

RESEARCH PAPER

Effect of pomegranate peel extract and Thyme leaves extract on growth of *Escherichia coli* isolated from infant diarrhea

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ABSTRACT:

This study were done in Al Batoul Teaching Hospital Baquba, Diyala Province, Iraq from 25/ 9/2016 to 1/2/2017, two hundred Stool samples were collected in sterile container from infants suffered from diarrhea with the age less than two year. All stool samples were cultured on MacConkey agar to isolation and identify *Enteropathogenic Escherichia coli* and then sub cultured of expected isolates on the Eosin Methylene Blue agar medium (EMB) , identification of bacteria on the base of morphological characteristics (colony , shape , Gram stain), and biochemical tests were done according to standard procedures, then confirmation of diagnoses were performed by by Api20E and VITEK 2. Sensitivity test of *Escherichia coli* isolates to fifteen antibiotics were performed to select the multi drug resistance isolates for investigation the inhibition effect of pomegranate peel extract and Thyme leaf extract on those isolates ,the results showed a high antibacterial effect of alcoholic extracts of pomegranate peel followed by the hot and cold water extract, were the rate of diameters of the inhibition zone at the concentration 100 mg / ml 21.92 , 18.02 and 14.02 mm respectively. The inhibitory activity of Thyme leaf extract at concentration of 100 mg /ml for alcoholic, aquatic extract hot and cold water extract were 11.3,9.8 and 3.22 mm respectively. We concluded that both alcoholic extracts of pomegranate peel and Thyme have a good inhibitory effect against growth of multi drugs resistance pathogenic *Escherichia coli* .

KEY WORDS: *Escherichia coli* , infants diarrhea , plant extracts ,pomegranate thyme.

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1. INTRODUCTION:

Infants diarrhea is one of the important illnesses with high morbidity and mortality in children with a symptoms of having

More five loose or liquid stool per day, it remains the second leading r in dehydration and cause of death in children younger than five years^[1,2]

Several studies indicate that the average number of diarrheal cases per year in children younger than five years in developing countries is 3.2 - 4.8 million cases occurring during the first year of life and gradually decreases at 4 years^[3] .There are many causes of infectious diarrhea, which include viruses such as *Rotaviruses* ,

bacteria such as *Escherichia coli*, *Salmonella* and *Shigellae* etc, and parasites such as *Entamoeba histolytica* others, each of these microbial pathogens causes disease in a different mechanism, infectious diarrhea is frequently referred to as gastroenteritis^[4].

Escherichia coli is a normal flora where it is present in the human intestine and mammals. However some strains have the characteristics of virulence factors, causing serious diseases which have been described as a cause of diarrhea in children^[5] , The *Enteropathogenic Escherichia coli* (*EPEC*) is a major pathogen causing diarrhea , the infection is associated with one or more of the parasitic and viral pathogens of gastroenteritis such as *Entamoeba histolytica* and *Balantidium coli*^[6].

Severel al studies performed in iraq for isolation of infectious agent cause infants diarrhea one of

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this study performed in Duhok province/ Kurdistan region on 522 infants diarrhea samples the result show that *Escherichia coli* the most prevalent enteric bacteria were form 305 (58.43 %),and in mixed infection were *E coli* and *E histolytica* (64.34 and 62.01 % respectively ^[7] , other study in Iraq carried for isolation and detection of *Escherichia coli* from 40 Iraqi patients with chronic diarrhea reveal that *Escherichia coli* form 16 (40 %) ^[8]

Recently medicinal herbs and plants has been widely used in treatment because of its low side effects, the few concentrations are acceptable to the body in its natural form and are highly synthesized in the secondary metabolites of plants effective against the pathogens and characterized by their high stability and excellent biological performance ^[9,10] .

Thyme plant one of the important medicinal plants because their active chemical ,constituents against growth of bacteria include Thymol , carvacrol , Terpinene , pycmene ^[11,12] , so that the thyme benefit in treatment of different diseases as antirhematic , antiseptic , bactericidal , hypertensive , urinary tract infection , respiratory , gastric , bronchitis , spasm , others ^[13,14] .

The second important medical plant use in current studypomegranate peel due to the active peel constituents as ellagic acid ,Flavonols , gallotannins , and hydrolysable tannins, such as punicalagin, have the most activities ^[15,16,17] ,All parts of the pomegranate fruit were recorded in treatment of several diseases including cancer, inflammatory,atherogenic, diabetes, hepatoprotective, and antioxidant, and antioxidant activity, etc^[18,19] , in additon the high quantity of pomegranate peeavailable as a waste produce from manufacture processes of pomegranate juice which considered as environmental problem . The aim of our study was to conducting general stool analysis for infants infected with diarrhea associated with amoebic dysentery, isolation of *Enteropathogenic Escherichia coli* from infants with less than two year oldsuffered from diarrhea associated with amoebic dysentery, and study inhibitory effect of two plant extracts (pomegranate peel extract and Thyme leaf extract on the growth of bacteria .

Materials And Methods

Collection Of Samples

Two hundred of stool samples were collected from infants suffering from Amoebic dysentery diarrhea with age less than two year from Al Batoul Teaching Hospital, Baquba, Diyala Province, Iraq. Collection of samples were carried during the period 25/ 9/ 2016 to 1 / 2 /2017.

Isolation And Identification Of *Escherichia coli*

Stool samples were collected from infants infected with diarrhea, general stool examination were done to detect consistency, color, mucus, blood and parasites specially *Entamoeba histolytica* , then Stool sample were cultured on MacConkey agar and Eosin Methylene Blue (EMB) for isolation of *Escherichia coli* . All isolates were identified on the basis of colony characteristics, Gram stain, biochemical tests ^[20]. confirmation of identification were achieved by using the Api20E ^[21] and VITEK 2 systems ^[22].

Antibiotic Susceptibility Test

The resistance of *Escherichia coli* isolates to Antibiotics were

investigated against fifteen antibiotics according to the standard Kirby-Bauer (disk diffusion method) , antibiotics include Tetracycline(75 µg) , Meropenem(10 µg) , Ampicillin_ Sulbactam(10_10 µg) , Doxycycline(30 µg) , , Cefoxitin(30 µg) , Ciprofloxacin(5 µg) , Gentamycin(10 µg) , Augmentin(20_10 µg) , Piperacillin(100 µg) Tetracycline(30 µg) (Chloramphenicol (30 µg) , Amikacin(30 µg) , Cefixime(5 µg) , Cefuroxime (30 µg) , Ceftriaxone (30 µg) , spread (100 ul) of bacterial isolate suspension with 5×10^8 cell / ml concentration by sterile cotton swab on the surface of Muller Hinton agar medium , seven antibiotics disc were placed in each plates and incubate for 24 hour at 37 C recorded the inhibition zone of growth , the determination of resistant isolates occur depending on the (CLSI 2014)^[23] .

Plant extracts:

pomegranate peel and thyme leaf were selected as a test plant , ten grams of plant powdered was dissolved in 100 ml of 70% ethanol solvent for

preparation of alcoholic extract by procedures as described in [24], ten grams of plant powdered was dissolved in 100 ml of distilled hot water and distilled cold water for preparation of hot water extract and cold water [25, 26]. For the preparation of four plant extract concentrations from raw extracts to screening antibacterial testing , dissolved (1) gm in 10 ml of distilled to obtain solution with 100 mg / ml concentration ,then we prepare solutions with 20 ,40,60,80,100 mg /dl concentration , sterilize by use 0.22 Millipore filter and stored at4C in refrigerated conditions till use in the study [27] .

Inhibition effect of Plant extracts :

Suspension (100 ul) of testing bacterial isolates with 5×10^8 concentration were spread by sterile cotton swab on Muller Hinton Agar medium Well diffusion method were used to investigate the plant extracts activity, transport 50 ul from each concentration of plant

extracts by sterilize micropipette to wells with 6 mm diameter (makes by sterile cork bores) , Then plates incubated for 24 hours at 37 C , the

Table (1) : Biochemical test for *Escherichia coli* isolates identification

Biochemical tests						
Test	VP	MR	Indol	catalase	Oxodase	Citrate utilization
Result	-	+	+	+	-	-

(+) = positive (-) = negative , vp = voges-proskauer , MR =methyl red

The API_20E and VITEK 2 were then used to confirmation ,the results were compatible with the results of the biochemical tests .

The results of percentage of susceptibility 50 bacterial solates to antibiotics in table (2) showed highly resistance to Ticarcillin(98%) , Cefixime(88%) , Cefuroxime (90%) , and

diameter of inhibition zone per millimeters were measured [28].

Statistical analysis :

Data were analyzed by using (Chi-square) to compare between percentages and (T test) to compared between numerical data (means±SD), (Duncan test) also has been used to compared between means of numeric data at level of significance of P=0.05 was applied to test.SPSS v.21 and Excel 2013 programs were used to analyze current data [29].

Results and Discussion

In this study,50 isolates of *Escherichia coli* .were isolated from 200 stool sample among infants diarrhea associated with amoebic dysentery . culture on Mac Conky agar and subculture on eosin methylene blue for confirmation, the results of the biochemical tests of *Escherichia coli* showed that all isolates (50 isolates) yielded positive results for catalase, methyl red, and indol, while negative results with Voges-Proskauer (VP),Citrate Utilization and Oxidase test ,table (1)

Ceftriaxone (84%) . Tetracycline(56%) were as the highly effective antibiotics were

Meropenem (92 %) (, Ampicillin_ Sulbactam(56 %) , Doxycycline(58%) , , Cefoxitin(78 %) , Ciprofloxacin(56 %) , Gentamycin(64%) , Augmentin(54%) , Piperacillin (70 %) (Chloramphenicol (62 %) , Amikacin(64 %) ,

Table (2) Susceptibility of (50)*Escherichia coli* isolates to antibiotics

Antibiotics	Sensitive		Intermediate		Resistance	
	no	%	no	%	no	%
Tetracycline	22	44	0	0	28	56
Doxycycline	29	58	6	12	15	30
Augmentin	27	54	13	26	10	20
Ciprofloxacin	28	56	12	24	10	20

Piperacillin	35	70	7	14	8	16
Meropenem	46	92	4	8	0	0
Chloramphenicol	31	62	10	20	9	18
Cefixime	3	6	3	6	44	88
Ceftriaxone	3	6	5	10	42	84
Amikacin	35	70	9	18	6	12
Gentamycin	32	64	5	10	13	26
Cefuroxime	3	6	2	4	45	90
Ticarcillin	1	2	0	0	49	98
Cefoxitin	39	78	5	10	6	12
Ampicillin_ Sulbactam	28	56	10	20	12	24

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The results of inhibitory effect of plant extracts on the growth of selective multidrug resistance isolate of *E. coli* bacteria was show that alcoholic extract of pomegranate peel was superior on water extracts (hot water extract and cold water extract) in inhibiting the growth of *E. coli*, the highest inhibitory effect of alcoholic extract was 21.92 mm at 100 mg /ml, less inhibitory effect at 20 mg / ml, (4.86) . As for water extracts (hot and cold water extract), the inhibitory activity was 18.02 and 14.02 mm respectively at 100 mg / ml concentration and less inhibitory effect at 20 mg / ml concentration, the inhibitory effect of water extracts (hot and cold water extract) at this concentration was 2.11 and 0 mm

respectively. Significant differences were observed in the values of the inhibition effect of the alcoholic and aquatic extracts at concentrations (20, 40, 60, 80,100) mg / ml .In table (3) and figure (1) The results reveal that the pomegranate peel extract (alcoholic and water extracts) have a good inhibitory effect against *E.coli* in comparison to antibiotics ,The study showed that the relationship between the concentration of the plant extract and the inhibitory effect was positive, increasing the concentration of the extract the bacteria become more sensitive to it.

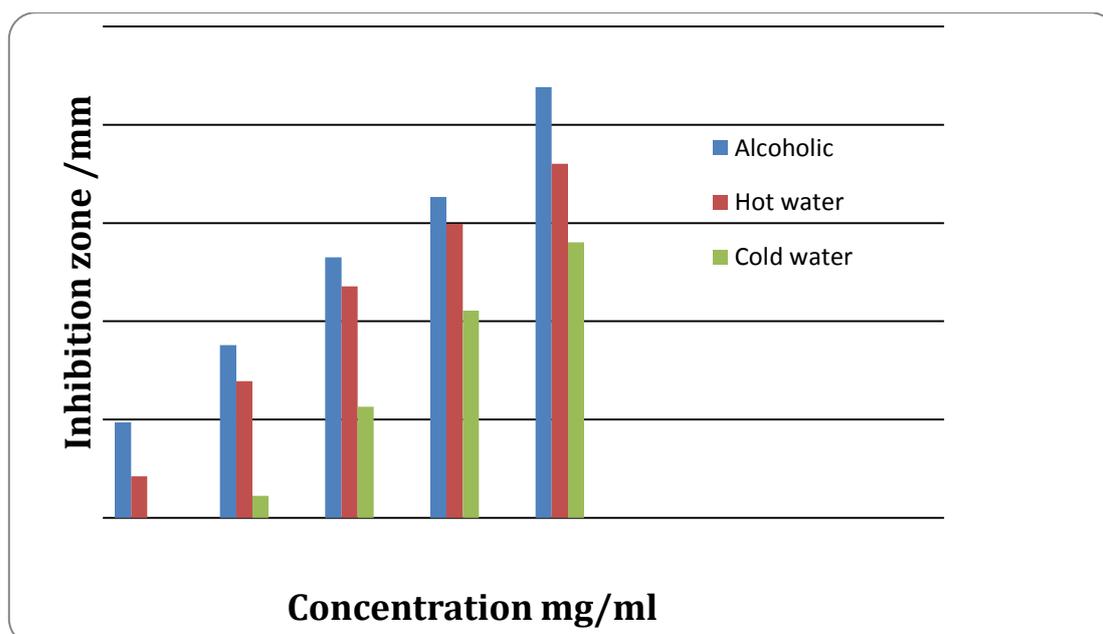


Figure (1) : inhibition zone / mm of four concentrations of pomegranate peel extracts

Table (3) :The inhibitory effect of Pomegranate peel on *E.coli* growth

Plant	Extracts	Inhibition zone/mm				
		Concentration mg/ml				
		20	40	60	80	100
Pomegranate	Alcoholic	4.86 ^d	8.78 ^c	13.26 ^b A	16.32 ^b A	21.92 ^a A
	Hot water	2.11 ^d	6.96 ^c	11.78 ^b	14.96 ^a A	18.02 ^a A
	Cold water	0	1.12 ^d	5.66 ^c	10.54 ^b	14.02 ^a A

* Small letters are used to compare horizontally between variables.

*Capital letters are used to compare vertically between variables.

* Different letters indicate significant differences between variables.

The chemical constituents of Pomegranate peel contain Tannin, and others which affects the nature of proteins in the bacteria, or may damage the plasma membrane, and changing its functional properties and inhibiting the growth of bacteria, so that the fruits and peel used in the treatment of abdominal, diarrhea and dysentery diseases and also reduces the level of cholesterol in the blood [30].

Current result similar to the finding of previous research that recorded the pomegranate peel extracts has potential effect more than ampicillin antibiotics on the growth of *Escherichia coli*, *salmonella*, and *staphylococcus* [31], the present finding was also agree with a results of [32,33].

Table (4) show the results of this study also showed that the inhibitory activity of Thyme leaf extract at a concentration of 100 mg / ml for Alcoholic, aquatic extracts (hot and cold water extract) 11.3, 9.8 and 3.22 mm respectively, and no inhibitory effect of alcoholic and aquatic extracts at 20 ,40 mg / ml concentration, The aqueous and alcoholic extracts of Thyme leaf did not exhibit any action or low effect on *E. coli* bacteria with the following concentration (60 and 80 mg/ml), no significant differences were

observed in the values of the inhibition zone of the alcoholic and aquatic extracts at concentrations (100) mg / ml figure (2) .

Hence it is probable that the minimal antibacterial effect recognized in current study in low concentrations may be due to the lower contents of bioactive components ., This result was agreed with a result of [34]. and the results of other previous study showed that the oil from *T. vulgaris* exhibited extremely inhibition effect on the all of antibiotics resistant clinical strains of *Staphylococcus*, *Enterococcus* and *Escherichia genus* were isolated from different infection infections of oral cavity, respiratory, genitourinary tracts and from hospital environment of hospital [35], the results of comparative study were showed that thyme oil has inhibition effect higher than Rose , pentaury and ozon oil on the growth of micro organisms [36]. The mechanism of inhibition effect due to the damage of : cytoplasmic membrane , active transport , coagulation of cell components , proton motive force , and electron flow [37].

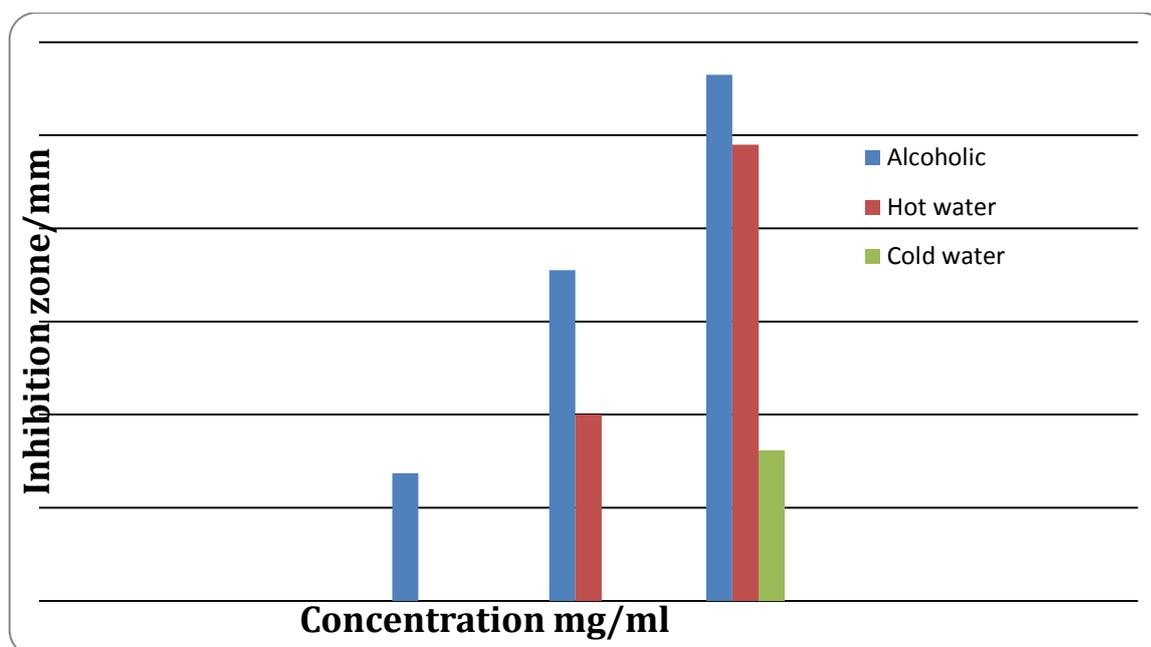
Table (4) The inhibitory effect of Thyme leaf extract on *E.coli* growth

Plant	Extracts	Inhibition zone/mm				
		Concentration mg/ml				
		20	40	60	80	100
Thyme	Alcoholic	0	0	2.74 ^c B	7.1 ^b B	11.3 ^a B
	Hot water	0	0	0	4 ^b B	9.8 ^a B
	Cold water	0	0	0	0	3.22 B

* Small letters are used to compare horizontally between variables.

*Capital letters are used to compare vertically between variables.

* Different letters indicate significant differences between variables.

**Figure (2) : inhibition zone / mm of four concentrations of Thyme extracts**

Conclusions

From the finding of current study we concluded that, both pomegranate peel extract and Thyme leaf extract have good inhibitory effect bacterial growth .of ,and alcoholic solvents was better than water solvent for types of extracts. The plant extracts considered a good effectively on

bacterial growth alternative when determine the toxin of plant .

Recommendations

We recommended to identify and extraction of the main chemical constituents of two plants and investigate their effects to the same or others pathogenic bacteria

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