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ANTIMICROBIAL EFFECTS OF SOME ESSENTIAL OILS AGAINST UROPATHOGENIC *ESCHERICHIA COLI* STRAINS

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INTRODUCTION

In these times, urinary tract infections are the most widespread bacterialinfections and *E. coli* is the versatile pathogen causing bacteremia (Foxman B., 2010, Miller LG. et al., 2004, Bonten M., et al. 2021).

The commensal in the human microbiome, E coli strainst is a Gram-negative bacteria that can produce, under favorable conditions, a serious range of urinary, gastrointestinal and, last but not least, systemic infections with serious health consequences (Ramos S. et al., 2020, Artimov L., 2020).

Bacteria expand their limits in the fight against antibiotics. Multi-resistant bacteria have become a serious problem in treating them (Giamarellou H., 2005, Siranosian B.A., 2022). Extended-Spectrum Beta-Lactamases (ESBLs) have grown due especially to inadequate antibiotic treatments. Extended-Spectrum Beta-Lactamases (ESBLs) are enzymes produced by *Enterobacteriaceae*, including *Escherichia coli strains*.These enzymes can break down the antibiotics resistance, especially beta-lactam antibiotics, such as penicillin's, cephalosporin's, and the monobactams. This is why ESBLs bacteria are also a threat to the development of new strategies to combat it. *E. coli* stains is the most frequently implicated in urinary tract infections (UTI)and are becoming increasingly resistant to antibiotics (Giamarellou H., 2005, Foxman B., 2010, Schmiedel J. et al., 2014, Zangane Matin F., et al. 2021).

In our study, evaluating 408 urocultures were evaluated over a period of 6 months,the incidence of this bacteria was 76.27%.The discovery of new methods to combat these bacterial strains it is of great interest because it is increasingly difficult to treat certain urinary tract infections. Phytotherapeutic alternatives in the treatment of mild urinary infections can be the essential oils of some aromatic plants or combinations of them, as we observed in the situations explained in the figures 5-7.

Mentha piperita is one of the oldest medicinal plants used for medicinal tea (Stanescu U. et al., 2021). *Thymus vulgaris* and *Oreganum vulgare*is known for his traditional use in the treatment of antibacterial properties(Rehman N.U. et al., 2021, Mancuso M., 2020, Bokhari N. et al.2016, Lu M. et al., 2018).
Peppermint oil has antimicrobial properties, disrupting the lipid fraction of the microbial plasma membrane. *Thymus spp.* also possesses such antibacterial properties (Rehman N.U. et al., 2021), as well as *Oreganum vulgare* (Lopez L.N., 2017, Lu M. et al., 2018), being a well-known and valuable plant for obtaining essential oils.
This study aimed to evaluate if the *Mentha piperita oil, Origanum vulgare oil and Thymus vulgaris oil* have antibacterial properties and have a potential treatment in urinary infections.

MATERIALS AND METHODS

Essential oils of *Mentha piperita, Oreganum vulgare, and Thymus vulgaris* were used, being tested with absorbent paper discs, made by hand. All this essential oil products is made by Fares, Romania.

These handcraft discs that were used in this study, were designed to mimic the classical antibiotic test, being sterilized, impregnated with oils, and tested using the classical disc diffusion method for antibiotic susceptibility testing. The way to see the antimicrobial effects of these essential oils, on sterilized handcraft discs that are impregnated with 5 µl of each essential oil we wanted to test it.

As a working methodology we used a reference *Escherichia coli* strain ATCC 25922, on Muller Hinton agar (Carl Roth GmbH, Germania), which were tested for quality control with the following antibiotics discs (Oxioid, Thermo Fisher brand, United Kingdom): Ampicilin 10µ (AMP), Amoxycillin/clavulanic acid 30 µg(AMC), Ceftazidime30 µg(CAZ), Imipenem10 µg(IPM),Norfloxacin10 µg(NX), Nitrofurantoin300 µg(F), Amikacin30 µg(AK) by procedure Kirby Bauer disc diffusion test.

The results of the antibiograms were valid according to CLSI (The Clinical & Laboratory

Standards Institute, 2012), which allowed us to continue our research.The discs were tested on the *Escherichia coli* ATCC 25922 strain (control) and wild strains taken from patients in a clinical laboratory.

The identification of the E coli strains was done with the help of selective agar medium like Cisteine-Lactose Electrolyte-Deficiente Agar (CLED agar from Oxioid[th], UK) and differential identification media like: Motility, Indole, and Urease (MIU) test, Triple, Sugar, Iron agar (TSI) and Simmons Citrate agar.

RESULTS AND DISCUSSIONS

In this study, as shown in table 1, 408 urocultures were evaluated over a period of 6 months, of which we chose to deepen the reaction and incidence of the most common bacteria that cause urinary tract infection, namely *Escherichia coli*.

From the total of urocultures analyzed (n=408), 69,60% were negative, 15,93% were evaluated as polymorphic flora who representing a contamination of the urine collection, that being present after incubation of more than 2 types of bacterial colonies, makingimpossible any proper identification.

From the total of urocultures 3,43% bacteria strains, other than *Escherichia coli*, are belonging to the genera: Staphyloccocus, Enterococcus, even other Enterobacteriaceae were isolated.What interested us, was the presence of a 11,02% urocultures with *Escherichia coli* strains.

From the analysis of classical antibiograms (Fig 1) it was found that AMC, AK, IMP, NX, F, were the most sensitive of the antibiotics, while CAZ and AMP showed resistance to the tested strains.

The urinary tract infection is a major problem for many women nowadays, as shown by the research, *Escherichia coli* Causing Recurrent Urinary Tract Infections: Comparison to Non-Recurrent Isolates and Genomic Adaptation in Recurrent Infections (Nielsen K. L. et al., 2021)

Of all the *Escherichia coli* strains tested we identified that 75,55% multi-resistant ESBLstrains. The Clinical & Laboratory Standards Institute (CLSI) issued recommendations for ESBLscreening and confirmation for isolates of *Enterobacteriaceae* species for example in our study *Escherichia coli* (fig 3).

After performing the antibiograms (Fig 3), wetested the handcrafts discs fill up with 5 µl oil of *Mentha piperita*, *Oreganum vulgare* and *Thymus vulgaris* essential oil, both on the ATCC 25922 control strain *Escherichia coli* and for clinical strains isolated from the patients urocultures.

Tabel 1. Spectrum of the urocultures analyzed

Uroculture results	Ian	Feb	Mar	Apr	May	Jun	Total
ESBL	9	8	7	5	4	1	34
Polymorphic flora	7	3	9	22	7	17	65
<1000 UFC/mL	33	58	45	48	47	53	284
Escherichia coli	9	8	7	5	9	7	45
Other bacteria	4	2	2	1	2	3	14
TOTAL	53	71	63	76	65	80	408

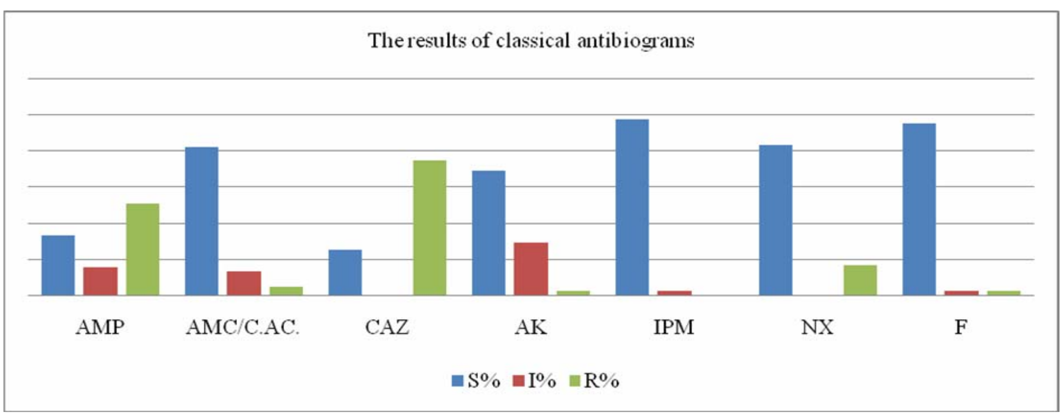

Fig. 1. Classical antibiograms from urocultures

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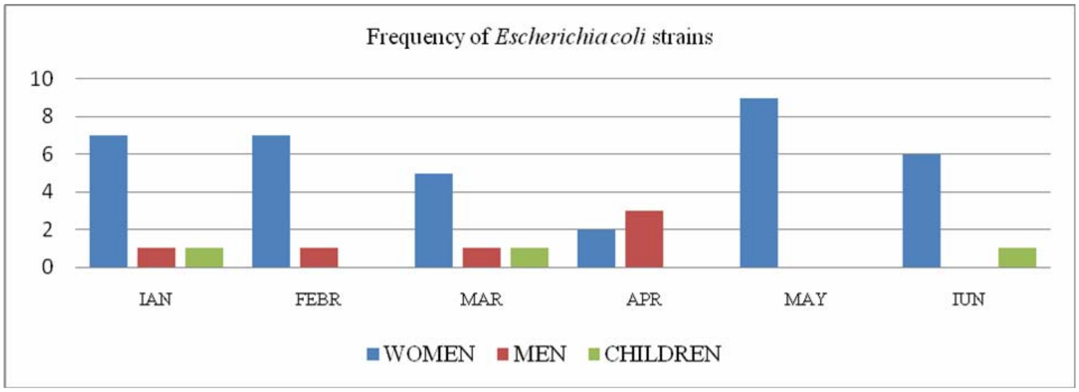

Fig. 2. Frequency of *E.coli* infections

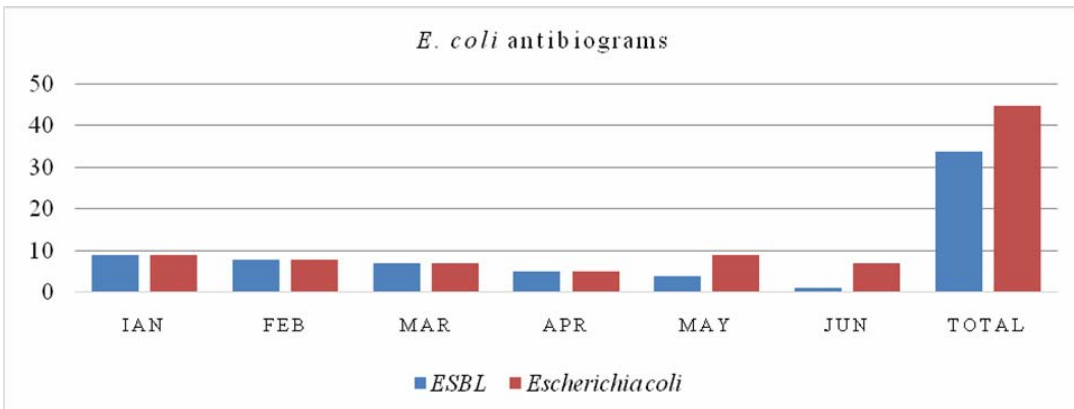

Fig. 3. Monthly distribution of the antibiograms

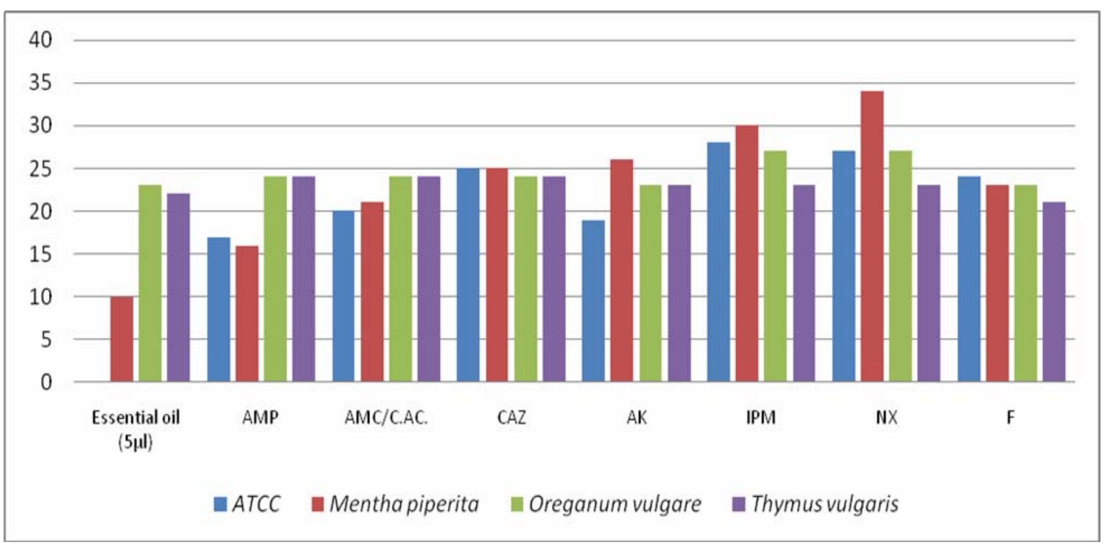

Fig. 4. The ATCC 25922 antibiogram

The ATCC 25922 has been used as a control strain for both the classical antibiotics and the improved antibiotics with the selected oils. The first observation to mention is that the ATCC 25922 strain of *Escherichia coli* was within the quality control range, according to CLSI, which proves the veracity of the study.

A quality control was also performed to see the inhibition range of the essential oils on the ATCC strain by impregnating 5 µl of each essential oil (*Mentha piperita*, *Oreganum vulgare* and *Thymus vulgaris)* on the handcraft discs.

The results obtained were the following: in the case of simple discs with 5 µl *Oreganum vulgare* we found a diameter of 23 mm, at *Thymus vulgaris*a 22 mm and for *Mentha piperita* a 10 mm.

While antibiotics discs improved with *Mentha piperita* oil had an increase in AMC, AK, IPM, NX and oils *Oreganum vulgare* and *Thymus vulgaris* showed an increase in AMP, AMC, AK compares to the unimproved ones, confirming that it has antibacterial properties. After making the antibiotics of the classic patients (Fig 5, 6, 7) we improved the same antibiotics with the chosen essential oils. The empty discs witch was improved by 5 µl of the selected oils were added, showed an inhibition zone for *Mentha piperita* with a 20 mm, for *Thymus vulgaris* an 25 mm and for *Oreganum vulgare* 24 mm.

Increases from normal antibiotics have been reported in *Mentha piperita* oil to all the antibiotics chosen but with the highest values observed at CAZ, AK and NX.

Oreganum vulgare essential oil was most effective at AMP, AK si IPM, while *Thymus vulgaris* oil had significant increases at AMP si AK.

In the second case, the paper discs wich was impregnated with 5 µl of *Mentha piperita* oil showed an increased of 8mm, to *Oreganum vulgare* an increased of 21mm and for *Thymus vulgaris* with 23mm. According to the graph resistance to AMP (6 mm) was observed. After improving AMP with *Oreganum vulgare* and *Thymus vulgaris* oils was noticed an increased sensitivity with 20 mm respective 22 mm in diameters.

In the third case, our discs that have been improved with selected oils showed an increased for *Mentha piperita* by 10mm, on *Oreganum vulgare* with 21mm and for *Thymus vulgaris* by 20 mm.

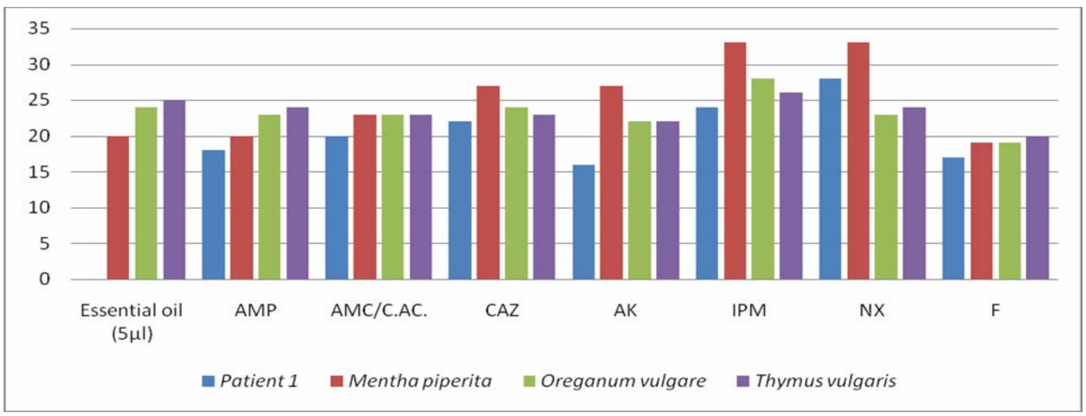

Fig. 5. Antibiogram spectrum analyzed at pacient 1

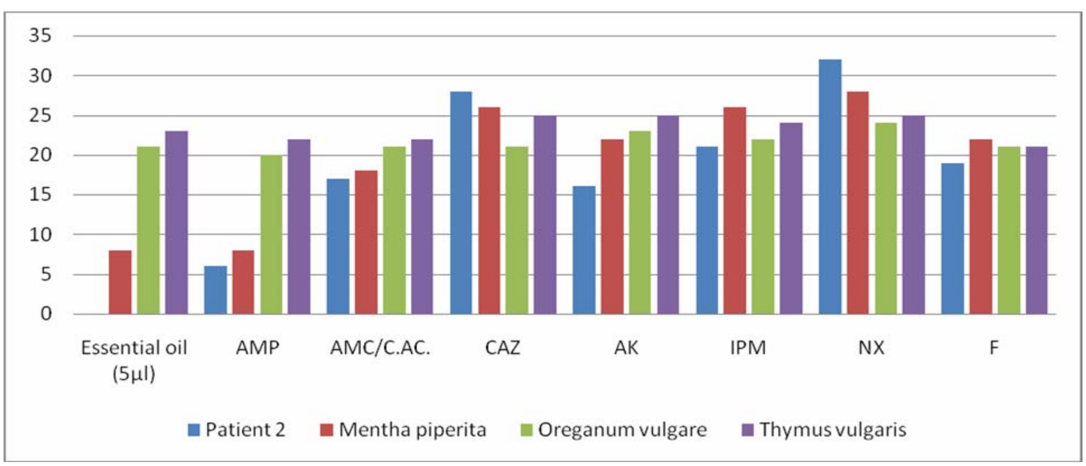

Fig. 6. Antibiogram spectrum analyzed at pacient 2

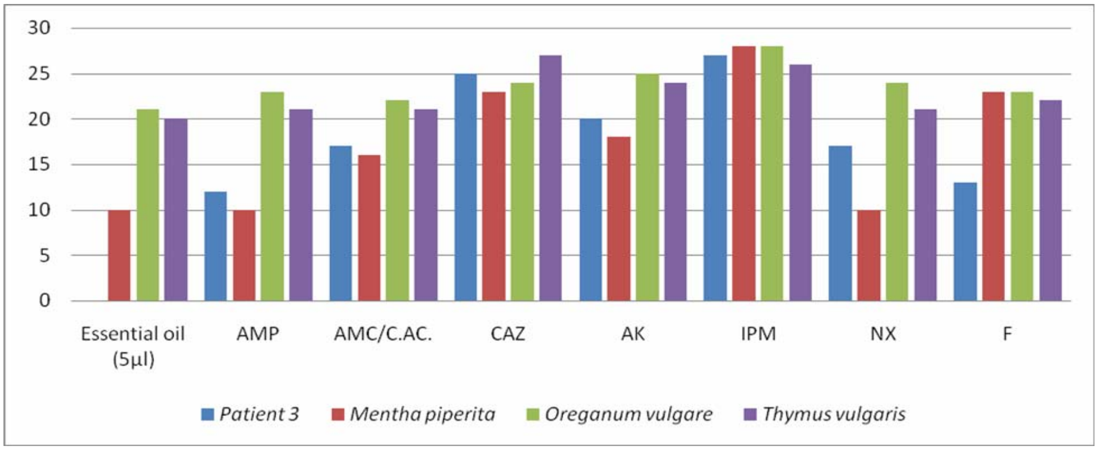

Fig. 7. Antibiogram spectrum analyzed at pacient 3

The analyzed oils were successful for F antibiotic with a significant increase respectively with *Mentha piperita* by 23mm, for *Oreganum vulgare* with 23mm diameter and with *Thymus vulgaris* 22mm, compare to the classic antibiotic by 13 mm.

We have reported on the *Oreganum vulgare* and *Thymus vulgaris* oils an increase of sensitivity to AMP, AMC, AK and NX.

CONCLUSIONS

Results of the experiments reveal an increase in sensitivity to antibiotics improved with essential oils to the both ATCC 25922 and wild strains of *Escherichia coli*.The results obtained in experiment were the following: in the case of handcraft discs with 5 µl *Oreganum vulgare* we found a diameter of 23 mm, at *Thymus vulgaris* a 22 mm and for *Mentha piperita a* 10 mm. Statistical modeling has shown that the average has an increase over classical testing with simple antibiotics.However, of the seven antibiotics tested, two of them CAZ and NX showed no substantial increases following improvement with essential oils.

In the case of the AMP antibiotic the average results for the classic antibiotic are 12 mm inhibition zone and for improved antibiotics with *Mentha piperita* 12,66mm, *Oreganum vulgare* 22 mm and with *Thymus vulgaris* 22,33mm.

Another antibiotic with remarkable results is AMC with an average for classic testing of 18 mm, while in *Mentha piperita* 19 mm, *Oreganum vulgare* 22 mm and for *Thymus vulgaris* 22mm.

The most relevant effects were obtained in AK with a mean for classical testing of 17.33 mm and in *Mentha piperita* 22,33mm, *Oreganum vulgare* 23,33 mm and *Thymus vulgaris* 23,66mm.

For the IPM case the classical testing an average of 24 mm, while in the case of essential oils *Mentha piperita* an average of 29mm, for *Oreganum vulgare* a value of 26mm and for *Thymus vulgaris* an average of 25.33 mm was recorded.

The classicaltesting for F has an average of 16.33 mm and the antibiotics improved by *Mentha piperita* has an 21.33 mm diameter, for *Oreganum vulgare* 21 mm and *Thymus vulgaris* has 21 mm.

ABSTRACT

Now, there is a big interest on the discovering and developing a new antimicrobial product with efficient actions on pathogen bacteries. Also, a lot of attention has been focused on the antimicrobial activity screening and methodology of this.

A lot of study aims antibacterial activity of the plants like phytotherapeutic alternatives of the antibiotics resistance (Giamarellou H., 2005, Foxman B., 2010, Schmiedel J. et al., 2014, Zangane Matin F., et al. 2021)..

In this paper, evaluated a number of 408 urocultures and antibiograms and tested some essentials oils from: *Mentha piperita, Origanum vulgare and Thymus vulgaris.*

The results showed that all the essentials oils tested has a good antibacterial activity in urinary infections, compared with antibiotics.

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