION

Coffee is one of the most widely consumed beverages worldwide, lauded for its pleasant flavor and aroma, its pharmacological characteristics and most importantly its role as a stimulant on mental and physical activity. Recently, the scientific and popular interest concerning its significance on health has increased due to the beneficial pharmacological properties established in clinical and epidemiological studies[1.]*P.aeruginosa* is a leading cause of nosocomial infections and is responsible for 10 percentages of all hospital-acquired infections worldwide.

It continues to pose a therapeutic challenge because of the high rate of morbidity and mortality associated with it and the possibility of development of drug resistance during therapy. Standard antibiotic regimes against *P.aeruginosa* are increasingly becoming ineffective due to the rise in drug resistance. With the scope for developing new antibiotics being limited, alternative treatment, options are gaining more and more attention[2]. A number of recent studies reported complementary and alternative treatment options to combat *P.aeruginosa*infections[2].

P. aeruginosa is a leading cause of hospital-acquired pneumonia and chronic lung infections in cystic fibrosis patients. Iron is essential for bacterial growth, and *P. aeruginosa* expresses multiple iron uptake systems, whose role in lung infection [3].The most frequent pathogens that cause the corneal ulcers are *P. aeruginosa* and *Staphylococcus aureus*[4]. Coffee extracts were investigated concerning their antimicrobial potential against different human pathogenic organisms by the disk diffusion method. The antimicrobial activity against pathogens such as *Staphylococcus aureus, Enterococcus faecalis, Staphylococcus epidermidis, P.aeruginosa, E. coli and Candida albicans*[1].

II. MATERIAL AND METHODS

The standard bacteria, *P.aeruginosa* (ATCC 27853) were already available at the laboratory of medical microbiology, College of Applied Medical Sciences at Shaqra University.

Study Design: Experimental research designs

Study Location: laboratory of medical microbiology, College of Applied Medical Sciences at Shaqra,Shaqra University

Study Duration: March 2017 to June 2017.

Material:

1. Test Organism:
2. The standard bacteria, *P.aeruginosa* (ATCC 27853) were already available at the laboratory of medical microbiology, College of Applied Medical Sciences at Shaqra, Shaqra University.
3. Green Coffee Bean :
4. Powdered Green coffee bean was obtained commercially from Shaqra store.
5. Muller-Hinton Agar (Oxoid):
6. It was commonly used for antibiotic susceptibility testing and determined Antibacterial activity of Green coffee bean extract on P. aeruginosa (ATCC 27853).
7. Antibiotic Disc (Oxoid) :
8. Imipenem (10 µg) ,CLA(5µg) ,Piperacillin(100µg) , Augmentin(30 µg) , Fusidic acid(10 µg), Metronidazole (5µg), Penicillin G(10 units) and Clindamycin(2 µg).
9. Nutrient Agar (Oxoid):
10. Nutrient agar used for cultivation *P. aeruginosa* (ATCC 27853).
11. Nutrient Broth:

Nutrient broth used for cultivation *P. aeruginosa* (ATCC 27853))

Procedure methodology

Preparation of inoculum[5].

Bacteria inoculum were prepared by sub-culturing a loopful of each strain in nutrient agar at 37°C for 24 hr. Colonies from the overnight cultures were picked with sterile loop and inoculated into sterile test tubes with 3 ml nutrient broth. The turbidity of bacterial suspension was adjusted to 0.5 McFarland's standard giving a bacterial load of about 1 × 108 CFU/ ml.

Antimicrobial Susceptibility[6].

Antimicrobial susceptibility was done by Kirby Bauer technique.

Antibiotic susceptibility testing was done as follows: 4 to 5 well-isolated colonies of the same morphological type from an agar plate were selected from 18 - 24 hour old culture. Top of each colony was touched with a wire loop and transferred to a tube containing 4 to 5 mL of peptone water. This suspension was incubated at 37˚C for 2 hours. Turbidity of suspension was adjusted to 0.5 McFarland standard. A sterile non toxic swab was dipped into this suspension & then rotated & pressed several times on inside wall of test tube above the level of fluid to remove excess inoculums. Dried surface of Muller Hinton agar was inoculated with the swab three times at 60°C from previous stroke. Plate was allowed to dry for about 5 minutes to remove the excess moisture. Antibiotic discs were placed appropriately on agar surface (minimum 24 mm distance between discs & 15 mm distance between disc & margin of plates). On one 120mm plate 6 antibiotic discs were placed. Once the disc was placed care was taken not to move the disk. Then plate was put in incubator at 37˚C in inverted position. Incubation was done for 16-18 hours aerobically. Zone of inhibition was measured to the nearest millimeter using scale.

Preparation of Green Coffee Bean Extract[7].

Aqueous extracts of coffee were obtained by a coffee brewing procedure based on a previous study by Antonio et al. Preparation of 30% extract was done by percolating 100 ml of pre-boiling (95ºC) sterile water through 30 g of ground coffee. A filter paper was used to filter the extracts. After preparation of 30% aqueous extract of coffee, further dilution was done using sterile water to obtain the concentrations of 20%, 10% and 5%.

Disc Diffusion Method[8]**.**

The antibacterial activity of Green coffee bean extract was determined by disc diffusion method. Aseptically swab the organism onto a Muller Hinton plate. Swab in three directions to ensure complete plate coverage. Let the plate stand for 5 minutes. Cut 5-mm squares on agar. Pour Green coffee bean extract. Incubate the plate inverted at 37°C for 24 to 48 hrs. Measure the zones of inhibition in millimeters, using a ruler on the underside of the plate. Record the zone size**.**

III. RESULT

Results in table 1,figure 1 and photo1 shown that the interpretation of antibiotic sensitivity test for *P. aeruginosa* (ATCC 27853) were sensitive to Imipenem (10µg) CLA (5µg) and Piperacillin (100µg) while strains were resistance to Augmentin (30µg) and Fusidic acid(10µg), Metronidazole(5µg), Penicillin G (10 units), and Clindamycin (2µg) .

Table no 1: Showsthe interpretation of antibiotic sensitivity test for *P. aeruginosa* (ATCC 27853).

antibiotic disc	**Strain**
	P. aeruginosa(ATCC 27853)
Imipenem(10 µg)	**S**
Piperacillin(100 µg)	**S**
CLA(5µg)	**S**
Clindamycin(2 µg)	**R**
Augmentin(30 µg)	**R**
Penicillin G(10 units)	**R**
Fusidic acid(10 µg)	**R**
Metronidazole(5 µg)	**R**

*S= sensitive
* R= resistance

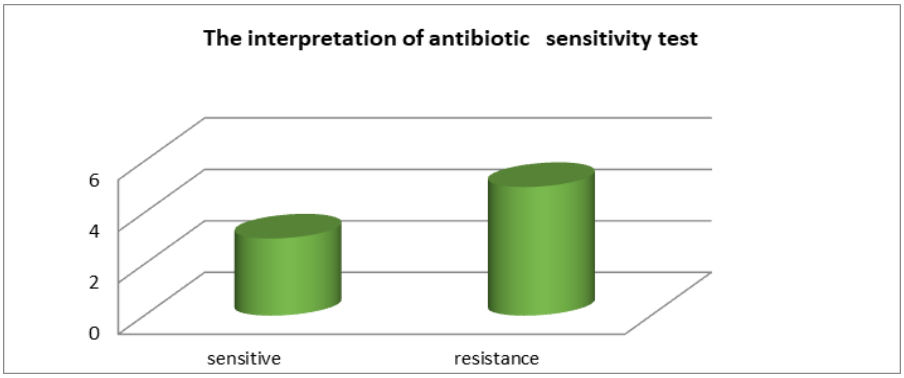

Figure1: The interpretation of antibiotic sensitivity test for *P. aeruginosa* (ATCC 27853)

Photo1: Interpretation of antibiotic sensitivity test for *P.aeruginosa* (ATCC 27853).

The results in table 2 and photo2 showed that the green coffee extract concentration increased with increased inhibition zone compared with the control on the one hand and with concentrations on the other hand. The inhibition zones were 0, 20, 13, 0 and 0 mm of green coffee extract 0, 0.3,0. 2, 0.1 and 0.5mg/ml, respectively.

Table no2: Records the Inhibition zone (mm) of green coffee aqueous extract against multidrug resistant *P.aeruginosa* (ATCC 27853).

green coffee extract (mg/ml)	control	0.3	0.2	0.1	0.5
Inhibition zone (mm)	**0**	**20**	**13**	**0**	**0**

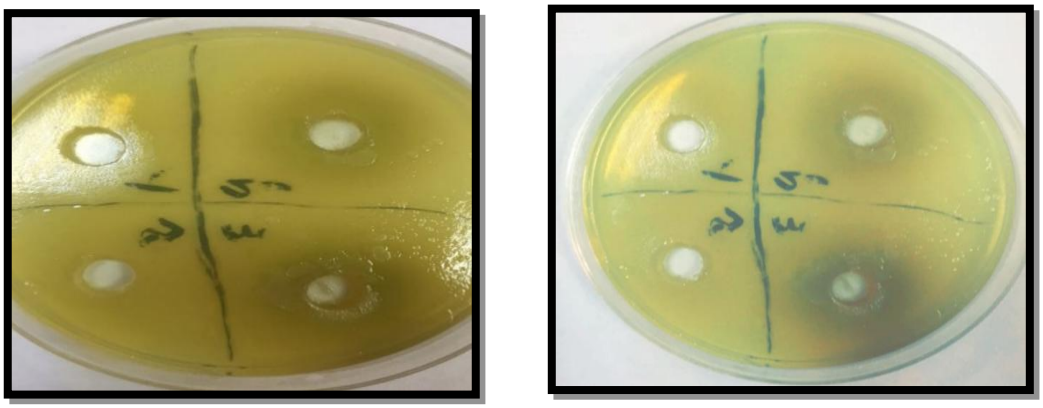

Photo2: Inhibition zone (mm) of green coffee aqueous extract against *multidrug resistant P. aeruginosa (ATCC 27853)*

Result in table 3and figure 2 revealed that Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) green coffee extract against multidrug resistant *P. aeruginosa* (ATCC 27853) were 0.2 mg/ml and 0.25 mg/ml respectively.

Table no3: Shows MIC and MBC of green coffee bean aqueous extract against multidrug resistant *P. aeruginosa*(ATCC 27853)

strains	MIC	MBC
Pseudomonas spp.	0.2 mg/ml	0.25mg/ml

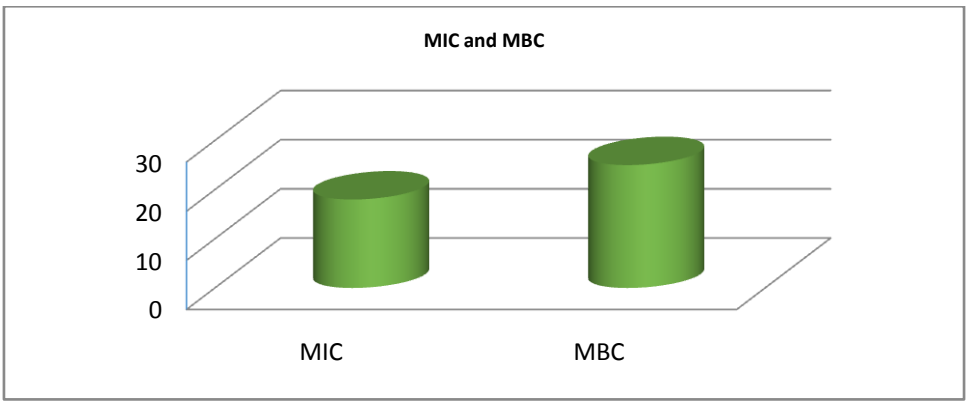

Figure 2: MIC and MBC of green coffee bean extract against multidrug resistant *P. aeruginosa* (ATCC 27853)

IV. DISCUSSION

P.aeruginosa is recognized as the etiological agent of several community- and healthcare-associated bacterial infections difficult to eradicate[9]. This Gram negative bacterium has developed strains with remarkable survival, disseminations and resistant mechanisms to the first election antibiotics such as β-lactam because its outer membrane functions as a barrier to several substances[10].

P.aeruginosa (ATCC 27853) in present study were resistant to Clindamycin Augmentin, Penicillin G, Fusidic acid , Metronidazole and this result agree with previous studies carried out by Al Zaidi , Igbalajobi et al, and Kaur et al,[11-13]have shown similar high degree resistance of *P. aeruginosa* to ceftazidime.

P.aeruginosa (ATCC 27853) in present study show sensitive to Piperacillin, CLA, Clindamycin and Imipenem . This result agree with Ahmed[14] who reported that 25% of *P.aeruginosa* (ATCC 27853) resistance to Imipenem while Lakum et al[15] reported that 21.6% of *P.aeruginosa* rom resistance to Imipenem and Kaur et al[13] revealed that 33% of *P.aeruginosa* isolated resistance to meropenem& 17.8% resistance to Imipenem.

After the emergence of multi-drugs resistant pathogens, the research for new remedy alternatives has led to the recognition of the potential of medicinal plant extracts for treating the infections associated to these types of microorganisms[16].Moreover, there is a synergistic effect of antimicrobial plant extracts with commonly used antibiotics; this effect has become the foundation of a multi targeted approach used against multi-drugs resistant bacteria[17].

Chlorogenic acid as the active ingredient present in the unroasted green coffee Arabica beans is responsible for its antimicrobial property. Polyphenol is an organic compound found in this extract is responsible for its antioxidants property. Leptin is another compound found in this extract is responsible for regulating the energy intake and expenditure[18].

Pruthviraj et al[19] demonstrated that the caffeine extracted from the leaves and leaf buds of Camellia sinensis (green tea), and beans of Coffea Arabica (coffee) inhibits the growth of gram-negative bacteria like *E. coli,P. mirabilis, Klebsiella pneumonia, P.aeruginosa.*

The present study investigated the antibacterial potential of green coffee Arabic beans extract against multidrug *P.aeruginosa* strains. The results clearly indicated the antibacterial activity of green coffee Arabica beans extract against all strains used in this study. Bacterial sensitivity to antimicrobial drugs classified as resistant, if an induced zone of inhibition by an antimicrobial drug is less than 8 mm; intermediate, if it is between 8-11 mm and sensitive, if it exerted an inhibition zone diameter of 12 mm or more[20] According to this report, all test *P.aeruginosa* strains (inhibition zone was 20,13mm) were sensitive to green coffee bean extract, this result agree with Deressa et al[5] who found that Coffee and cinnamon extracts, and honey have demonstrated a broad spectrum antibacterial effect against *E. coli*, Citrobacter (29 mm).species, *Staphylococcus epidermidisa* (31 mm), *P.aeruginosa* (ATCC 27853) (27 mm), and *Staphylococcus aureus* (ATCC 2923) (35 mm).

In current study MBC of green coffee Arabica beans excreta was 0.25mg/ml and MIC was 0.2 mg/ml and this result agree with Tien et al[8] they found that coffee at a concentration of 20% and 15% showed activity against*P. Gingivalis, P. Intermedia* and disagree with Sura[21]reveledthat MBC and MIC of Coffee against *S. mutans* was 200 mg/ml and was 600mg/ml respectively.

V. CONCLUSION

Green coffee Arabica bean extract have bactericidal and bacteriostatic effect against multidrug-resistant *P.aeruginosa* (ATCC 27853).

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