Effect of seed oil *Ricinus communis* on *E. coli* isolated from Recurrent Urinary Tract Infections

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Abstract
This study was conducted in Wasit governorate for the period between February 2012 to February 2013 to determine the impact of Castor seed oil at different concentrations 100, 75, 50 and 25% in inhibition of the growth of *E. coli* isolated from 52 male and female patients (2-70 yrs) attending Al Zahra Hospital and Al Karama Hospital in Kut city. Oil was extracted from seeds of Castor had the ability to inhibit *E. coli* isolated from patients presented with recurrent urinary tract infections . Zone of inhibition accomplishing was 9.06 mm in diameter.HPLC analysis revealed that the content of α-linolenic in Castor seed oil (18.90 µg\ml) was higher than other fatty acids followed by oleic. Perhaps this is why it able to inhibit *E. coli*; which affect the cellular components in bacteria.
Key words: Castor oil, *Escherichia coli*, Urinary Tract Infections

تأثير زيت بذور الخروع *Ricinuscommunis* في بكتريا *E. coli* المعزولة من التهابات المجاري البولية المتكررة

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الخلاصة:
أجريت هذه الدراسة في محافظة واسط للمدة مابين شباط 2012 وشباط 2013 لتحديد تأثير زيت بذور الخروع بتراكيز مختلفة 25 و 50 و 75 و 100% في تثبيط نمو بكتريا *E. coli* المعزولة من 52 مريضا من الاناث والذكور باعمار تتراوح 2−70 سنة الراقدين في مستشفى الزهراء والكرامة في مدينة الكوت. الزيت المستخلص من بذور الخروع له القدرة في تثبيط بكتريا *E. coli* المعزولة من المرضى الذين يعانون من التهابات المجاري البولية المتكررة وبقطر تثبيط 9.06 ملم . كشف تحليل HPLC أن محتوى حامض الفا−لينولينيك في زيت بذور الخروع (18.90 ميكروجرام \ مل) كان أعلى من الأحماض الدهنية الاخرى ويليه حامض الأوليك. ولعل هذا هو السبب في كونه قادر على تثبيط *E. coli*؛ اذ يؤثر في مكونات الخلية في البكتيريا.

1. Introduction

The demand for herbal medicinal plant is growing very fast in recent years, because of the patient's resistance to antibiotics and its side effects. The screening of plant extracts and plant products for antimicrobial activity has shown that higher plants represent potential source of antibiotic prototypes [1].

Castor oil (ricinus oil, phorboyl, tangantangan oil) is natural oil derived from the seeds of the castor bean*Ricinuscommunis* by cold pressing (for medicinal use) or hot pressing (for industrial purposes). Chemically, castor oil is a triglyceride characterized by a high content of ricinolein (a glyceride of 12-hydroxy-9-octadecenoic acid). It has the approximate fatty

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acid composition of ricinoleic acid (87%), oleic acid (7%), linoleic acid (3%), palmitic acid (2%) and stearic acid (1%), with trace amounts of dihydroxystearic acid [2,3]. The cathartic action of orally ingested castor oil traditionally has been attributed to irritant or stimulatory effects of ricinoleic acid on the gastrointestinal smooth muscle; the ricinoleic acid is liberated in the small intestine by the action of pancreatic lipase [4,5,6]. Some of its physical and chemical properties were examined and compared with those of standard oils: olive, sunflower, cotton seed, Linseed, soybean, coconut, palm and castor.

The refined oil was found to show good to moderate activity against disease causing bacteria viz. *Shigella dysenterial, Salmonella typhi, S.aureus* and fungal pathogens viz. *Macrophomin aphascolma, Alternaria alternata, Curvularia lunata.*

Escherichia coli is present in the intestinal tracts of both humans and animals, is released into the environment through faecal material and is therefore used as an indicator of faecal contamination [7]. *E. coli* is also a reservoir for antibiotic resistance genes [8]. In the natural environment the resistant bacteria and resistance genes from animal or environmental origin might transfer to humans [9,10]. Use of antibiotics is one of the factors contributing to resistance [11-13]. Antibiotic resistant bacteria in soil and aquatic environment [14] and antibiotic resistant *E. coli* from human and animalsources have been previously documented [15].

The present work aimed to investigate the inhibitory effect of castor oil on *E. coli* isolated from patients suffering from urinary tract infections (UTI).

2. Material and Methods

General Urine Examination

Colour, consistency, specific gravity and pH of urine was recorded. Afterward, it was centrifuged for 15 minutes at 3000 rpm. Supernatant was removed for chemical tests involved glucose, ketone, protein and bile pigments. On the other hand, the residual was taken for direct examination to detect the presence of pus cells, red blood corpuscles (RBC), epithelial cells, bacterial cells and others**.**

Isolation and identification

Mid-stream urine specimens were collected from 52 male and female patients (2-70 yrs) attending Al Zahra Hospital and Al Karama Hospital in Kut city.A loopful of mid-stream urine specimens were streaked on MacConkey agar and Blood agar plates and incubated at 37°C for 24 hours. Conventional biochemical tests were employed foridentification of *E. coli* and the results were confirmed by analytical profile index (API) 20E.

Plant collection

Fresh seed (3g) of castor bean(Local), were collected from the University of Baghdad and University of Kut/ College of Agriculture. Once completely dried it was grounded to a powder form.

Preparation of extract

Powdered seed were extracted in Hexane 40% (w/v) at room temperature (25°C) [16]. The mixture was left for 24 hours and filtered using Whatman no.1 filter paper. The filtrates were then evaporated under reduced pressure and dried using a rotary evaporator at 55°C. The process was repeated to obtain extract from solvent (hexane). Dried extracts from the solvent was stored in labeled sterile screw capped bottles at -20 °C and analysis of the oil was conducted by HPLC at the ministry of Sciences and Technology. Dilutions by hexane to different concentrations (25%, 50%, 75% and 100%) of oil were prepared [12,14].

Inhibitory effect of fixed oil on *E. coli* isolates

Well diffusion method described by Ikeagwu*et al*. [17] was followed to detect inhibitory the activity of plant fixed oil by spreading bacterial suspension (1.5×10^8 cfu/ml) over Mueller Hinton agar plates using sterile cotton swab. Then wells with a diameter of 6 millimetres (20 mm apart from one another) were made on the surface of previously cultured Mueller Hinton agar plate. Wells were filled with 50 microlitre from each fixed oil plates were incubated aerobically at 37ºC for 24 hr, after incubation period, the diameter of inhibition zones around wells were recorded in millimetres. Tests were performed in triplicate.

Statistical analysis

Statistical analysis by factor design (RCBD) experience and three replicates and compared calculations adoption rates less significant difference at the level of probabilistic test (LSD). Differences were considered significant when $P < 0.05$.

3. Results and Discussion

General urine examination was carried out for 52 urine specimens collected from patient suffering symptoms of UTI. The results revealed that 31 (11.8 %) patient were suffered from

recurrent urinary tract infections. According to a study conducted by [18], the result shows infection of 89.5% from 310 urine samples and results of Hamlemant [19] showed infection of 87% from 300 urine samples.

Depending on morphological and cultural characteristics of resultant growth on solid media, a primary identification was adopted. White large colonies grown on blood agar and simultaneously appeared pink on MacConkey agar were identified primarily as *E. coli*. This primary identification was confirmed by conventional biochemical tests and api 20E system.

Table 1 summarizes the effect of castor oil on *E. coli* isolated from recurrent urinary tract infections by zone of inhibition accomplishing was 9.06 mm in diameter. The interaction between oil type and its concentration was significant ($P < 0.05$), given that oil at 100% concentration implies the highest inhibitory effect (figure 1). The antibacterial activities of the plant oils are in general agreement with previously reported studies on the oils of thyme and castor on *E. coli* [20], nutmeg, Soy bean and castor on *E. coli* [21-24],oregano on *E.coli* [25,26]

Table 1- Inhibition zones (mm in diameter) caused by castor oil against *E. coli* isolated from recurrent urinary tract infections

fixed oil / specimens	25%	50%	75%	100%	Control	Mean
1	6.0	6.0	6.0	6.5	6.0	6.12
2	6.0	6.16	6.66	8.0	6.0	6.7
3	6.0	6.0	6.23	7.0	6.0	6.3
4	6.3	7.0	11.0	13.66	6.0	9.5
5	6.0	6.0	6.0	6.23	6.0	6.05
6	6.0	6.0	6.0	6.0	6.0	6.0
7	6.0	6.0	6.0	6.0	6.0	6.0
8	6.0	6.0	6.0	6.0	6.0	6.12
9	6.0	6.0	6.0	6.0	6.0	6.0
10	6.0	6.0	6.0	6.0	6.0	6.0
Mean	6.03	6.1	6.53	7.08	6.0	
LSD	OIL = 0.16		CONs = 0.10		OIL ×CONs = 0.33	

Each datum is the mean of 10 specimens

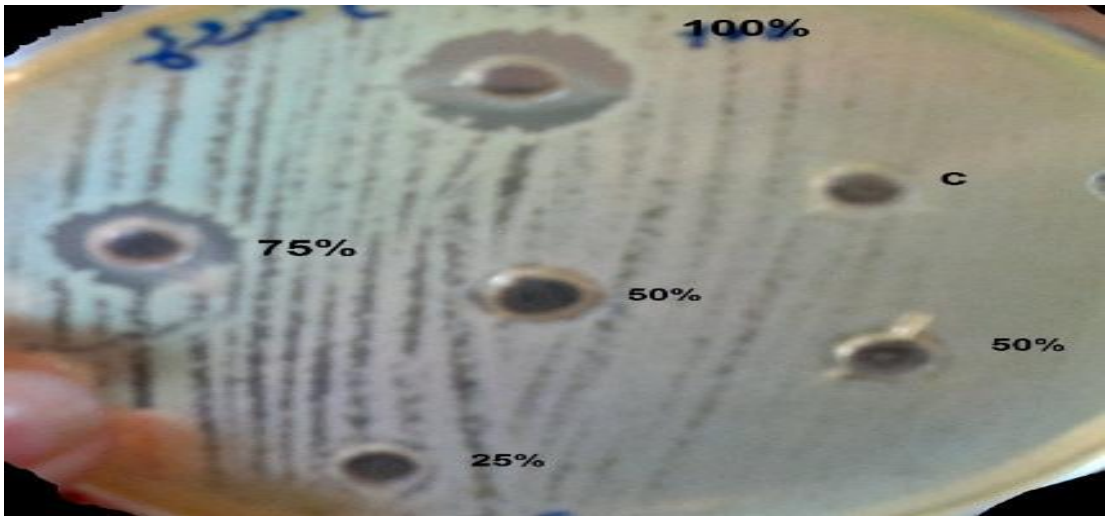

Figure 1- inhibition zoneof castor oil on *E. coli*

HPLC analysis

Through figure 2 and table 2, it can be noticed that castor oil contains a high proportion of fatty acids α-liolenic C18:3 (omega 3) followed by Oleic C18:1 have been found to inhibit protein biosynthesis, as well as inhibit the activity of several enzymes involved in essential metabolic processes in the target bacteria species (enzyme in amino acid metabolism). which have the oxidation ability of some vital cell components

[25]. The linolenic acid content (18:3) of oil is fairly low, giving the oil a strong oxidative stability. Fatty acids are not randomly distributed in natural oils, which could be the reason for the inhibition of *E. coli* isolates tested in this study. According to Scarth (2000) the fatty acid composition is 87% oleic acid, 5% linoleic acid, and 7–8% total saturated acids.

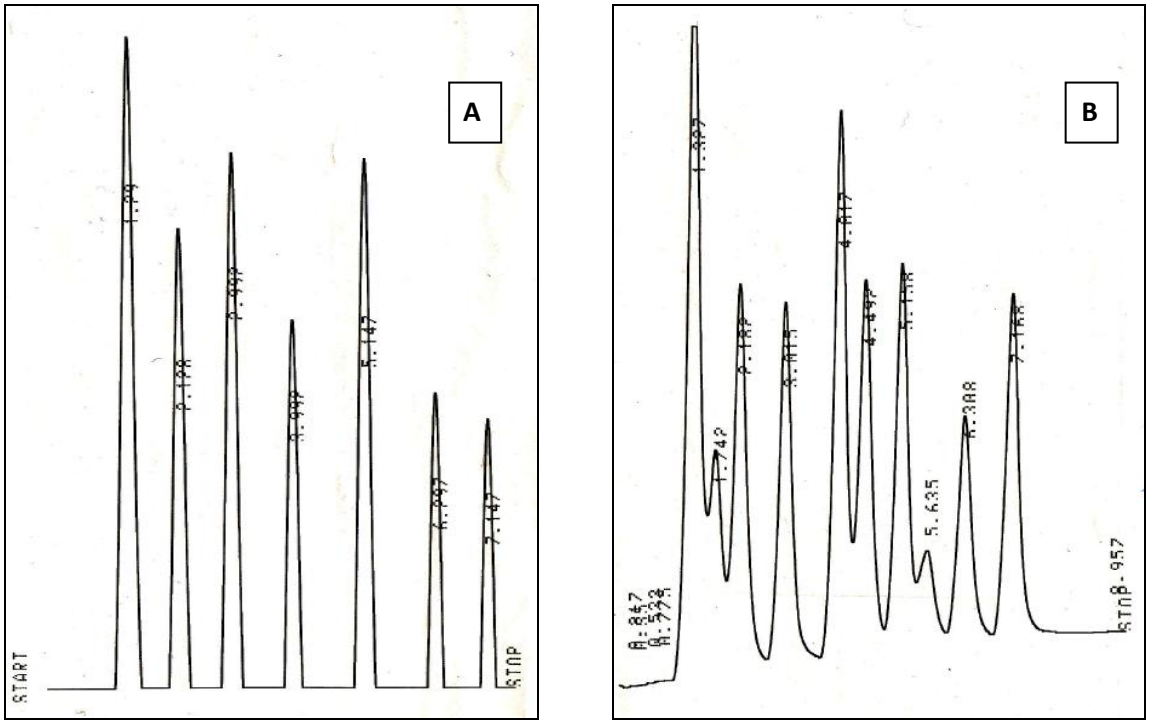

Figure 2-HPLC analysis. A) castor oil. B) standards

Table 2- The sequences of the eluted material of the Castor oil

Seq.	Subjects	Retention time minute	area	Concentration (µg\ml)
1	a-liolenic C18:3 omega 3	1.32	69653	18.90
2	Stearic acid C18:0	1.74	21149	5.74
3	Myristic acid C14:0	2.18	39427	10.70
4	Linoleic C18:1 omega 6	3.01	37084	10.06
5	Oleic C18:1	4.01	51784	14.05
6	Palmetic C16:1	4.49	38209	10.37
7	Arachidic acid C20:3	5.15	38745	10.51
8	Salicylic	5.63	11977	3.25
9	Valerianic	6.30	25092	6.81
10	Succinic	7.16	35290	9.57

4. Conclusion and Recommendations

It can be concluded that the castor seed oil has a disincentive effect on *E. coli* isolated from recurrent urinary tract infections. The recommendation is to take advantage of the plant varieties in Iraq's. Future research and private varieties containing a high proportion of saturated fatty acids and unsaturated preferably test oils different concentrations.

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