Annals of Biology **38** (1) : 150-155, 2022

Impact Score : 0.32 (Scopus)

Development of Hand Sanitizers Using *Teucrium polium* Leaves Extract to Increase the Antimicrobial Efficiency

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(Received : January 18, 2022; Accepted : March 14, 2022)

ABSTRACT

The present research aimed at increasing the antimicrobial efficiency of alcohol-based hand sanitizers by mixing them with ethanol and hexane crude extracts of *Teucrium polium* leaves. Bacterial samples were collected from the palm of the hands of 50 people to both sexes and it was found that *Escherichia coli* and *Citrobacter freundii* were present (76 and 68%). Ethanol and hexane of leaf extracts were used at concentrations (50, 25, 12.5, 6.25 and 3.125 mg/ml). The inhibitory activity was studied using the agar well diffusion method and the new mimic method which simulated the process of wiping hands with sterilizer. The results of the first method showed that the ethanol and hexane extract of the leaves of this plant had a different inhibition effect for both bacteria, where the low concentrations of 3.125 mg/ml showed the best inhibition compared to the rest of the concentrations with inhibition diameter 21 and 23 mm for the ethanol extract and 28 and 25 mm for the hexane extract. The results of the second method confirmed that where the concentration of 3.125 mg/ml strong inhibition, and when mixing hand sanitizer with these concentrations of extracts and using both methods, the inhibitory effect increased better than using hand sanitizer alone or extracts alone and in preference to concentration 3.125 mg/ml. It can be concluded that mixing the extracts of *T. polium* plant with the alcohol-based hand sanitizer led to an increase in its effectiveness in inhibiting the growth of *E. coli* and *C. freundii*.

Key words : Hand sanitizer, *E. coli, Citrobacter freundii*, *Teucrium polium*, mimic method

INTRODUCTION

Hands are playing important role in microbial transmission in the same person's body or between persons and between persons and touched things (Edmonds-Wilson *et al.*, 2015). Alcohol-based hand sanitizers have an inhibitory ability against many Gram-negative and Gram-positive bacteria (Gold and Avva, 2018). But with the excessive and increasing use of hand sanitizers, many types of bacteria may show resistance mechanisms against sanitizers. Studies of other pathogens have also shown increased tolerance when exposed to low concentrations of alcohol (Pidot *et al.*, 2018). Natural plants are an important source of many pharmaceutical materials from antiquity to the present, this is used by people in the treatment of many diseases because it contains a large number of bioactive compounds. Medicinal plants are a substantial source of secondary metabolites that have various biological activities. These secondary metabolites have an important source of antimicrobial agents (Newman and Cragg, 2020). *Teucrium polium* extracts were used as antimicrobial materials and in folk medicine treatments. This plant species is used as antibacterial, antipyretic, antifungal, anti-rheumatic, carminative and antispasmodic properties. The volatile oil of *T. polium* showed medication effects against *Staphylococcus aureus* (Motamedi *et al.*, 2015). The ability of this plant extract particularly from aerial components was improved to inhibit the growth of *Acinetobacter baumannii* and candida species (Khazaei *et al.*, 2018). Also, methicillin-resistant *Pseudomonas aeruginosa* and *E. coli* Q157 : H7 growth was repressed exploitation the phytochemical from *T. polium* (Sevindik *et al.*, 2016).

Therefore, the present study aimed at increasing the antimicrobial efficiency of alcohol-based hand sanitizers by mixing them with ethanol and hexane crude extracts of *T. polium* leaves.

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MATERIALS AND METHODS

Swabs were taken from the palm of the hand of 50 adults (both genders) at the University of Technology, Iraq using the transport medium. Samples were transported in an ice box to the laboratory. The 50 samples are cultured in the brain heart infusion broth for 24 h. A six-fold serial dilution was prepared for each sample. Each activated sample in the sixth dilution was cultured on nutrient agar, MacConkey agar and Chromogenic media (*Pseudomonas aeruginosa* chromogenic agar, *vibrio* chromogenic agar, *E. coli*-Coliforms Chromogenic Medium) and then incubated at (37°C) for 24 h. Biochemical tests and microscopic examinations were also performed. Bacterial species were identified, and isolated by replanting each bacterial species separately on nutrient broth.

T. polium plant was obtained from the herbal market in the Baghdad city identified at the Environment Research Centre/University of Technology, Iraq. The leaves of this plant were washed, dried away from sunlight, and then ground by an electric grinder. 150 g of the plant powder was soaked in 600 ml of ethanol and another 150 g was soaked with hexane for three days and filtered with Whatman filter paper No. 4. The solution was dried using a rotary evaporator. The powder obtained was kept at 4°C in refrigeration using an opaque case to avoid light until use. Dilutions of the extract were made as follows : 50, 25, 12.5, 6.25 and 3.125 mg/ml.

Two types of alcohol-based hand sanitizer from different origins were used. Different concentrations of plant extracts were mixed with the hand sanitizer.

In the test of bacterial sensitivity to the plant extract, 0.1 ml of the bacterial suspension for each type of microorganisms was spread on Muller Hinton solid medium using Cork borer, then four holes were made with a diameter of 6 mm for each hole and :

0.1 ml of a plant extract with different concentrations as mentioned in the above paragraph.

0.1 ml different concentrations of plant extracts were mixed with each type of the alcohol-based hand sanitizer.

0.1 ml of each alcohol-based hand sanitizer was added as a positive control.

Distilled water was also added to one of the pits as a negative control.

The dishes were left for 15 min and then incubated at 37°C for 24 h. The Inhibition Zone was measured using a ruler.

Newmimic method was used for the purpose of simulating the process of wiping hands with hand sanitizer and knowing its effect on the microorganisms in the palm of the hand *in vitro*. During this method, sterile cotton swabs were immersed in the bacterial suspension that had been titrated with 0.5 McFarland's standard (1.5 × 108). They were grown on Muller- Hinton agar plates in three directions to obtain homogeneous growth and then left in the laboratory for 5 min. The culture media were placed in the incubator for 2 h. The culture media was wiped with 0.1 ml of hand sanitizer. The culture media were wiped with 0.1 ml of hand sanitizer and mixed with different concentrations of plant extract. The dishes were returned to the incubator and left for 22 h. The level of bacterial growth was measured in solid culture media as (++) : thick growth, (+) : clear growth, (-) : clear inhibition and (- -) : strong inhibition depending on the density of bacterial growth on Muller-Hinton agar.

RESULTS AND DISCUSSION

The type of bacteria isolated and their percentage from the palm of the hand of 50 adults are shown in Table 1. The percentage of *E. coli* and *C. freundii* was 76 and 68, respectively, while the percentage of *P. aeruginosa* and *Klebsiella* was 24 and 16, respectively. All *E. coli* isolates produced lactose fermenter bright pink colonies, also *Citrobacter* appeared pink lactose fermenter colonies, while all *Klebsiella* isolates gave a mucous lactose fermenter colony on MacConkey agar. *Pseudomonas* was produced by non-lactose fermenting opaque colonies on MacConkey agar. Chromogenic agar was used for the purpose of confirming a bacterial diagnosis and identifying species. *E. coli* Coliforms

Table 1. Type of bacteria isolated and their percentage from the palm

Type of bacteria	No. of persons	Percentage
E. coli	38	76
C. freundii	34	68
P. aeruginosa	12	24
Klebsiella	8	16

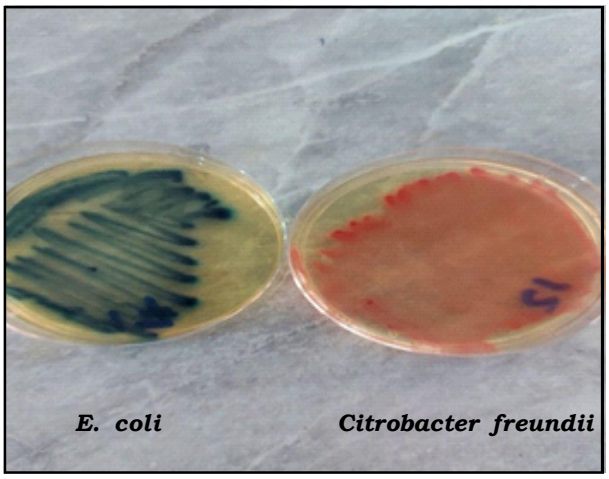

Fig. 1. *E. coli* and *Citrobacter freundii* on *E. coli* coliforms chromogenic medium.

Chromogenic Medium was used in which *E. coli* colonies appeared in blue colour and *C. freundii* appeared in dark pink colour (Fig. 1). The highest percentages of bacteria isolated from the palm of the hand were selected (*E. coli* and *C. freundii*).

The results of diffusion method 1 are shown in Tables 2, 3 and 4. Table 2 shows the diameters of bacterial growth inhibition rates by plant extracts. *E. coli* showed sensitivity to the ethanol extract (50 to 12.3 mg/ml) by *T. polium* ranging between 14 to 21 mm, and *C. freundii* showed sensitivity to the same extract in the range from 16 to 23. mm. *E. coli* showed sensitivity to hexane extract at rates ranging between 18 to 25 mm and in *C. freundii* from 25 to 28 mm (Table 2).

Table 3 shows the diameters of bacterial growth inhibition by mixing plant extracts and hand sanitizer-1, where the mixture of ethanol extract (50 to 12.3 mg/ml) with hand sanitizer-1 gave inhibition diameters against *E. coli* ranging from 18 to 29 mm and the inhibition diameters against *C. freundii* from 24 to 34 mm. The mixture of hexane extract (50 to 12.3 mg/ml) with hand sanitizer-1 gave inhibition diameters against *E. coli* ranging from 26 to 36 mm and against *C. freundii* with inhibition diameters between 31 to 39 mm. The positive control in this method was hand sanitizer-1.

The diameters of bacterial growth inhibition by the mixture of ethanol extract (50 to 12.3 mg/ml) with hand sanitizer-2 against *E. coli* ranged from 16 to 23 mm and the inhibition diameters of the same mixture against *C. freundii* gave 18 to 21 mm (Table 4). The mixture of hexane extract (50 to 12.3 mg/ml) with hand sanitizer -2 gave inhibition diameters against *E. coli* ranging from 18 to

Table 2. Antibacterial effect of the ethanol and hexane extracts of *T. polium* on the growth of the isolated bacteria

Bacteria isolates	Ethanol extracts conc.					Hexane extracts conc.					Control
	50	25	12.5	6.25	3.12	50	25	12.5	6.25	3.12	D.W.
E. coli	14	12	17	22	21	18	21	21	24	25	0
C. freundii	16	15	21	21	23	25	26	26	26	28	0

Table 3. Antibacterial effect of the ethanol and hexane extracts of *T. polium* mixed with hand sanitizer (1) on the growth of the isolated bacteria

Bacteria isolates	Ethanol extracts conc. mixed with hand sanitizer (1)					Hexane estracts conc. mixed with hand sanitizer (1)					Control (+) hand sanitizer-1
	50	25	12.5	6.25	3.12	50	25	12.5	6.25	3.12	
E. coli	18	18	22	25	29	26	29	33	35	36	9
C. freundii	24	24	26	29	34	31	33	35	38	39	10

Table 4. Antibacterial effect of the ethanol and hexane extracts of *T. polium* mixed with hand sanitizer (2) on the growth of the isolated bacteria

Bacteria isolates	Ethanol extracts conc. mixed with hand sanitizer (2)					Hexane estracts conc. mixed with hand sanitizer (2)					Control (+) hand sanitizer-2
	50	25	12.5	6.25	3.12	50	25	12.5	6.25	3.12	
E. coli	16	15	18	19	23	18	19	22	23	27	8
C. freundii	18	17	17	19	21	24	25	27	27	29	8

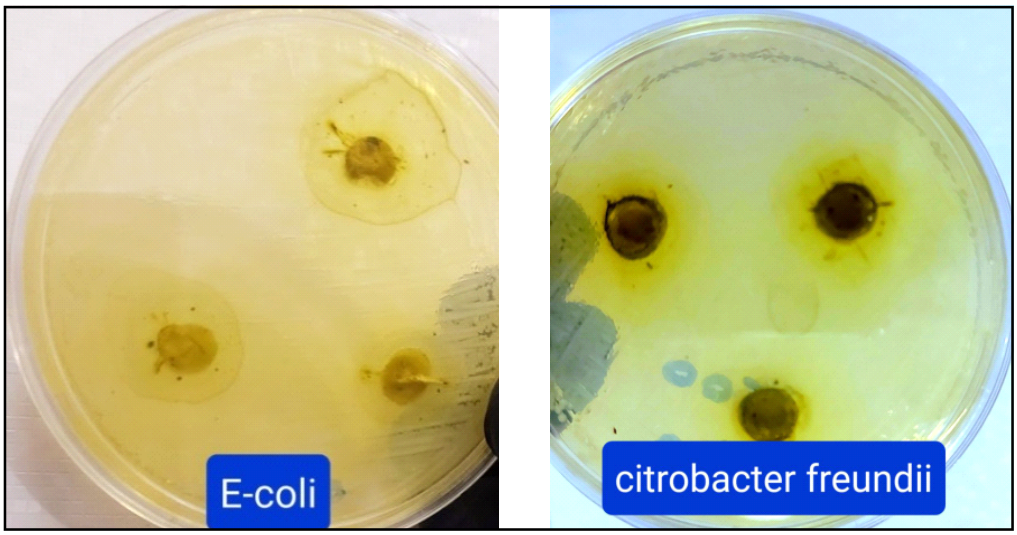

Fig. 2. The results using method (1).

27 mm and against *C. freundii* with inhibition diameters between 21 to 29 mm. The positive control was hand sanitizer-2 (Fig. 2).

The results of modified method 2 are shown in Tables 5, 6 and 7. In Table 5, the estimation of antibacterial effect of ethanol and hexane extracts of *T. polium* plant at concentrations (from 50 to 3.12 mg/ml) was done, where (++) was adopted for dense bacterial growth and (+) sign for clear bacterial growth and (- -) for strong inhibition and (-) for clear inhibition, and the ethanol extract showed a clear inhibition of *E. coli* at a concentration of 3.125, 6.25, 12.5 and a clear inhibition against *C. freundii* at a concentration of 3.125. Hexane extract showed a clear inhibition against *E. coli* at a concentration of 6.25 and 12.5 and a strong inhibition against the same bacteria at a concentration of 3.125. It also had a clear inhibition against *C. freundii* at a concentration of 6.25, 12.5, 25 and 50, while it showed a strong inhibition against the same bacteria at a concentration of 3.125.

The second method by mixing the hand sanitizer-1 with ethanol and hexane extract at concentrations from 50 to 3.125 mg/ml, the mixture showed clear inhibition in most of the concentrations against both bacteria except at concentration 50 where it showed clear growth, while 6.25 and 3.125 of ethanol and hexane extract showed strong inhibition (Table 6).

According to Table 7 using the second method and by mixing the hand sanitizer-2 with ethanol and hexane extract at concentrations

Table 5. Antibacterial effect of the ethanol and hexane extracts of *T. polium* on the growth of the isolated bacteria

Bacteria isolates	Ethanol extracts conc.					Hexane extracts conc.				
	50	25	12.5	6.25	3.12	50	25	12.5	6.25	3.12
E. coli	++	++	-	-	-	+	+	-	-	--
C. freundii	++	++	+	+	-	-	-	-	-	--

(++) : thick growth, (+) : clear growth, (-) : clear inhibition and (- -) : strong inhibition.

Table 6. Antibacterial effect of the ethanol and hexane extracts of *T. polium* mixed with hand sanitizer (1) on the growth of the isolated bacteria

Bacteria isolates	Ethanol extracts conc. mixed with hand sanitizer (1)					Hexane extracts conc. mixed with hand sanitizer (1)					Control (+) hand sanitizer-1
	50	25	12.5	6.25	3.12	50	25	12.5	6.25	3.12	
E. coli	+	-	-	--	--	-	-	--	--	--	-
C. freundii	+	+	-	--	--	-	-	-	--	--	+

Table 7. Antibacterial effect of the ethanol and hexane extracts of *T. polium* mixed with hand sanitizer (2) on the growth of the isolated bacteria

Bacteria isolates	Ethanol extracts conc. mixed with hand sanitizer (2)					Hexane extracts conc. mixed with hand sanitizer (2)					Control (+) hand sanitizer-2
	50	25	12.5	6.25	3.12	50	25	12.5	6.25	3.12	
E. coli	++	+	-	-	-	-	-	-	-	-	+
C. freundii	++	-	-	-	-	-	-	-	-	-	+

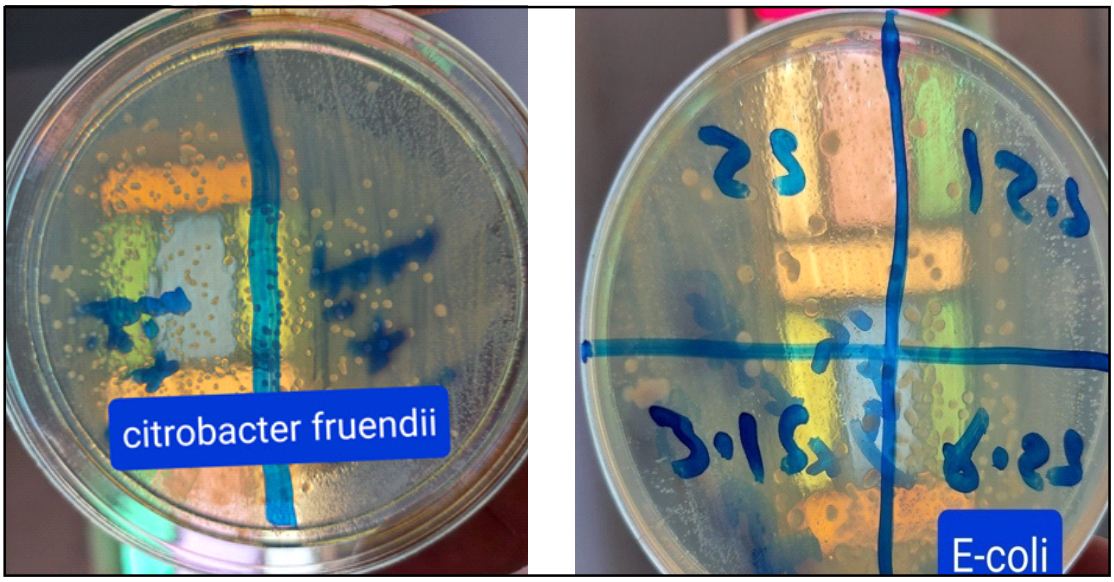

Fig. 3. The results using method (2).

from 50 to 3.125 mg/ml, like Table 6, the mixture showed clear inhibition in most of the concentrations and low concentrations had the best results (Fig. 3).

The human hand, especially the palm of the hand, played a major role in the transmission of germs to the rest of the body parts of the individual or between individuals or from inanimate surfaces to people (Edmonds-Wilson *et al.*, 2015). In this research, as a result of the high percentage of *E. coli* and *C. freundii* within the microorganisms isolated from the palm of the hand of 50 adults, which were selected to test the ability of two types of alcohol-based hand sanitizers to inhibit and to develop hand sterilizers using plant extracts; *T. polium* was selected for its anti-bacterial properties, as its aerial parts contained saponin, ethyl phenol, monoterpenoid and sesquiterpenes (Elmasri, 2015). Also it contained flavonoids. The antibacterial mode of action of many terpenoids associated with many of the terpenoids had the ability to inhibit the resistance of bacteria to antimicrobial agents by inhibiting biofilm formation, inhibiting microbial growth and inhibition of efflux pump (Zengin and Baysal, 2014).

The mechanisms of flavonoids as antibacterial agents were disruption of DNA synthesis, obstructing the functions of the cytoplasmic membrane, inhibition of energy metabolism, and disruption of the adhesion process and biofilm formation. Hand sanitizer which was based on alcohol had ethanol or isopropanol, or n-propanol, alcohol´s antimicrobial properties linked to its capacity to dissolve the lipid in cytoplasmic membranes and the protein denaturation of microbes (World Health Organization, 2015). Therefore, the synergistic effect between alcohol-based hand sanitizer and ethanol and hexane extract of *T. polium* leaves gave a higher inhibitory effect than using hand sanitizer alone, especially with low concentrations of plant extracts preventing bacteria from causing resistance to this sterilizer. If various plant extracts were used it had a clear effect on the growth of bacteria;

the lower concentration of the plants extract allowed a dual antibacterial effect.

CONCLUSION

It was found that mixing the extracts of *T. polium* plant with the alcohol-based hand sanitizer led to an increase in its effectiveness in inhibiting the growth of *E. coli* and *C. freundii*, which opened the way for the use of medicinal herbal extracts to develop the effectiveness of hand sanitizers and other disinfectants against various types of pathogenic microorganisms.

ACKNOWLEDGEMENTS

The authors thank Al-Muthanna University and Environment Research Center/University of Technology, Iraq for their valuable support and scientific assistance.

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