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. col). 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 proteine 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 vacules 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-
cyclopentanediene, 1-methoxy-ciclohexene, corylone,
palmitic acid, 5,8-dimethoxypsoralen, terpineol and
umbelliferone, the compounds tested against multi-drug
resistant bacterial[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-
geranyloxypsoralen, 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
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 inocu-
ated. The C-p C-ees and liquid medium were added in
"24Well Tissue Culture" (Micro Titration Plate) and incu-
bated 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 mini-
imum inhibitory concentration of the C-p C-ees by the opti-
um 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 ob-
tained 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-eens against Staph. Aureus

<table>
<thead>
<tr>
<th>Time</th>
<th>Control 2000 ppm</th>
<th>Control 4000 ppm</th>
<th>Control 8000 ppm</th>
<th>Control with Staph. aureus 2000 ppm</th>
<th>Control with Staph. aureus 4000 ppm</th>
<th>Control with Staph. aureus 8000 ppm</th>
<th>Mean of time</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 hr</td>
<td>1.29 i</td>
<td>1.19 j</td>
<td>1.29 f</td>
<td>1.27 x</td>
<td>1.25 w</td>
<td>1.15 a</td>
<td>1.27 e</td>
</tr>
<tr>
<td>6 hr</td>
<td>1.62 b</td>
<td>1.62 b</td>
<td>1.62 b</td>
<td>1.28 y</td>
<td>1.28 y</td>
<td>1.28 y</td>
<td>1.27 e</td>
</tr>
<tr>
<td>9 hr</td>
<td>1.60 f</td>
<td>1.60 f</td>
<td>1.60 f</td>
<td>1.24 z</td>
<td>1.24 z</td>
<td>1.24 z</td>
<td>1.24 e</td>
</tr>
<tr>
<td>12 hr</td>
<td>2.25 d</td>
<td>2.25 d</td>
<td>2.25 d</td>
<td>2.20 e</td>
<td>2.20 e</td>
<td>2.20 e</td>
<td>2.20 e</td>
</tr>
<tr>
<td>15 hr</td>
<td>2.5c</td>
<td>2.5c</td>
<td>2.5c</td>
<td>2.5d</td>
<td>2.5d</td>
<td>2.5d</td>
<td>2.5 d</td>
</tr>
<tr>
<td>18 hr</td>
<td>2.13 e</td>
<td>2.13 e</td>
<td>2.13 e</td>
<td>2.12 d</td>
<td>2.12 d</td>
<td>2.12 d</td>
<td>2.12 d</td>
</tr>
</tbody>
</table>

Mean of treatment: 1.87 f, 2.47 e

LSD C. 0.05 = 0.009
LSD C. x Time x 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-eens against Staph. aureus, the measures of the bacterial cell counts was 0.46 at 3hr for 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 sores throat[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*

<table>
<thead>
<tr>
<th>Time</th>
<th>Control</th>
<th>Control with E. coli</th>
<th><em>C. limon</em></th>
<th><em>C. aurantiifolia</em></th>
<th><em>C. sinensis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ppm</td>
<td>ppm</td>
<td>ppm</td>
<td>ppm</td>
<td>ppm</td>
</tr>
<tr>
<td>3 hr</td>
<td>1.58 g</td>
<td>1.58 g</td>
<td>0.22 t</td>
<td>0.15 i</td>
<td>0.15 i</td>
</tr>
<tr>
<td>6 hr</td>
<td>1.62 f</td>
<td>1.62 f</td>
<td>0.28 ab</td>
<td>0.38 c</td>
<td>0.38 c</td>
</tr>
<tr>
<td>9 hr</td>
<td>1.30 b</td>
<td>1.30 b</td>
<td>0.34 y</td>
<td>0.56 rs</td>
<td>0.56 rs</td>
</tr>
<tr>
<td>12 hr</td>
<td>2.25 d</td>
<td>2.25 d</td>
<td>0.38 x</td>
<td>0.59 qr</td>
<td>0.59 qr</td>
</tr>
<tr>
<td>15 hr</td>
<td>2.3 e</td>
<td>2.3 e</td>
<td>0.47 uv</td>
<td>0.61 pq</td>
<td>0.61 pq</td>
</tr>
<tr>
<td>18 hr</td>
<td>2.15 v</td>
<td>2.15 v</td>
<td>0.59 qr</td>
<td>0.64 no</td>
<td>0.64 no</td>
</tr>
</tbody>
</table>

**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 multidrug resistant bacterial[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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References

Biographies

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