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Molecular detection of *Escherichia coli* in ulcerative colitis and the potential therapeutic activity of *Artemisia annua* extract in Sulaimaniyah province.

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ABSTRACT

Background: *Escherichia coli* is part of a huge bacterial family, Enterobacteriaceae, which are gram-negative bacilli, rod-shaped bacteria that are usually found in the gastrointestinal tract of humans. *E. coli* is a motile, non-spore-forming, facultative anaerobic bacterium that ferments lactose quickly and produces an indole ring, oxidase-negative, catalase-positive colonies that appeared pink in color on MacConkey agar at optimum temperature 37°C. Its main virulence factors are toxins such as Shiga toxin-1 and Shiga toxin-2. E. coli frequently colonizes the colonic mucosa; it can cause chronic active colitis. Artemisia species are widely distributed throughout the world and are used worldwide for food, seasoning, and traditional medicinal treatment. A. annua extract has demonstrated diverse biological activities and contained antimicrobial compounds with selective activity against E. coli and other gram-negative bacteria.

Aimof the study: To detection pathogen E.coli in ulcerative colitis and the therapeutic potential activity of A. annua extract against isolated bacteria.

Patients and methods: This study was designed to include 74 children below 4 years old and 100 adult with a suspected of ulcerative colitis, who underwent biopsy sampling in the Teaching Hospital for Gastroenterology and Hepatology in Sulaimaniyah between Marc 2024 to December 2024. blood agar and MacConkey agar was employed for the cultivation of the bacteria at 37C for 24 hours. Prepared A.annua extract, then well diffusion method was used for detect the therapeutic potential activity of different concentration of A.annua against isolated bacteria.

Results: A significant difference ($P \leq 0.01$) has been found in value of the activity of different concentration of A. annua extract on the isolated bacteria compared with negative control. We have been recorded increased the mean level of both concentration of A.annua (20 and 40)mg/ml, with pathogenic bacteria compared with control.

Conclusion: The effective methods for detection pathogen *E.coli* in ulcerative colitis is Molecular method to detect the *E.coli* nucleic acid and the Stx1 gene that is responsible for colitis; *A. annua* extract showed significantly higher effectiveness against *E.coli.*

Keywords: *E.coli*, Stx1 gene, PCR, , *Artemisia annua*

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INTRODUCTION:

Within the category of inflammatory bowel disease (IBD), the two most common chronic inflammatory conditions are Crohn's disease (CD) and ulcerative colitis (UC). Fever, fatigue, diarrhea, bloody stools, weight loss, and stomach discomfort are among the many commonalities between these two conditions(1). The main causes of bloody diarrhea are Shiga toxin-producing *E. coli* and non-typhoid salmonella, listed in decreasing order of frequency of incidence(2). It is still largely unknown how widespread the problem is, how antimicrobial resistance (AMR) affects human health, and how much it costs the healthcare sector and society at large(3). *E. coli* is the most important bacterial food-borne agents in the world and the primary causes of intestinal illness in people(4). Resistance genes can be transferred between bacteria that typically live in the human colon; this type of transfer becomes a major issue when these innocuous bacteria turn into pathogens(5). Antibiotic effectiveness is at risk due to the rapid evolution of resistant microorganisms occurring globally(6). *E. coli* is one of the better natural flora in the digestive systems of humans and animals; certain strains can cause the intestinal tract disease known as gastroenteritis(7). *E. coli* facultative an aerobic and gram-negative, belongs to the family of bacteria known as Enterobacteriaceae(8). According to STEC virulence genes, Stx can function in the intestinal lumen and move to the lamina propria after clinical exposure. Purified Stx proteins induce ribotoxic stress, inflammation, and death in a variety of cell types. Stxs are released when STEC colonization occurs, and they adhere to intestinal epithelium but not to healthy intestinal cells(9). Since ancient times, plants have been a major source of therapeutic chemicals that have helped to maintain human health(10). Asia is home to the wild *A. annua*, a plant in the Asteraceae family(11). Around the world, ethano-pharmacological medicine relies heavily on the genus Artemisia(12). Within the Compositae (Asteraceae) family, the genus Artemisia has over 500 species commonly referred to as "Wormwood," "Mugwort," or "Tarragon." In North America, Asia, and Europe, most Artemisia species are found in temperate zones. However, It was found that The essential oil was shown to reduce the phenotypic expression of five soluble virulence factors: lecithinases, lipases,

Age group (Years)	No.of tested sample	Positivesample No. and (%)	Breast feeding No. and (%)of positive	Mixed feeding No. And (%) of positive	Formula feeding No.and(%) Of positive	Rural patient isolated M.O.%	Urban patient isolated M.O.	Age-specificimmunization status
1 – 5	74	9(12.16)	2(14.28)	2(14.28)	5(35.71)	5(55.55)	4(44.44)	9(100)
5 - 60	100	5 (5.0)	-	-	-	3 (60)	2(40)	5 (100)
□χ2 = 2.79		P = 0.078		P > 0.01		Non Significant		

DNase, hemolysins, and gelatinase. Antibacterial and antifungal qualities are among the several biological activities exhibited by other compounds derived from Artemisia species.The present investigation determined that the antimicrobial compounds present in the essential oil of A. annua exhibited antimicrobial activity against strains of yeast and bacteria, these compounds specifically inhibited the expression of virulence factors that are associated with cells and soluble substances(13). Numerous antibiotic-resistant bacterial strains have decreased the efficiency of several therapeutic antibiotics or, in some cases, completely eliminated their effectiveness (14).

Patients and Methods: One hundred and Seventy four biopsy samples were obtained from the patients attending to the Teaching Hospital for Gastroenterology and Hepatology in sulaimaniyah.the samples were inoculated in to transport media(tryptone soy broth) then transferred

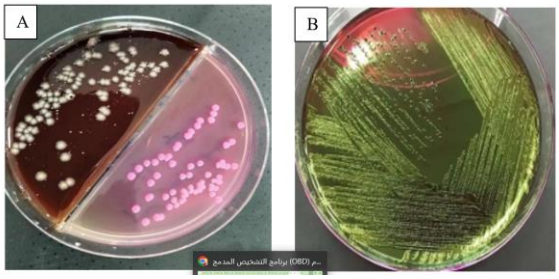
to the laboratory and inoculated on the blood agar and macConkey gar and incubated for 24 hr at37°C.

Ethical Approval: The Ethical Commission in the Kurdistan religion Ministry of Health certified this research. Teaching Hospital for Gastroenterology and Hepatology in Sulaimaniyah, Health Administration(934 at 2024.3.25). informed permission was provided by the Ministry of Health and the University of Tikrit/College of Medicine.

Statistical analysis: Statistical data analysis was carried out using SPSS v. 23 program Categorical variables were presented as frequencies and percentage and correlation test used . Quantitative data were expressed as mean ± standard deviation. A NOVA was used to compare between means of variables value of P value <0.001 was regarded as statistically significant.

Result: Fourteen(8.0%) *E.coli* were isolated,The results revealed high percentage of isolation among GIT biopsy samples collected from children formula feeding 5(35.71%), 2(14.28%) positive mixed feeding 2(14.28%) isolated from breast feeding and 5(35.71%) from adult over five years old. **Table (1):** Number and percentage of children that breastfeeding, mix feeding, formula feeding and adult with number and percentage of Urban and Rural patient.

Culture characteristic: Out of 174 patients with probable colitis, 14 biopsy samples (8.0%) were isolated as E. coli and the bacteriology culture test yielded positive results. The colonies were spherical, big, and white on blood agar; they were pink on MacConkey agar (Fig.1). All E. coli isolates were identified by Gram's staining, biochemical tests, colony shape, and additionally relying on the vitek2 compact system for confirmation after the samples were cultured on MacConkey medium and incubated for 24 hours under conditions conducive to the development of these bacteria.



(Figure 1) A- E.coli on each of blood agar (left)and MacConkey agar(right). B- E.coli on E.M.B

Biochemical tests: MacConkey agar and Blood agar were used to cultivate all of the E. coli isolates. The light pink agar became dark pink as a result of the lactose sugar being fermented by the E. coli bacteria in this medium (1.A). The pink, spherical, smooth colonies that have been recognized as cultural characteristics are consistent with the cultivation features on MacConky agar media and the analysis of E. Coli(15). Eosin Methylene Blue (EMB) agar medium is used as the differential medium for the microorganisms. On this medium, the growth of E. coli is shown by a green metallic sheen (figure 1.B). All E. coli isolates were non-hemolytic and grew in spherical, gray-white, moist, opaque, convex, and complete colonies on blood agar. At 37 °C, the E. coli on EMB agar were incubated for a whole day. Gram staining of the isolates under a microscope showed rod-shaped, Gram-negative bacteria, as previously reported by(16)., who had previously published similar observations about the properties of E. coli cultures on blood agar. Catalase tests were positive and oxidase tests were negative for every E. coli isolate. To differentiate between isolates of E. coli, the Indole test—which produces a red Indol ring—is an essential diagnostic tool. A good outcome was shown by the E. coli isolates' diffusion activity in the motility test. Both the Simmon citrate and urease tests yielded negative results since there was

no color change. A negative result for the Veges-Proskour test was suggested because there was no noticeable color change upon application of the reagent. E. coli isolates on triple sugar iron (T.S.I.) agar ferment sugar and release CO2, which causes the medium to turn yellow.

Table (2) Biochemical tests of *E. coli.*

No.	Biochemical tests	Reactions
1	Oxidase	-
2	Catalase	+
3	Motility	+
4	Indol production	+
5	E.M.B.	Green-metallic sheen
6	Simmon Citrate	-
7	Methyl – red	+
8	Urease	-
9	Voges-Pruskour	-
10	T.S.I.	A/A , +gas
11	liquefy gelatin	-

(-) Negative result, (+) Positive result , A/A(ferment all sugar) , +gas = CO2.

Freedom The tested isolates were identified as E. coli through further biochemical testing performed with the VITEK2 compact system to validate characterization.

Polymerase chain reaction(PCR):

Molecular approaches to the detection of E. coli 14 positive biopsy samples had their genomic DNA extracted directly; these samples were phenotypically positive, underwent molecular identification, and were then utilized for conventional PCR to determine the presence of an E. coli infection. The 2% Agarose gel electrophoresis of 13 samples (92.85%) of biopsy tissue containing entire DNA recovered from bacterial growth in colon biopsies immediately revealed PCR results for 347 bp of Stx1 (encoding Shiga toxin 1) when compared to the molecular ladder (100-2000). Electrophoresis on 2% agarose at 5 volt/cm2 for 1:30 hours produced the result.as shown in Figure (2).

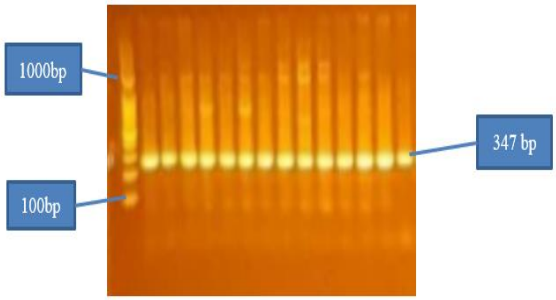


Fig. (2): Stx1 gene , which has the size (347 bp).

Antimicrobial sensitivity tests:

Sensitivity tests towards (18) different antibiotics were done for 14 *E. coli*, isolates by using VITEK2 Compact System, The results for the selected isolates appeared different resistant patterns.

Table (3) E.coli VITEK2 Compact System results for antibiotic resistant.

Isolated microorganisms	(IZD mm) of 20mg/ml Artemisia plant	(IZD mm) of 40mg/ml Artemisia plant	(IZD mm)of Ethanol 50% (control)
E.coli 1	14	16	0
E.coli 2	14	18	0

Antibiotic	*E.coli*	
	Resistant	%
Ampicillin	13	92.85
Amoxicillin / Clavulanic acid	13	92.85
Ticarcillin	12	85.71
Pipracillin / Tazobactam	12	85.71
Cefalotin	6	42.85
Cefoxitin	2	14.28
Cefotaxime	13	92.85
Ceftazidime	9	64.28
Ertapenem	1	7.14
Imipenem	2	14.28

Amikacin	2	14.28
Gentamicin	2	14.28
Tobramycin	5	35.71
Nalidixic Acid	12	85.71
Ciprofloxacin	9	64.28
Ofloxacin	8	57.14
Nitrofurantion	5	35.71

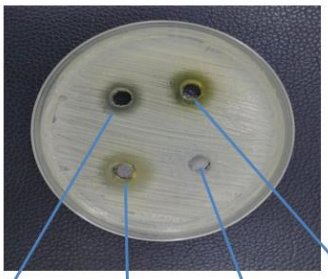
The susceptibility of E.coli to different concentration of Artemisia annua extraction solution, using agar well diffusion technique:

Antimicrobial activity of A. annua plant extraction solution using agar well diffusion assay ,was used to evaluate antimicrobial activity of A.annua extaction. Target strains were inoculated in mueller hinton agar at 37°C for 24hr. serial dilutions were made to find a density of about 107 cells/ml. Antimicrobial activities was carried out by spreading 0.1 ml of the bacterial cells in the agar surface. Four wells were created on the surface of the culture and plates were incubated at 37°C for 24 hr. Inhibition zones were measured with a digital caliper.

Table 4: The susceptibility result for (20 and 40) mg Artemisia annua/ml Ethanol 50% on the E.coli show different inhibition zone diameters(IZD).

* IZD= Inhibition zone diameter

The obtained results showed that the tested isolates bacterial were completely susceptible to the antimicrobial effect of *A.annua* extract for both concentrations (20 and 40mg/ml) these observations suggest that Artemisia plant extraction solution may be effectively used as alternative antimicrobial agent for microbial infections.



Ampicillin , Aa (40mg/ml) , Ethanol 50% , Aa(20mg/ml)

Fig.(3): Effect of *A.annua* on *E. coli* at 37°C for 24hrs.

Table 5: Inhibition rates of Artemisia plant extract on *E. coli* with time.

E.coli	Zero time	After 60 min	After 120 min	After 240 min	After 480min
	Bacterial count (CFU/ml)				
20mg/ml A.annua	10000000	5444444	117762	5877	126
40mg/ml A.annua	10000000	3423433	25433	2642	0
X2 = 26.185	P = 0.000014	P < 0.01	Highly Significant		

The result of exposure to *A.annua* extract solution for both concentrations (20 and 40)mg/ml at 37°C with different time periods on *E.coli* isolates, mean value of two isolates (E1, E2) viability may be seen in (table 5) and (fig. 3) The inhibition rate studies presented that the viability of the all number of *E.coli* isolates were drastically reduced with the (20mg/ml)concentration to a non-existing level with (40mg/ml) concentration after (480) minutes of exposure at 37°C.This demonstrate that A. annua has an antimicrobial activity across the tested *E.coli* isolates. The data of the inhibiting rate for the 40mg/ml *A.annua* extract solution on the all number of viable CFU/ml for *E.coli* isolates indicate a significant decrease in activity (P< 0.01) with (0) viable CFU/ml remaining after (480) minutes of exposure at 37°C compared with (10000000) viable CFU/ml at zero time.

Discussion:

In the table (1) the results show that the percentage of E. coli was isolates with a percentage of (8.0%). This result was

disagreement with the locally result that obtained by(17). with a percentage of 24%. All E.coli isolates were identified by Gram's staining and biochemical tests. The bacteria were observed as *E. coli* is gram-negative, straight, rod-shaped, non-sporing, non-acid fast, and bacilli that exist in single and pairs. Cells are typically rod-shape(18). also 52 patient under 4 years old breastfed from total 74 patient (70.27%), exclusively breastfed infants are exposed less to many pathogens than are infants who are not breastfed or mixed feedings, after passive immunity(IgA) wanes(19). The results revealed high percentage of isolation among GIT biopsy samples collected from children formula feeding 5(35.71%), mixed feeding 2(14.28%), 2(14.28%) breast feeding and 5(35.71) isolated from adult (table-1). This may be because lactoferrin, oligosaccharides, and breast milk secretory IgA play a dynamic role in enhancing the immune response against harmful bacteria and preventing adhesion. The results of the local study, which found a significant percentage of microbes isolated in samples taken from children who were formula-fed (78.6%), mixed-fed (8.35%), and breastfed (13.3%), were almost in agreement with this one. The present study concludes that natural breastfeeding is important for children to eliminate intestinal infections, particularly diarrheagenic E. coli (20). The result appeared that the higher number of Age-specific immunization status (100%) which its agreement with the result of (21). who obtained (96.3%). The odds of diarrhea were higher among rural people than urban ones and this was consistent with the findings in Sulaimaniyah and other outside location, this could be attributed to the fact that the lack of access to water and sanitation facilities in the rural was more than in the urban areas (22).

Molecular methods for E.coli detection:
The genomic DNA was extracted directly from 14 positive biopsy samples were phenotypically positive underwent molecular identification, was used for (conventional PCR) to determine E.coli infection. Whole DNA extracted from bacterial growth in colon biopsies directly were subjected to 2% Agarose gel electrophoresis of DNA from biopsy tissue 13 samples (92.85%)this result because many *E. coli* are carrier Stx1 gene which responsible for colitis infection and the remain one bacteria didn't have this gene but may be carrier another pathogenic gene that have cause to colitis,gel electrophoresis directly showing PCR products for 347 bp of Stx1 (encoding shiga toxin 1)when compared to the molecular ladder (2000-100).It has been demonstrated that the 16S rRNA PCR assay can correctly identify a variety of bacteria in a variety of sample types, The detection of 16S rRNA by PCR is a quick and accurate method. They noted that utilizing 16S rRNA identified all isolates as E. coli(23).

Susceptibility of *E.coli* isolates for antimicrobials: the results in the (table 2) show high resistance (92.85%) for AmoxicillinClavulanic acid and Ampicillin and giving an indication that the studied *E.coli* isolates have the capacity to resist β-lactam antibiotics by producing β-lactamase enzymes or by decrease the permeability of the outer membrane(24). The resistance for Ticarcillin high showed a rate of (85.71%). This was approximately dis agreement to the results of(25). with a rate of (98%). The results also showed that *E.coli* isolates were highly resistant to Pipracillin / Tazobactam (85.71%), also in agreement with the result of (26). that obtained(96%). Ciprofloxacin(64.28%), These results were in agreement with the results of (24). who obtained result rate (100%) resistance for this antibiotic. these bacteria carry the

resistance genes for this antibiotic, The results also indicate lower resistance for aminoglycoside Nitrofurantion(35.71%), Amikacin,Imipenem,Gentamicin(14.28%), Trimethoprim/sulfamethoxazole (28.57%), The resistance may refer to antibiotic alteration by cellular enzymes which is considered as the most common resistance mechanisms or may be due to mutations in the ribosomal proteins, This was agreement with a local study done by(27). (In Arabic) found that (31.8%) of his *E.coli* isolates were resistant to Amikacin , whereas Gentamicin resistance was agreement with the results of(27). with a resistance rate of (31.8%). The results also showed high resistance to Cefotaxime with a rate of (92.85%). These results were nearly agreement with the results obtained by(28).with a rate of (98%) resistance. The resistance for Nalidixic Acid showed a rate of (85.71%). This was nearly agreement to the results of(29). who obtained a rate of higher resistance (91%). also results for Tobramycin resistance (35.71%) were agreement with the result done by(25). who found that (39.6%) of his research *E.coli* isolates were resistant to Tobramycin . The resistance mechanism for this antibiotic was by decreasing the antibiotic accumulation inside the cell and throwing it outside the cell, The percentages of *E.coli* resistance to Nitrofurantion antibiotics were (35.71%), This is nearly agreement with the local study results of(27). in Iraq, she found (36.3%) resistance for Nitrofurantion respectively. The result for Cefalotin resistance (42.85%) which agreed with the results of(30). The result for Ceftazidime resistance was (64.28%), which in agreed with the results of (28). with a resistance rate of (27.4%)for this antibiotic.The result for Ofloxacin resistance was (57.14%), which agreed with the results of (31). with a resistance rate of (61.9%)for this antibiotic. The result for Cefoxitin resistance was (14.28%), which is agreed with the results of(25). with a resistance rate of (18.8%) for this antibiotic. The resistance for Ertapenem showed a rate of (7.14%). This was approximately agreement to the results of(25). with a rate of (4.2%)., The results are considered clear evidence for the best capacity of Artemisia plant extract solution in treatment of various *E.coli* infections. They are agreement with the result that obtained with(32),(33). That a.annua have a grate inhibition rate for all *E. coli.*

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