Interaction of Folk Medicinal Plants with Levofloxacin against *Escherichia Coli*

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**Abstract**

The present study was conducted to assess the *in vitro* activities of folk medicinal plants in combination with levofloxacin against TG1 and mutant KAM3-1(ΔacrB-ΔtolC) *Escherichia coli* strains. Plants were chosen based on their traditional use in combination with antibiotics among laymen. Standard protocols were followed to examine the antimicrobial activity of plant extracts and levofloxacin against *E. coli* in term of their minimum inhibitory concentrations (MICs) and to evaluate the plant extracts-levofloxacin interaction using checkerboard method. Among the twelve plants investigated, *Thymus vulgaris*, *Zingiber officinale*, *Teucrium polium*, *Matricaria chamomilla* and *Curcuma longa* had the best antimicrobial activities against *E. coli* strains with MIC values at 250 μg/ml. It is noteworthy to mention that other folk plants extracts revealed no effects against *E. coli* strains. Furthermore, additive interactions were observed between levofloxacin and *T. polium* or *T. vulgaris* against *E. coli* wild-type TG1 strain. There was no antagonism being observed in this study. The detection of additive interaction between the extracts and levofloxacin demonstrates the prospective of these folk medicinal plants as a source of compounds to modulate antibiotic resistance.

**Keywords**: Interaction, *Escherichia coli*, levofloxacin, antibiotics, Minimum inhibition concentration, checkerboard
INTRODUCTION
The expansion of bacterial resistance to the currently used antibiotics has necessitated the need to look for novel antibacterial agents. Universally, *Escherichia coli* is a critical source of food borne illness and a threat to public health. The growing incidence of antibiotic-resistant *E. coli* isolates is a worldwide health concern and are currently linked with higher morbidity, mortality and usually higher care expenditure compared with strains vulnerable to antibiotics. Fluoroquinolones have been broadly used in the genitourinary infections’ management, particularly in cases of acute uncomplicated cystitis. Although fluoroquinolone resistance was infrequent among urinary tract pathogens, resistance in *E. coli* has developed and keeps rising. Levofloxacin has an inhibitory activity against different uropathogens, including *E. coli*. Moving on now to consider that some medicinal plants produce multidrug resistance inhibitors which improve the activities of antibiotics against multidrug resistant bacteria pathogens. It is this finding that encouraged efforts in screening of medicinal plants for possible interaction with standard antibiotics against resistant bacteria as this would cover the approach for possible isolation of multidrug resistance inhibitors of plant origin. A study of this type would also help to show the class of antibiotics that could be accurately combined with certain herbal medications in ethnomedicine against certain infections. In our previous works, we have investigated the effects of numerous plants in Jordan, traditionally thought to have some antimicrobial activity on their potential antibiotic resistance-modifying activity of different antimicrobials. In this study, *in vitro* activity of twelve folk medicinal plants was investigated for their ability to potentiate, modify or minimize the activities of levofloxacin against *E. coli* strains. This study may highlight if traditional use of these plants for bacterial infections represent a real effect or not.

MATERIALS AND METHODS
Plant material
Twelve plants were selected based on their traditional usage as folk medicine either alone or in combination with antibiotics. The plants were purchased from traditional herbal market in Amman, Jordan [Table 1]. The taxonomic identification of plants was verified by comparing the voucher specimens with those of established identity which are located in the Herbarium of the Faculty of Science, The University of Jordan and with the help of a plant taxonomist. A voucher specimen was placed at the Department of Pharmaceutical Sciences, School of Pharmacy, The University of Jordan (Phytochemistry lab.).

Preparation of plant extracts
Plant materials were dried, grounded to a fine powder (40g each) and then extracted

<table>
<thead>
<tr>
<th>Botanical species</th>
<th>Family</th>
<th>Part used</th>
<th>Voucher Specimen</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Camellia sinensis</em> L. (Kuntze)</td>
<td>Theaceae</td>
<td>Leaves</td>
<td>CS-118</td>
</tr>
<tr>
<td><em>Curcuma longa</em> L.</td>
<td>Zingiberaceae</td>
<td>Rhizome</td>
<td>CL-918</td>
</tr>
<tr>
<td><em>Achillea tomentosa</em> L.</td>
<td>Asteraceae</td>
<td>Arial parts</td>
<td>AT-119</td>
</tr>
<tr>
<td><em>Cinnamomum zeylanicum</em> (Syn. <em>Cinnamomum verum</em>)</td>
<td>Lauraceae</td>
<td>Bark</td>
<td>CZ-202</td>
</tr>
<tr>
<td><em>Zingiber officinale</em> (Rosc.)</td>
<td>Zingiberaceae</td>
<td>Rhizome</td>
<td>ZO-118</td>
</tr>
<tr>
<td><em>Thymus vulgaris</em> L.</td>
<td>Lamiaceae</td>
<td>Leaves</td>
<td>TV-119</td>
</tr>
<tr>
<td><em>Matricaria chamomilla</em> L.</td>
<td>Asteraceae</td>
<td>Flower</td>
<td>MC-020</td>
</tr>
<tr>
<td><em>Pimpinella anisum</em> L.</td>
<td>Umbellifers</td>
<td>Fruits</td>
<td>PA-202</td>
</tr>
<tr>
<td><em>Salvia rosmarinus</em> (Spenn.)</td>
<td>Lamiaceae</td>
<td>Leaves</td>
<td>RO-718</td>
</tr>
<tr>
<td>(Syn. <em>Rosmarinus officinalis</em>)</td>
<td>Lamiaceae</td>
<td>Leaves</td>
<td>AJ-619</td>
</tr>
<tr>
<td><em>Artemisia jordanica</em> (Danin)</td>
<td>Asteraceae</td>
<td>Arial parts</td>
<td>TP-319</td>
</tr>
<tr>
<td><em>Teucrium polium</em> L.</td>
<td>Lamiaceae</td>
<td>Leaves</td>
<td>SO-001</td>
</tr>
</tbody>
</table>

Syn.: synonym
using a Soxhlet with 500 ml of 95% ethanol for 4 hrs. A rotary evaporator was used to concentrate the extract under reduced pressure and heating below 40°C. The dried crude extract was weighed and dissolved in ethanol to get 10 mg/ml concentration, and stored in amber tubes and placed under 4°C for extra experiments.

**Bacterial strains and growth conditions**

*E. coli* wild-type TG1 strain (ATCC 25922) and a mutant strain KAM3-1(ΔacrB-ΔtolC) were used for this study, maintained routinely at 37°C on Luria-Bertani medium for 24 hrs. Bacterial subcultures were suspended in nutrient broth, supplemented with 15% glycerol and stored at -70°C for further use.

**Antimicrobial tests**

**MICs Determination**

MIC of plant extracts as well as levofloxacin (Dar Al-Dawa Pharmaceutical Manufacture, Amman, Jordan) was determined by 96-well micro-dilution method. Agar diffusion assay was not performed because it could not always be reliable for plant extracts as the natural active compounds are not polar enough to easily diffuse in the aqueous agar matrix and since MIC is required for the following assays, broth dilution was performed. Experiments were carried out in triplicate in accordance with the recommendations of the National Center for Clinical Laboratory Standards. Ethanol was used as a negative control while levofloxacin was used as a positive control. The 1st well was aliquoted with 180 μl of Mueller-Hinton broth (MHB) (Biolab, Budapest, Hungary) whereas, 100 μl of MHB was added to the 2nd - 10th well. In the 1st row of the plate, 20 μl from the stock solution of plant extracts (10 mg/ml) was added. Then, 2-fold serial dilutions were done using a micropipette. Each well was inoculated with 100 μl of approximately 1 McFarland standard of freshly grown bacteria, making the final concentration in the wells in the range (1000–7.81 μg/ml). Regarding levofloxacin, the final concentration range used to determine MIC was (1- 0.00781 μg/ml). The MIC values were obtained visually after incubation of the inoculated 96 well plates at 37°C for 24 hours, as the minimum concentration that led to bacterial growth inhibition.

**Determination of combination interaction using microdilution checkerboard method**

The checkerboard broth microdilution method was used for determination of the effect of combinations of levofloxacin and the selected plant extracts with efficient MIC on 96-well plates with a final volume of 200 μl in each well, as described the literature. Briefly, 2-fold dilutions of both levofloxacin and plant extracts was used for the assay before testing. Stock solutions of 10 μg/ml of levofloxacin and 10 mg/ml of plant extracts were used. The levofloxacin was serially diluted over the x-axis while the plant extracts was diluted over the y-axis [Fig. 1]. The used inoculum concentration and incubation conditions were as in MIC determination method. Control sample containing the solvent with MHB and the bacteria was used. Each experiment was performed in triplicate.

As mentioned earlier, the combination MIC was determined with the minimum concentration at which no turbidity was observed. The data resulted by the checkerboard method were evaluated in terms of the fractional inhibitory concentration index (FICI) and calculated for each combination using the formula in [Fig. 2]:

**RESULTS AND DISCUSSION**

Plants have been used by many cultures to treat many conditions since they are natural, non-toxic, and inexpensive sources of drug. It is well known that many communities use herbal brews during antibiotics treatment believed that this well augment the effects of these antibiotics. Levofloxacin is among the recent antibiotics used for treatment of different infectious diseases mainly those induced by *E. coli*. It is noteworthy to mention that many people increase their consumption of certain plants brew during levofloxacin therapy as well as other antibiotics. Based on this and to examine possible drug-herb interaction, it was important to examine scientifically the possible interaction between levofloxacin and the most common plant used in combination with it.

The addition of certain herbal remedies and diverse antibiotics may affect the activity of these antibiotic. This study investigated the
antimicrobial activity and interaction between different plant extracts and levofloxacin.

**Antimicrobial activity of tested plants**

The results of the antimicrobial activity of the tested plant extracts are presented in [Table 2]. Among all plant extracts studied, *T. polium, T. vulgaris, C. longa, M. chamomilla* and *Z. officinalis* showed the lowest MIC values against the E. coli wild-type TG1 (250 μg/ml). These findings are in accordance with previous studies\(^ {18-22}\). On the other hand, other plant extracts didn’t show any noteworthy inhibition against this strain (MIC >1000 μg/ml).

On the other hand, The mutant strain *E.coli* KAM3-1 (∆acrB-∆tolC) showed increment susceptibility profile for levofloxacin. Moreover, the susceptibility pattern of the mutant strain showed similar results for the wild type when the tested plants examined.

While many of these plants’ antimicrobial activity was studied earlier, their interaction with levofloxacin is not widely explored yet.

**Interaction of plant extracts with levofloxacin**

In this study, Checkerboard Assay was performed to study the possible synergistic activity between levofloxacin and the ethanolic extract of selected medicinal plants. Among these plants five were chosen based on their inhibitory activity

**Table 2. Antimicrobial activity of plants against E.coli strains (μg/ml)**

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>E. coli wild-type (TG1)</th>
<th>E. coli KAM3-1 (∆acrB-∆tolC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. sinensis</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>C. longa</td>
<td>250</td>
<td>250</td>
</tr>
<tr>
<td>A. tomentosa</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>C. zeylanicum</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>Z. officinalis</td>
<td>250</td>
<td>250</td>
</tr>
<tr>
<td>T. vulgaris</td>
<td>250</td>
<td>250</td>
</tr>
<tr>
<td>M. chamomilla</td>
<td>250</td>
<td>250</td>
</tr>
<tr>
<td>P. anisum</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>R. officinalis</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>A. jordanica</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>T. polium</td>
<td>250</td>
<td>250</td>
</tr>
<tr>
<td>S. officinalis</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>Levoflaxacin</td>
<td>0.03125</td>
<td>&lt;0.0078</td>
</tr>
</tbody>
</table>

*Experiments were conducted in triplicate.

**Fig. 1.** Checkerboard method for Determination of the effect of the combination on *E. coli*  

**Fig. 2.** FICI calculation formula

The results were analyzed as follows: synergy, FICI of ≤0.5; additivity, FICI of >0.5 to ≤1; no interaction (indifference), FICI of >1 to ≤4; antagonism, FICI of >4.\(^ {13,16}\)
against *E. coli* wild-type TG1. An overview of FIC index details is shown in [Table 3]. The antimicrobial interaction of levofloxacin in combination with *T. polium* or *T. vulgaris* against *E. coli* strains showed additive effect. On the other hand, no interaction in *C. longa, M. chamomilla* and *Z. officinalis* combinations was detected. Moreover, neither synergistic nor antagonistic effects were observed when these plants combined with levofloxacin.

A possible explanation for the obtained results [Table 3] might be that these plant extracts have numerous phytochemicals which might inhibit bacterial growth by different mechanisms. Various Studies have investigated the ability of many plant-derived compounds to affect the antimicrobial resistance pattern. It has been shown that many of these compounds exert their antimicrobial activity over membrane disturbances, such as polyphenols. Some of these compounds can also produce a specific peptidoglycan inhibiting antibiotics activity by targeting the same site in the cell wall. Among pathogenic bacteria, efflux pumps are accountable for a significant degree of resistance to antibiotics. Some phytochemicals were identified to boost the efficacy of antimicrobial agents by inhibiting bacterial multidrug resistance efflux systems.

Previous studies revealed that triterpenes, flavonoids and tannins were present in *T. polium* and *T. vulgaris* extracts. Flavonoids, one of the broadest classes of phenolic compounds, has been found to permeabilize the outer membranes of Gram-negative bacteria, which might contribute to enhanced uptake of levofloxacin. In addition, some flavonoids have demonstrated an inhibitory activity against *E. coli* DNA gyrase, which would improve the antibacterial activity of levofloxacin. On the other hand, Alkaloids that are present in *T. polium* might disrupt the bacterial outer membrane, affect the cell division by inhibiting the nucleic acid synthesis, and inhibiting the efflux pump or down regulation of efflux pump genes, working together to improve levofloxacin activity.

Therefore, the various modes of action of the medicinal plants from the antibiotics seems to be a significant aspect in the augmented bactericidal activity noted when used in grouping. Meanwhile the combinations of plant extracts with antibiotics might inhibit Gram negative bacteria, and some combinations might be additive, synergistic or indifferent, these combinations may be applied and favorable in inhibiting bacteria.

Moreover, using such antibiotics in combination will reduce the prescribed dose lower than when used alone, which may extra minimize the incidence of adverse effects induced by these antibiotics. Testing crude extracts for such activities is the initial step in recognizing those compounds in plants that have strong potentials for use in combination with antibacterial. Further isolation and purification of the crude extracts might show an improvement in bioactivity than the crude extracts.

**CONCLUSION**

Additive interaction was observed when levofloxacin combined with *T. polium* or *T. vulgaris* against *E. coli* wild-type strain while no interaction was noted with *C. longa, M. chamomilla* and *Z. officinalis*. Further clinical studies are required to ensure the effectiveness of plant extracts in vivo. Our study suggests the possibility of concomitant use of levofloxacin and plants in the treatment of infections caused by *E. coli* or at least that the concurrent administration of these plants with levofloxacin will not diminish the antimicrobial activity of it.

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**Table 3.** antimicrobial interaction of plant extracts and levofloxacin combination

<table>
<thead>
<tr>
<th>Plant extracts</th>
<th>FIC of plant</th>
<th>FIC of levofloxacin</th>
<th>FICI</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. longa</em></td>
<td>0.062</td>
<td>2</td>
<td>2.062</td>
<td>Indifference</td>
</tr>
<tr>
<td><em>T. polium</em></td>
<td>0.015</td>
<td>0.499</td>
<td>0.515</td>
<td>Additive</td>
</tr>
<tr>
<td><em>M. chamomilla</em></td>
<td>0.015</td>
<td>2</td>
<td>2.015</td>
<td>Indifference</td>
</tr>
<tr>
<td><em>T. vulgaris</em></td>
<td>0.015</td>
<td>0.499</td>
<td>0.515</td>
<td>Additive</td>
</tr>
<tr>
<td><em>Z. officinalis</em></td>
<td>0.125</td>
<td>1</td>
<td>1.125</td>
<td>Indifference</td>
</tr>
</tbody>
</table>

*Experiments were conducted in triplicate.*
ACKNOWLEDGMENTS

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS’ CONTRIBUTION

All authors listed have made a substantial, direct, and intellectual contribution to the work, and approved it for publication.

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ETHICS STATEMENT

Not applicable.

DATA AVAILABILITY

All datasets analyzed in the study are included in the manuscript and presented as tables.

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