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A COMPARATIVE ASSESSMENT OF THE ANTI-BACTERIAL ACTIVITY FOR THE CITRUS-PEEL CRUDE-ETHANOL EXTRACTS AGAINST SOME PATHOGENIC BACTERIA

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Abstract:*In vitro trail evaluated the anti-bacterial activity for Citrus-peel crude-ethanol extracts (C-p C-ees) of (C. limon, C. aurantiifolia and C. sinensis) against (Staph. aureus and E. coli). The activity of C-p C-ees against Staph. aureus was 0.46 at 3hr for concentration 2000ppm of C. limon 0.46 and concentration 8000ppm of C. aurantiifolia at 3hr was 0.44 for concentration 4000ppm of C. sinensis. After 12hr, C. limon of 8000ppm was 0.12, C. aurantiifolia for 4000ppm 0.15. The level for (4000 and 8000ppm) of C. sinensis 0.14. At 18hr all were (0.04, 0.06 and 0.04; 0.04, 0.08 and 0.09), concentration (2000, 4000 and 8000ppm) of (C. sinensis and C. limon). The effect of C. aurantiifolia at same time and concentrations were (0.14, 0.11 and 0.2). The activity for C-p C-ees against E. coli were (0.52, 0.53 and 0.57; 0.56, 0.56 and 0.62; and 0.56, 0.72 and 0.64) at 3hr for concentration (2000, 4000 and 8000ppm) of (C. limon, C. aurantiifolia and C. sinensis). At 12hr were (0.22, 0.33 and 0.34; 0.28, 0.28 and 0.34; and 0.27, 0.38 and 0.39) for concentrations (2000, 4000 and 8000ppm) of (C. limon, C. aurantiifolia and C. sinensis). At 18hr were (0.04, 0.13 and 0.18; 0.1, 0.15 and 0.16; and 0.06, 0.1 and 0.1) for same concentrations of C. Spp, these reductions were significant comparing the control treatments. That showed the C-p C-ees effects of (C. limon and C. sinensis) similar on E. coli than C. sinensis. That concluded an important role in the clinical medicine.*

Keywords: *Citrus-peel crude-ethanol extracts (C-p C-ees), C. aurantiifolia, C. limon, C. sinensis, Staph. aureus, E. coli, PPM, Anti-bacterial.*

Introduction

The peel *C.* essential oils (*C.* EOs) was economically good as fruit juice industry waste[1], were a mixture of over hundred compounds into three fractions: Terpene hydrocarbons, oxygenated compounds and non-volatile compounds. *C. Spp* EOs had anti-bacterial effects[2], the mixture of volatile compounds consisted of mono-terpene hydrocarbons[3], were sources of natural products used alternative for infectious diseases treatment[4], they more effective to control bacteria[5]. They showed anti-bacterial potential[6], there had activities against most important food-borne bacteria[7]. They directed towards food-borne bacteria, employed in the food preservation, referred as Generally Recognized As Safe (GRAS)[8]. The use of them provided "natural" alternative to foods chemical preservation[9], in plant products had applications in food, cosmetic and aroma-therapy[10].

C. fruit peels possessed anti-bacterial properties and managed bacterial infections[11]. The *C.* EOs anti-bacterial had interest in food industries[12]. The EOs were effective on bacteria via compounded protopine and corydaline alkaloids, lactons, polyacetylene, acyclic sesquiterpenes, hypericin and pseudo-hypericin[13-14]. The antagonistic of EOs extracted by hydro-distillation from the *C.* fruit peel evaluated against food-borne bacteria *Staph. aureus*[15]. *C. Spp* considered an important source of poly-methoxylated flavonoids a class of secondary plant metabolites. Anti-bacterial activities was important in rising prevalence of drug resistance pathogenic bacteria[16]. The evolution of multiple drug resistant pathogenic bacteria had search for new sources of anti-bacterial[17]. New anti-bacterial agents, with lipo-phylic properties, were in plants vacuoles and wax cuticle played a role as anti-bacteria[18-19]. Ethyl acetate extracts from all *C.* peels showed anti-bacterial activities, broad spectrum inhibition against all G-positive bacteria as *Staph. aureus*. The major components were limonene, citronellal, b-pinene, b-pinene, sabinene and citronellal[20].

C. aurantiifolia EOs used as pharmaceutical forms[21-22], they had the alarming incidence of antibiotic resistance bacteria[23]. They exhibited anti-bacterial against *Staph. aureus*[24]. Their peel EOs reported the hexane extract activity against streptomycin resistant bacteria[25-26]. They

used as an anti-septic, for colds, coughs and sore throats[27]. The active extract were: 5-geranyloxypsoralen; 5-geranyloxy-7-methoxycoumarin; 5,7-dimethoxycoumarin; 5-methoxypsoralen; and 5,8-dimethoxypsoralen. In addition, the hexane extract allowed identification of 44 volatile compounds, were 5,7-dimethoxycoumarin, 3-methyl-1,2-cyclopentanedione, 1-methoxy-ciclohexene, corylone, palmitic acid, 5,8-dimethoxypsoralen, terpineol and umbelliferone, the compounds tested against multi-drug resistant bacteria[28].

C. limon peel acted on the pathogenic bacteria as prevention and treatment of oral bacteria[29-30]. The anti-bacterial of *C. Spp* peels wax and hexane extracts tested against (*E. coli* and *Staph. aureus*). Two poly-methoxylated flavonoids and a coumarin derivative isolated from *C*-p C-ees, as anti-bacterial and 6,7-dimethoxycoumarin from *C. limon*[31]. *C. lemon L* peel oil showed strong anti-bacterial effects[32]. The 4 anti-bacterial ethanol and n-hexane extracted from *C. lemon* peel were active against the oral bacteria, identified 8-geranyloxypsolaren, 5-geranyloxypsolaren, 5-geranyloxy-7-methoxycoumarin, and phloroglucinol 1-β-D-glucopyranoside[33]. The antagonistic activity of the EOs hydro-distillation extracted from *C.* fruit peel evaluated against food-borne pathogen bacteria as *Staph. aureus*, were more effective against the G-positive bacteria, as of *C. limon*[15]. The Methanol and Ethanol extract of *C. limon*, had anti-bacterial activity due to phyto-chemicals present in peels, the extracts showed anti-bacterial activity against (*E. coli* and *Staph. aureus*)[34].

C. sinensis had anti-bacterial activities detected and developed of new potential anti-bacterial[35-36], the extract prevented colds, flu and helping to fight bacterial infections[37], *C. sinensis* EOs had anti-bacterial activities[38]. The anti-bacterial effects of peel extract demonstrated potent anti-bacterial activity[39]. They found effective against G-negative bacteria[40], they extracted by n-hexane, physico-chemical properties of the oils and soaps. The anti-bacterial activities assessed for the two oils and soap produced against most of the G-positive and negative bacteria were compared with antibiotics. The *C. sinensis* EOs demonstrated growth inhibitions against *Staph. aureus.* That obtained physico-chemical and anti-bacterial properties. The *C. sinensis* EOs provided a synergy for suitable raw materials for the cosmetic and pharmaceutical industries[41]. The *C. sinensis* EOs Methanol and Ethanol extract evaluated for anti-bacterial activity due to phyto-chemicals present in fruit's peels. The anti-bacterial activity of Methanol and Ethanol peel extract of *C. sinensis L* evaluated on (*E. coli* and *Staph. aureus*). Methanol extract showed maximum inhibition for *Staph. aureus*[34].

This work aimed to compare the anti-bacterial activity of C-p C-ees derived from the three *C. Spp* fruit peels in serial concentrations against some types of pathogenic bacteria as (*Staph. aureus* and *E. coli*).

Material and Methods

Preparation of the *C*-p C-ees: The peel of (*C. limon*, *C. aurantiifolia* and *C.* sinensis) were collected freshly in a quantity of 50g for each. Fresh peels were extracted with 100ml of 100% Ethanol by "Soxhlet Apparatus" for 6hr at 60°C. The resulted *C*-p C-ees were evaporated to dryness using a "Rotary Evaporator" at 40°C. The concentrations were prepared from each *C*-p C-ee of the three *C. Spp* in (2000, 4000 and 8000 ppm) and added respectively into mineral based liquid medium containing Sodium Chloride 5.0g/L, Yeast Extract 3.0g/L and Peptone 5.0g/L[42].

Preparation of the tested pathogenic bacteria: The tested pathogenic bacteria *(Staph. aureus* and *E. coli*), these were cultured for 24hr on "Nutrient Agar Media" and then inoculated. The *C*-p C-ees and liquid medium were added in "24Well Tissue Culture" (Micro Titration Plate) and incubated at (30-35°C)**.** The *C*-p C-ees were examined for its effects against the tested bacteria after (3, 6, 9, 12, 15 and 18hr)[43].

Demonstration of the turbidity: Determination of minimum inhibitory concentration of the *C*-p C-ees by the optimum density were measured the turbidity photo-metrically. Using the "Spectro-photo-meter" at 450 Nano-meter (nm)[44].

Data analysis: The statistically methods used the obtained data subjected to analysis of "Variance" by (ANOVA) as a "Factorial in Complete Block Design"[45], then followed by the "Duncan's Multiple Range Test" to compare the means[46].

Results and Discussion

Table 1: The activity for the C-p C-ees against *Staph. Aureus*

Time	Control			Control with Staph. aureus			C. species									Mean of time
							C. limon			C. aurantiifolia			C. sinensis			
	2000 ppm	4000 ppm	8000 ppm	2000 ppm	4000 Ppm	8000 ppm	2000 ppm	4000 ppm	8000 ppm	2000 ppm	4000 ppm	8000 ppm	2000 ppm	4000 ppm	8000 ppm	
3 hr	1.58 i	1.58 i	1.58 i	3.41 a	3.41 a	3.41 a	0.46 qr	0.52 lmn	0.52lm	0.47 pq	0.51lm	0.46pqr	0.51 no	0.44 rs	0.47op	1.29 a
6 hr	1.62 h	1.62 h	1.62 h	3.08 b	3.08 b	3.08 b	0.48 pq	0.54 klm	0.58 k	0.5mno	0.54 lm	0.52lm	0.49 op	0.41 s	0.49no	1.24 b
9 hr	1.30 j	1.30 j	1.30 j	2.04 g	2.04 g	2.04 g	0.50 no	0.54 klm	0.49no	0.5 nop	0.51lm	0.55kl	0.42 rs	0.4 s	0.41 s	0.95 c
12 hr	2.25 d	2.25 d	2.25 d	2.08 fg	2.08 fg	2.08 fg	0.17 vw	0.18 vw	0.12 za	0.16 wx	0.15 xy	0.25t	0.17 vw	0.14 yz	0.14wx	0.96 c
15 hr	2.3c	2.3c	2.3c	2.10 f	2.10 f	2.10 f	0.11 ab	0.1 zabc	0.11yza	0.15 xy	0.14 yz	0.23tu	0.07bcd	0.12 za	0.1 abc	0.95 c
18 hr	2.15 e	2.15 e	2.15 e	2.12 ef	2.12 ef	2.12 ef	0.04d	0.08 abcd	0.09abc	0.14 yz	0.11 ab	0.2uv	0.04 d	0.06 cd	0.04 d	0.90 d
Mean of treat-ments	1.87 b			2.47 a			0.31 d			0.33 c			0.27 e			LSD Time 0.05 = 0.012
LSD C. 0.05 = 0.009 LSD C. × Time × Concentration 0.05 = 0.041																

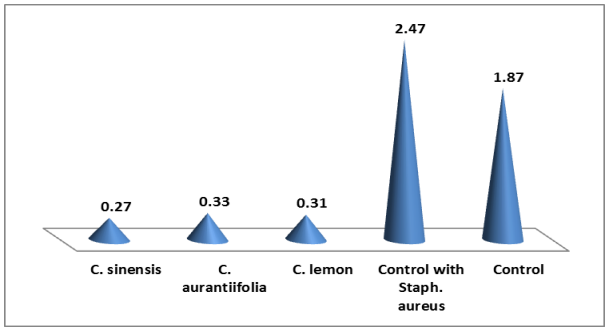

Diagram 1: The mean of treatment against *Staph. aureus*

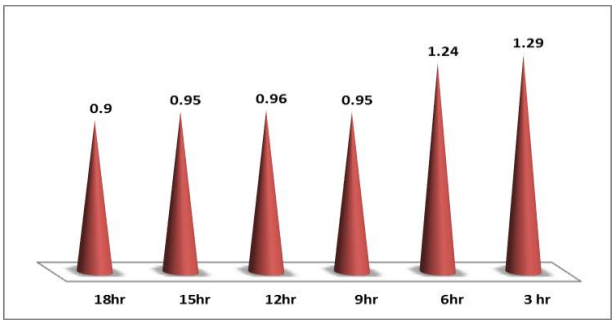

Diagram 2: The mean of time against *Staph. aureus*

Table 1 and diagrams (1 and 2) showed the activity for the *C*-p C-ees against *Staph. aureus*, the measures turbidity indicated the bacterial cell counts was 0.46 at 3hr for concentration 2000ppm of *C. limon* and it was in the same time 0.46 for concentration 8000ppm of *C. aurantiifolia*, but at 3hr was 0.44 for concentration 4000ppm of *C. sinensis*. The differences were significant if compared with negative and positive control after 3hr. After 12hr, the level of 8000ppm of *C. limon* scored turbidity 0.12 as well as at the same time, *C. aurantiifolia* at 4000ppm scored turbidity 0.15. On the other hand, the level of (4000 and 8000ppm) of *C. sinensis* gave the same turbidity 0.14. The reduction of turbidity was significant in all cases compared with controls in the same time. At 18hr all turbidity decreased in totally as (0.04, 0.06 and 0.04; 0.04, 0.08 and 0.09) for the concentration (2000, 4000 and 8000ppm) for (*C. sinensis* and *C. limon*), respectively. As well, the effects of *C. aurantiifolia* were at the same time and concentrations as (0.14, 0.11 and 0.2) of turbidity. The mean of *C. Spp* factors were (0.31, 0.33 and 0.27) for *(C. limon*, *C. aurantiifolia* and *C. sinensis*) respectively. The means indicated that turbidity significantly decreased with *C. sinensis* than others and showed the effects of *C. sinensis* was more on *Staph. aureus*. The results showed also that *C. sinensis* extract was stronger than other *C. Spp* in most cases.

C. aurantiifolia EOs had the alarming incidence of antibiotic resistance of medical importance bacteria[23], had anti-bacterial activity against *Staph. aureus*[24]. They used in traditional medicine as an anti-septic, for colds, coughs and sore throats[27]. *C. limon* peel EOs acted on the oral bacteria[30]. *C. lemon L* peel oil showed strong anti-bacterial activity[32], they active against the oral bacteria, exhibited high anti-bacterial activity[33]. The antagonistic activity of the EOs from the *C.* fruit peel evaluated against food-borne bacteria as *Staph. aureus*[15]. The *C. limon* Methanol and Ethanol extract evaluated for anti-bacterial activity, showed anti-bacterial activity against *Staph. aureus*[34]. *C. sinensis* had anti-bacterial activities[35-36], the extract prevented colds, flu and helping to fight bacterial infections[37]. All *C.* EOs had anti-bacterial activities[38-39]. The anti-antibacterial activities assessed for the two oils

and the soap produced against G-positive bacteria compared with antibiotics. The *C. sinensis* EOs demonstrated inhibitions against *Staph. aureus*[41]. The *C. sinensis L* Methanol and Ethanol peel extract evaluated the anti-bacterial activity on *Staph. aureus* showed maximum inhibition[34].

Table 2: The activity for the C-p C-ees against *E. coli*

Time	Control			Control with E. coli			C. species									Mean of time
							C. limon			C. aurantiifolia			C. sinensis			
	2000 ppm	4000 ppm	8000 ppm	2000 ppm	4000 ppm	8000 ppm	2000 ppm	4000 ppm	8000 ppm	2000 ppm	4000 ppm	8000 ppm	2000 ppm	4000 ppm	8000 ppm	
3 hr	1.58 g	1.58 g	1.58 g	3.20 a	3.20 a	3.20 a	0.52 t	0.53 st	0.57 r	0.56 rs	0.56 rs	0.62 op	0.65 n	0.72jk	0.64 no	1.31 b
6 hr	1.62 f	1.62 f	1.62 f	3.13 b	3.13 b	3.13 b	0.64 no	0.59pqr	0.59 qr	0.51 tu	0.64 no	0.63 no	0.82 i	0.82 i	0.81 i	1.35 a
9 hr	1.30 h	1.30 h	1.30 h	0.46 vw	0.46 vw	0.46 vw	0.58 qr	0.56 rs	0.61 pq	0.59 qr	0.58 qr	0.57 r	0.73 j	0.69 ki	0.68 im	0.72 d
12 hr	2.25 d	2.25 d	2.25 d	0.47 uv	0.47 uv	0.47 uv	0.22 de	0.33 yz	0.34 y	0.28 ab	0.28 ab	0.34 y	0.27 bc	0.38 x	0.39 x	0.73 c
15 hr	2.3 c	2.3 c	2.3 c	0.43 w	0.43 w	0.43 w	0.2 ef	0.3 za	0.25bcd	0.2 ef	0.24 cd	0.23 de	0.2 ef	0.32 yz	0.3 za	0.69 e
18 hr	2.15 e	2.15 e	2.15 e	0.58 qr	0.58 qr	0.58 qr	0.04 j	0.13 hi	0.18 fg	0.1 I	0.15 gh	0.16 gh	0.06 j	0.1 i	0.1 i	0.61f
Mean of treat-ments	1.87 a			1.38 b			0.39 d			0.40 d			0.48 c			LSD Time 0.05 = 0.008

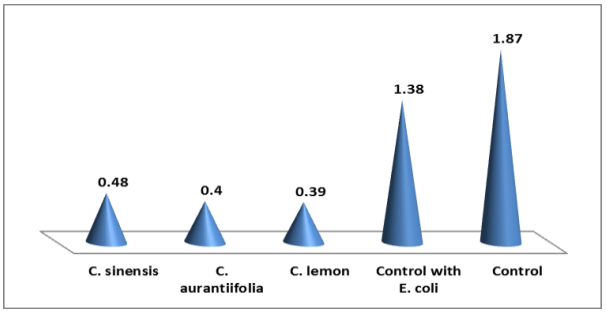

Diagram 3: The mean of treatment against *E. coli*

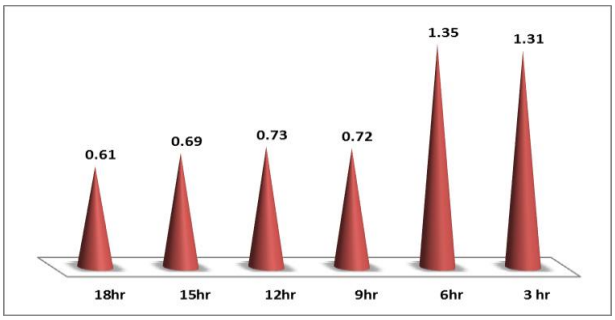

Diagram 4: The mean of time against *E. coli*

Table 2 and diagrams (3 and 4) showed the activity of the *C*-p C-ees against *E. coli* turbidity were (0.52, 0.53 and 0.57; 0.56, 0.56 and 0.62; and 0.56, 0.72 and 0.64) at 3hr for concentration (2000, 4000 and 8000ppm) for (*C. limon*, *C. aurantiifolia* and *C. sinensis*), respectively, which confirmed all *C. Spp* in all concentrations gave a high reduction of *E. coli* if compared with positive and negative controls. Likewise, the turbidity at 12hr were (0.22, 0.33 and 0.34; 0.28, 0.28 and 0.34; and 0.27, 0.38 and 0.39) for concentrations (2000, 4000 and 8000ppm) for (*C. limon*, *C. aurantiifolia* and *C. sinensis*), respectively. The reduction was significant comparing with both control treatments. At 18hr all turbidity were decrease in totally as (0.04, 0.13 and 0.18; 0.1, 0.15 and 0.16; and 0.06, 0.1 and 0.1) for the same concentrations of the same *C. Spp*, respectively, these reduction of turbidity was significant comparing with control treatments. The means of *C. Spp* effects were (0.39, 0.40 and 0.48) for *(C. limon*, *C. aurantiifolia* and *C. sinensis*) respectively. The means were indicated the turbidity were approximately similar in both *(C. limon* and *C. aurantiifolia*) on *E. coli*. The means of time were (1.31, 1.35, 0.72, 0.73, 0.69 and 0.61) for *(C. limon*, *C. aurantiifolia* and *C. sinensis*) at (3, 6, 9, 12, 15 and 18hr) respectively. The results showed the strong effect of both *(C. limon* and *C. sinensis*) were similar on *E. coli* than *C. sinensis* in most cases.

C. aurantiifolia EOs had activities against antibiotic resistance bacteria[23]. Their peel EOs reported activity against streptomycin resistant bacteria[25-26], used in traditional medicine (anti-septic, for colds, coughs and sore throats)[27]. The active extract had inhibition against multi-drug resistant bacteria[28]. *C. limon* acted on pathogenic oral bacteria[30]. The anti-bacterial of their wax and hexane extracts of *C. Spp* peels tested against *E. coli*[31]. *C. lemon L* peel oil showed anti-bacterial[32], against the oral bacteria. These 3compounds were effective extracted using ethanol and n-hexane[33]. Most of EOs were more effective against bacteria[15]. All the extracts showed anti-bacterial activity against *E. coli*[34]. *C. sinensis* EOs had anti-bacterial activities[35-36], the extract prevented colds, flu and helping to fight bacterial infections[37-39]. They found effective against G-negative bacteria[40]. The anti-bacterial had against G-negative bacteria compared with antibiotics[41]. The anti-bacterial activity of *C. sinensis L* Methanol and Ethanol peel extract evaluated on *E. coli*[34].

Conclusion

This study concluded that (*C. limon*, *C. aurantiifolia* and *C. sinensis*) considered as important members were due to the chemical compounds from the *C*-p C-ees. These compounds were responsible for the anti-bacterial activity observed in *C. Spp* against that two selected pathogenic bacteria *(S. aureus* and *E. coli*). A significant effects presented in *C. aurantiifolia*-p C-ees against *E. coli* than other *C. Spp* but, *C. sinensis*-p C-ees presented a significant activity against *Staph. aureus.* These results played an important role in the fields of clinical medicine. Thus, the utilization of these *C*-p C-ees for therapeutic purposes will be requiring the evaluation of their anti-bacterial activities.

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