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COMPARISON THE ANTIBACTERIAL AND ANTIBIOFILM ACTIVITY OF *CITRULLUS COLOCYNTHIS* SEEDS AQUEOUS EXTRACT AGAINST THE BACTERIA ISOLATED FROM HUMAN AND SHEEP ORAL CAVITY

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Abstract
The current study was comparison between the bacteria isolated from oral cavity of animal and human against *Citrullus colocynthis* seeds extract, bacterial isolates were obtained from Al-Muthanna University, College of Veterinary Medicine, the isolates were isolated and from sheep's mouth that located in the Salman area, same isolates were isolated from human's mouth such as *Raoultella ornithinolytica*, *Citrobacter freundii, Pseudomonas aeruginosa, Enterobacter cloacae*, The MIC of *C.colocynthis* seeds extract was ranged from 100 to 66 mg/ml ,while the antibacterial activity results showed generally the isolates from sheep was more resistance than human with significant difference at $p \leq 0.05$ in different concentration, However the higher antibacterial activity noted in 400 mg/ml , in addition *E.cloacae* showed more susceptibility toward the extract in 200 mg/ml, 300 mg/ml and 400 mg/ml, the anti biofilm formation activity showed the bacteria from sheep had biofilm formation stronger than human's isolates with significant differences at $p \leq 0.05$ but after exposure to the extract the biofilm formation was decreased with no significantly between human and sheep isolates at $p \leq 0.05$, from the other side the findings reported a there was a significant difference before and after the treatment at $p \leq 0.05$ in both animal's isolates and human's isolates. the *C. colocynthis* seedsextract showed antibacterial and antibioilm formation activity against the isolates from human and sheep source but the bacteria from sheep showed resistance to the extract higher than the bacteria from human source .

Keywords: *Citrullus colocynthis* seed extract, anti-bacterial, anti-biofilm, oral cavity ,MIC

Introduction

In addition to serving as a source of food, plants are used as the starting point for the creation of many different medications (1). Citrullus colocynthis is a desert plant that has a wide range of bioactive compounds, including as flavonoids glycosides, fatty acids, and alkaloids. The immune system is strengthened by plant medications. Indigestion and gastroenteritis, together with intestinal parasites, have been treated with the dried fruit pulp of C. *colocynthis*. C. colocynthis has excellent pharmacological properties, such as anthelmintic, anti-inflammatory, anti-diabetic, and anti-cancer actions, in addition to laxative and purgative effects. Many studies have been conducted on the fruit's antioxidant, antibacterial and anti-inflammatory qualities (2).

The popular cuisine has long made use of *C. colocynthis*. According to Kamran et al. (3), some of its therapeutic properties include antioxidant, anti-inflammatory, anti-diabetic, and antibacterial actions. *C. colocynthis* has anti-diabetic, antineoplastic, , hypolipidemic, profibrinolytic, fibrinolytic, anti-microbial, anti-allergic and immune-stimulating properties. The reproductive system and fertility are also impacted (4). Due to this constraint, a variety of other antimicrobials are in use, and consumer preferences are shifting toward food goods made from herbs and spices that are known to have antimicrobial characteristics (5). phytogenic additives are just a few of the many ingredients that have been chosen as antibiotic growth promoter alternatives (6). To replace antimicrobials, phytobiotics are added to the feed for chicken. Due to these substances' antibacterial, antifungal, anti-parasitic, and immunological stimulatory properties, they can be utilized as alternatives to antibiotic growth promoters, which will improve hens' ability to produce quality products (7). The seeds of *C. colocynthis* (CCS) contained ten bioactive substances. CCS are immune-stimulating, antimicrobial, and promote growth. According to Alzarah et al. (8), CCSP enhances production efficiency and reduces immunological suppression.

Material and Methods

The microorganisms used in study

Bacterial isolates were obtained from Al-Muthanna University, College of Veterinary Medicine, the isolates were isolated and from sheep's mouth located in the Salman area,same isolates were isolated from human's mouth, such as *Raoultella ornithinolytica*, *Citrobacter freundii, Pseudomonas aeruginosa, Enterobacter cloacae*.

Prepare the Aqueous Extract of plants (Seeds extract)

Citrullus Colocynthis fruit were collected from Al- Salman area and the seeds were separated then dried and converted into a powder .an electrical grinder was used. Denver analytic balance used to weigh 200 g of powder. After being dissolved in 1000 cc of distilled water. The mixture was shaken continuously for 12 hours with a shaker water bath at 60ºC on the second day (Memmert GmbH, Germany). In order to prepare three concentrations, the mixture was filtered using sterile gauze and incubated overnight in an incubator (GFL. GmbH. Germany) (9). Three concentrations were prepared 200, 300, 400 mg/ml.

Determination the Minimum Inhibition Concentration (MIC)of plants extract

Approximately 50 µl of the *C. Colocynthis* seed extract and 100 µl of bacterial suspensions (adjusted to 0.5 McFarland standard) were added to Eppendorf tubes and incubated for 24 hours. The Minimum inhibition concentration was then determined by looking at the tubes to see if there was any turbidity (10).

Determination Antibacterial activity

The antibacterial activity of plant extracts estimated bythe agar well diffusion method which the isolates were adjusted to 1.5×10^8 CFU/ml, and subcultured on Mueller-Hinton agar had wells (6 mm in diameter) that were filled by 0.1µl of prepared concentrations of different extract (200, 300, 400 mg/ml) (10).

Effect MIC of Extracts on Preformed Biofilm

The bacterial suspension 100 µl was added into wells (96-well microtiter plate), followed by incubation at 37°C for 24 hr, the formed biofilm was washed at least three time with PBS to remove the non-adherent cells after that 200 µl of MIC extract added. The plate was kept at 37°C for 24 hr, a well was left empty as a negative control in other side same steps were repeated but without MIC of extract as a positive control .The biofilm strength was calculated as following (11).

1. OD ≤ 0.11 = Non-biofilm-former (NBF)
2. 0.11 <OD ≤ 0.19 = Weak biofilm-former (WBF)
3. 0.188< OD ≤ 0.37= Moderate biofilm-former (MBF)
4. OD>0.37 = Strong biofilm-former.

Statistical analysis

The outcomes were presented as means ± standard deviation, and statistical analysis was performed on the mean differences using an analysis of variance (ANOVA) in accordance with LSD. When P<0.05, differences were deemed significant.

Results

Determination MIC of *C.Colocynthis* seeds **extract**

The MIC of extract was ranged from 100 to 66 mg/ml between the isolates as shown in table

Table (1): Showed the minimum inhibitory concentration of extracts

Isolates	(mg/ml) Sheep	mg/ml *Human*
R.ornithinolytica	≤100	78
C. freundii	≤90	75
P. aeruginosa	100	100
E. cloacae	≤95	66

Antibacterial activity of *C.Colocynthis* seeds extract.

The antimicrobial activity results generally indicated that bacteria that isolated from sheep's mouth were more resistance than human's isolates with a significant difference at p ≤ 0.05 in all studied concentrations, but the higher antibacterial activity was noted in 400 mg/ml against both the bacteria that isolated from sheep and human's mouth . Moreover *E. cloacae* showed susceptibility to the extract in 200 mg/ml, 300 mg/ml, and 400 mg/ml compared to other bacteria , as shown in tables 1, 2, and 3.

Table (2): The inhibition zone of the extract toward human's isolates

Isolates	200 mg/ml Mean±S.E	300 mg/ml Mean±S.E	400 mg/ml Mean±S.E
Raoultella ornithinolytica	5.4±0.13 b	7.00±0.16 b	10.5±0.31 b
Citrobacter freundii	4.93±0.40 b	3.66±0.76 c	6.13±0.47 d
Pseudomonas aeruginosa	2.79±0.06 c	6.69±0.07 b	12.23±0.15c
Enterobacter cloacae	6.40±0.22 a	8.96±1.45 a	15.24±0.3 a

Table (3): The inhibition zone of the extract toward sheep's isolates

Isolates	200 mg/ml Mean±S.E	300 mg/ml Mean±S.E	400 mg/ml Mean±S.E
Raoultella ornithinolytica	3.6±0.30 b	4.10±0.26 b	6.8±0.21 b
Citrobacter freundii	2.93±0.20b	2.86±0.46 c	4.13±0.47 c
Pseudomonas aeruginosa	3.70±0.76 b	5.69±0.47 a	7.63±0.25 b
Enterobacter cloacae	5.40±0.10 a	6.96±0.45 a	10.24±0.25 a

Table (4): Comparison between the inhibition zone of extract among the isolates

Isolates	Source	200 mg/ml	300 mg/ml	400 mg/ml

		Mean±S.E	Mean±S.E	Mean±S.E
R. ornithinolytica	Sheep	3.6±0.30b	4.10±0.26 b	6.8±0.21 b
	Human	5.4±0.13 a	7.00±0.16 a	10.5±0.31 a
C.freundii	Sheep	2.93±0.20a	2.86±0.46 c	4.13±0.47 b
	Human	4.93±0.40 b	3.66±0.76 a	6.13±0.47 a
P. aeruginosa	Sheep	3.70±0.76 a	5.69±0.47 a	7.63±0.25 b
	Human	2.79±0.06 b	6.69±0.07 a	12.23±0.15a
E. cloacae	Sheep	5.40±0.10 b	6.96±0.45 b	10.24±0.25b
	Human	6.40±0.22 a	8.96±1.45 a	15.24±0.3 a

Anti-biofilm formation activity

The Biofilm activity results showed the bacterial from sheep had biofilm formation stronger than human's isolates with significant differences at p≤0.05 but after exposure to the extract the biofilm formation was decreased with no significantly between human and sheep isolates at p≤0.05 from the other side the findings reported there was a significant difference before and after the treatment at p ≤ 0.05 in both animal's isolates and human's isolates as in figure (1).

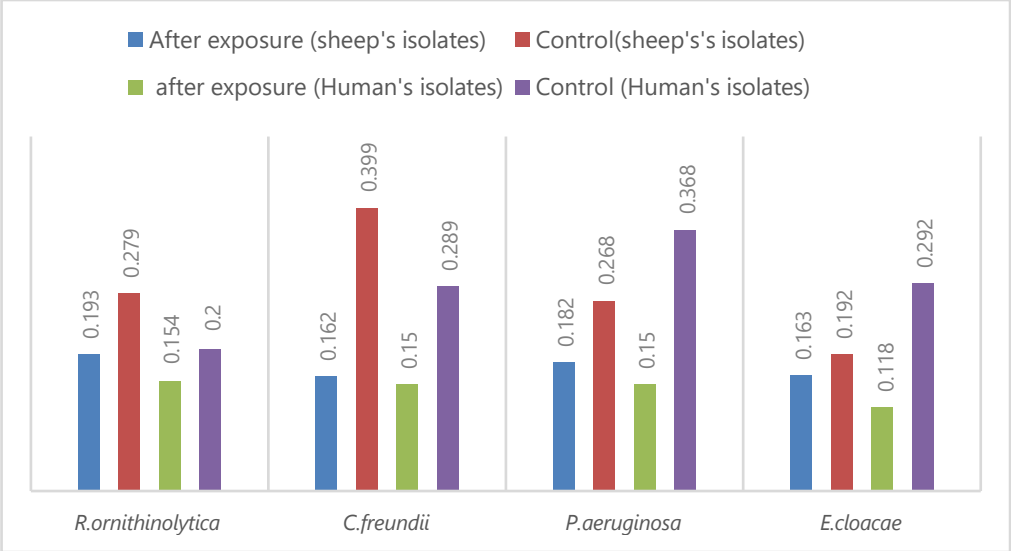


Figure (1): the biofilm formation of the isolates before and after exposure to seeds's extract

Discussion

The results were in agreement with numerous studies that assessed the antibacterial properties of the leaf and seeds of Citrullus colocynthis against gram positive and negative bacteria, but distinct in the response (12). The results supported by Priyavardhini et al (13)that *C. colocynthis* extracts displayed promising activity against all pathogens tested; additionally, the current results supported by Almalki(14) findings that the MIC values were found to be ranged from 100 to 150 g/ml for Gram positive bacteria and 100 to 250 g/ml for Gram negative bacteria, respectively.

In the same study, Almalki (14) found the antibacterial activity of the seeds of six different medicinal plants, including *C colocynthis*, *S aegyptiaca,* , *O. basilicum*, *H muticus. A lividus*, , and *R chalepensis*. With MIC values ranging from 100 to 250 g/ml, the MIC data showed varying levels of activity against Gram positive and Gram negative bacterial pathogens. The extracts of C. colocynthis, one of the plants, demonstrated promising efficacy against all of the pathogens tested; in particular, the MIC values were between 100 and 150 g/ml for Gram positive and between 100 and 250 g/ml for Gram negative. The MIC values of *C. colocynthis* towards *E. faecalis* ,S. *epidermidis*, *B. subtilis*, and *P. aeroginosa* were 100 µg/ml, *S. aureus* and *E. coli* were 150 µg/ml and towards *K. pneumoniae* 200 µg/ml respectively In addition, the results supported (15) which demonstrated that citrullus colocynthesis (L.) was effective against seven pathogenic bacterial strains, including *K. pneumonia , E. coli, P.mirabilis, Streptococcus spp, and S.aureus*. the use of

the antibiotic cefotaxime, the inhibitory effects of these extracts were compared. Alternatively, Bourhia et al. (16) examined the antimicrobial *C. colocynthis* for its possible antibacterial activity in the present investigation. In this regard, *E. faecalis* 471 and *L. monocytogenes* were resistant to the studied seed extract of C. colocynthis. The antibacterial efficacy of crude ethanolic extracts of Citrullus colocynthis Schrad's fruits, leaves, stems, and roots was investigated against Gram positive and Gram negative bacilli. Fruit and root extracts at double the strength were effective against Gram positive bacilli (Bacillus subtilis) whereas ethanol extracts of fruits, leaves, stems, and roots were shown to be potent against *B.pumilus* and *S.aureus*. According to Memon et al. (17), neither *E. coli nor P. aeruginosa*, two Gram-negative bacilli, responded to the treatment. The anti-biofilm of the compounds against bacteria suggested that the activity was concentration dependent, and the results of the antibiofilm formation activity agreed with (18). The new chemical was active against all tested concentrations in the case of *E. coli*, with *P. aeruginosa* showing the maximum property at 100 ug/ml. Several researches have clarified the antibacterial processes of the metabolites obtained from plants. According to research, active chemicals adhere to pathogenic bacteria' cell walls and produce an unfavorable environment for the outer cellular membranes, altering the contents of cells and allowing inner membrane contents to seep out (19). In comparison, plant components such, lectins,flavonoids, phenolics, tannins, quinines ,coumarins, terpenoids, , alkaloids, and polypeptides impede ATPase synthese, directly stimulating changes in bacterial cells' physiology that result in cell death (20).

The phenolic chemicals produced are known to lyse cell membranes and result in cell death (21). Due to their adaptation to *C. colocynthis* and the waste water, which resulted in acquired resistance to the phenolic chemicals, sheep isolates showed diminished sensitivity to the extract. Phenolic compounds were found to be the most frequent organic contaminants in wastewater, according to a research. The structure and operations of the microbial population may be affected by various substances throughout the wastewater treatment process. Nevertheless, the impact of phenolic compounds on the horizontal transfer of antibiotic resistance genes (ARGs) in wastewater treatment facilities (22).

Conclusion

The *C. colocynthis* seed extract showed antibacterial and antibioilm formation activity against the isolates from human and sheep source but the bacteria from sheep showed resistance to the extract higher than the bacteria from human source

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