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ОРИГИНАЛЬНОЕ ИССЛЕДОВАНИЕ

Antibacterial activity of Clove *Syzygium aromaticum* L. and synergism with antibiotics against multidrug-resistant uropathogenic *E. coli*

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Abstract. *Relevance.* Urinary tract infections pose a growing threat to humanity due to the rise of antibiotic resistance in uropathogens. Exploring natural sources for alternative treatments has become a prominent approach. *The aim* of the research was to investigate the antibacterial effects of clove (*Syzygium aromaticum* L.) against uropathogenic *Escherichia coli (E. coli). Materials and Methods.* The research was performed on three clinical multidrug-resistant uropathogenic *E. coli* isolates and *E. coli* ATCC 25922. Clove hydroalcoholic extract was obtained by cold maceration technique. To evaluate the antibacterial activity of the extract, agar well diffusion method was performed. Minimum inhibitory and minimum bactericidal concentrations of the extract were determined by microbroth dilution method. Light microscopy was used to investigate morphological changes in uropathogenic *E. coli* after exposure to clove extract. Checkerboard assay was used to assess synergism between clove extract and antibiotics. All obtained data were statistically processed. *Results and Discussion.* In well diffusion method, bacterial responses to clove extract were concentration-dependent with inhibition zone diameter of 7–10/10–15 mm for uropathogenic strains and *E. coli* ATCC 25922, respectively. Minimum inhibitory and minimum bactericidal concentrations of clove extract against uropathogenic strains were 25 mg/mL. The extract showed a lower minimum inhibitory concentration against *E. coli* ATCC 25922 (6.25 mg/mL) with minimum bactericidal concentration being 25 mg/mL. Minimum inhibitory and minimum bactericidal concentrations

ratio showed that clove extract tends to be bactericidal agent. Synergy test revealed that the combination of clove extract and nitrofurantoin or ciprofloxacin resulted in no interaction. However, minimum inhibitory concentrations of all tested agents in combinations exhibited varying degrees of decrease. Incubation of uropathogenic strains with the extract transformed them to unstable spherical L-form in percentage of 96–99 %. *Conclusion.* This study highlights clove as a potential natural antibacterial agent against multidrug-resistant uropathogenic *E. coli*, warranting further investigations into its antibacterial properties.

Key words: clove, *Syzygium aromaticum*, urinary tract infections, uropathogenic Escherichia coli, phytochemicals, antibacterial agent, antibiotic resistance

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Introduction

Since ancient times, spices have represented an essential part of traditional medicine for their prominent therapeutic properties. Among spices, clove stands out as a highly effective medicinal plant that has advantages over others [1], especially in terms of the high content of polyphenols and antioxidant compounds [2]. The scientific name of clove is *Syzygium aromaticum L*. and it belongs to the family Mirtaceae [3]. Cloves grow as medium-sized evergreen trees that are native to eastern Indonesia [4]. The dried flower buds are the commercial part of clove trees and can be used mainly in three forms: ground spice, whole buds and essential oil. Particularly, clove essential oil is the most commonly used form with a wide range of documented therapeutic effects [5]. Clove is traditionally used in many health conditions, including toothache, dental infections, burns, wounds, nausea, vomiting, bloating, disorders of the stomach, intestines and liver, nerves stimulation, food preservation and as an insecticide in agriculture [1, 3]. As per studies, several pharmacological activities of clove have already been validated, such as antibacterial, antifungal, antiprotozoal, antiviral, analgesic, antispasmodic, antioxidant, anti-inflammatory, antidiabetic, antidepressant, antiulcer, antithrombotic, antinociceptive, among others [3].

In microbiology, clove represents a substantial and promising antimicrobial agent as its efficacy against many pathogenic microorganisms has been intensively reported [6–11]. Moreover, in some countries clove is widely used to tackle malaria, scabies, tuberculosis, cholera, food-borne pathogens, worms, candida and viruses [12]. Many research have shown clove to be a highly effective antibacterial agent against many gram-negative and positive bacteria [3].

From the phytochemical point of view, clove contains a broad spectrum of active compounds to which its immense pharmacological activities are attributed. Clove is considered

one of the richest plant sources of phenolic compounds, such as flavonoids (e.g., kaempferol and quercetin), phenolic acids (e.g., gallic, hydroxybenzoic, hydroxycinnamic, ellagic, caffeic, ferulic, and salicylic acids) and tannins. Eugenol is the most abundant bioactive compound found in clove essential oil, other compounds in lower concentrations are eugenol acetate, carvacrol, thymol, cinnamaldehyde, α-humulene, β-cariofileno, β-pinene, limonene, benzaldehyde, farnesol and ethyl hexanoate [1, 3, 5].

Urinary tract infections (UTIs) are one of the most prevalent infections with significant mortality, morbidity and recurrence rate. The extensive use of antibiotics and the lack of clinical investigations have emerged in high resistance among uropathogens. Consequently, UTIs are a worrisome burden that significantly affects the quality of life, of individuals and societies alike. Among uropathogens, uropathogenic *Escherichia coli* (UPEC) are the most prevalent in hospitals and community with multidrug-resistance (MDR) being extensively reported [13]. The problem of antibiotic resistance is emerging alarmingly on a global level. Conventional antibiotics gradually lose their effectiveness and annually many patients die because of the exhaustion of antibiotic treatment options [14]. This issue is of keen interest to researchers and new approaches are being actively developed to treat resistant bacteria and to prevent the development of resistance [15]. Recently, medicinal plants are being intensively studied by researchers all over the world as they present promising natural alternatives to conventional antibiotics. Many plants have been validated to possess a potent antibacterial activity against a wide range of bacterial species, including MDR strains [16]. Searching two of major databases (Science Direct and Google scholar), we found few research on the antibacterial activity of clove against UPEC, thus this *in vitro* study aimed to investigate the antibacterial potential of clove extract against MDR-UPEC.

Materials and methods

Bacterial strains and inoculums preparation

The research was performed on three clinical MDR-UPEC isolates (UPEC 1, 2 and 3) and one reference strain *E. coli* ATCC 25922. Bacteria were provided by the laboratory of the department of microbiology named after V.S. Kiktenko, RUDN University, Moscow. UPEC strains utilized in this study were obtained from urine samples collected from patients (children aged 9 months to 18 years old) diagnosed with symptomatic UTIs, which were confirmed through laboratory testing. Bacteria were isolated and identified at the laboratory of the Russian Children's Clinical Hospital. All UPEC strains were resistant to tetracyclines, ceftazidime/clavulanic acid, ceftazidime, ceftriaxone, trimethoprim and ampicillin. UPEC 3 were additionally resistant to ciprofloxacin and imipenem.

For inoculums preparation, bacteria were cultured overnight in BHIB (Brain Heart Infusion Broth) (HIMEDIA®, Ref 173–500G) for 16–18 h at 37 °C, aerobically. Afterwards, the cultures were centrifuged (for 10 minutes at 3000 RPM in Eppendorf Centrifuge 5415 R), washed twice with phosphate buffer saline (PBS) and resuspended in NaCl (0.9 %). Finally, the turbidity of inoculums was adjusted photometrically to equal that of 0.5 McFarland standard.

Plant material and extraction

Dried clove buds (Russian Grocery Company "Indiana", Shchelkovo, Russia) were obtained from a supermarket in Moscow. To prepare a hydroalcoholic clove extract, cold maceration technique [17] was carried out as follows. Clove buds were first grinded to fine particles using electric blender after which they were placed in a flask with the addition of 80 % ethanol in a sample/solvent ratio of 1/10 (w/v). The flask was tightly closed to prevent evaporation and incubated with shaking (300 RPM), at 22 °C for 24h. Afterwards, the extract was filtered thrice by vacuum filtration (using Whatman filter paper № 1), then the filtrate was evaporated in rotary evaporator (IKA Werke, Staufen, Germany) at 40 °C. The obtained crude extract was in form of dark brown semisolid mass. Extract was stored in darkness at 4 °C.

Agar well diffusion method

Agar well diffusion method was performed to investigate the antibacterial effect of clove extract, as previously described [18]. Briefly, Muller Hinton Agar (MHA) (HIMEDIA®, Ref 173–500G) plates were seeded with fresh

bacterial inoculums, then cork borer (4 mm) was used to made wells in agar. Afterwards, 45 µL of clove extract were added into the wells in the following concentrations: 25, 50, 100 and 200 mg/mL in dimethyl sulfoxide (DMSO, VWR International LLC, USA) (10 % v/v in dH2O). DMSO (10 %) was added alone as a negative control. Plates were let to stand for 30 minutes until fully distribution of the extracts, and then incubated for 24 h at 37 °C. Following respective incubation period, diameters of inhibition zones were measured in mm.

Quantitative antibacterial assay

Quantitative antibacterial assay of clove extract was performed by determining minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) using previously described microdilution method [19]. Briefly, in a sterile U-bottom 96-well microplates serial twofold dilutions of clove extract were made in BHIB, followed by inoculation the wells with respective bacteria. Serial dilutions of 10 % DMSO served as negative control and all used solution except bacteria were included as sterility control. Plates were then incubated for 24 h at 37 °C. The lowest concentration that resulted in no visible bacterial growth was considered MIC. Further, all wells ≥ MIC were subcultured on MHA plates and incubated for 24 h at 37 °C. The lowest concentration with no growth on agar plates were evaluated as MBC. To determine whether the antibacterial effect of clove extract is rather bactericidal or bacteriostatic, MBC/MIC ratio was calculated for each strain. Values ≤ 4 indicate bactericidal effect whereas values > 4 indicates bacteriostatic effect [20].

Morphology

Light microscopy was used to investigate any morphological changes in general shape of UPEC after exposure to clove extract. Standardized concentrations (OD492 = 0.05) of overnight cultures were incubated with the extract at a final concentration of MIC/2 in BHIB for 24 hours at 37 °C, after which cultures were washed twice and resuspended in PBS. Finally, bacteria were simple stained with crystal violet 1 % and observed under a light microscope at 1,000×. In each sample, 100 random cells in random fields of view were observed. Control cultures consisted of bacteria incubated with 10 % DMSO. To investigate whither the morphological changes will persist in the 1st generation in absence of the extract, a subculturing in BHIB was performed and bacteria were observed as previously described. Images were obtained by Levenhuk M300 Base Digital Camera and Levenhuk ToupView (3.7.6273) software.

Checkerboard assay

To assess synergism between clove extract and antibiotics, checkerboard assay was performed [21]. Nitrofurantoin and ciprofloxacin (Sigma-Aldrich) were the tested antibiotics, and their MICs were firstly determined as described for clove extract. UPEC isolates were subjected to the assay. Briefly, 77 combinations of the tested agents were prepared in 96-microplates and inoculated with bacterial inoculum. Final plate setting is shown in Figure 1. Control plates (background plates) contained the same solutions except bacteria. Plates then were incubated at 37 °C for 20 h. The next day, wells were mixed, and optical density (OD) was read in a microplate reader at 492 nm. The mean of three reads was obtained and the percentage of bacterial growth was calculated as follows:

$$\frac{OD_{drug\ combination\ well} - OD_{background}}{OD_{drug\ free\ well} - OD_{background}} \times 100$$

The lowest concentration that inhibited the bacterial growth by more than 80 % was considered as MIC. Data obtained from checkerboard method were further analyzed by Loewe additivity-based model [22]. This model is a nonparametric approach commonly used to define the theoretical additive effects based on the fractional inhibitory concentration index (FICI). First, ΣFIC for each MIC was calculated as follows: ΣFIC = FIC (antibiotic) + FIC (plant extract); FIC (antibiotic) = MIC of antibiotic in combination/ MIC of antibiotic alone, and so for FIC (plant extract). In each plate, the lowest ΣFIC (ΣFIC_{min}) when the highest ΣFIC (ΣFIC_{max}) is smaller than 4 was considered as FICI. As all obtained ΣFICs were lower than 4, ΣFIC_{min} always expressed FICI. Results were then interpreted in accordance with the following criteria: synergy (FICI ≤ 0.5), no interaction (0.5 < FICI ≤ 4) or antagonism (FICI > 4).

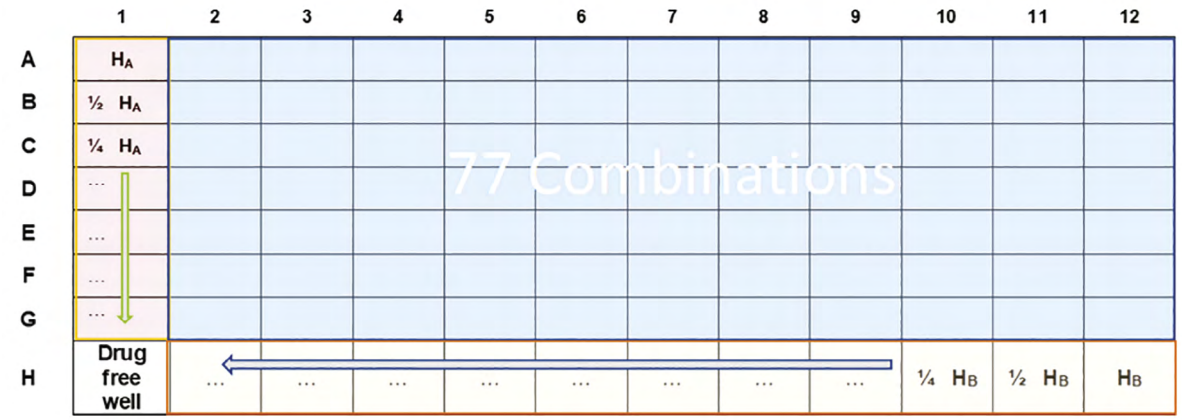

Fig. 1. Checkerboard final microplates setting [21]. H_A and H_B present the tested antibacterial agents

Statistical analysis

All trials were performed separately in triplicate. Three repeats for each tested case were included in each experiment. The obtained data were reported as the mean of all trials ± standard deviation (SD). Synergy assay was modeled using the Loewe additivity-based approach. Excel 2019 and XLSTAT 2023 were used to analyze data, calculate means and SD.

Results and discussion

Well diffusion

Well diffusion method was used to investigate the antibacterial activity of clove extract against *E. coli* strains. Clove extract exhibited antibacterial activity against all tested strains in a concentration dependent manner (Table 1, Fig. 2). UPEC isolates were sensitive only to the 100 and 200 mg/mL concentrations with inhibition diameters of 7–10 mm, while the lower two concentrations (25 and 50 mg/mL) resulted in no inhibition zone. In contrast, the standard strain *E. coli* ATCC 25922 showed sensitivity to all extract concentrations with diameters of 10–15 mm, which is quietly predictable. The correlation between antimicrobial effect and the concentration of plant extract is reported in literature [23]. Generally, our findings support those reported for clove extracts and essential oil against many pathogens. For example, an inhibition diameter of 16–20 mm was reported for clove essential oil (10 µL/disc) against gram negative and positive bacteria, including *E. coli*, *Salmonella* spp, *P. aeruginosa*, *Streptococcus* group D *and S. aureus* [24]. In another study, clove ethanolic extract showed a significant antibacterial activity against high level gentamicin resistant enterococci, with a dimeter of 25–26 mm, by well diffusion method [25]. Similarly, clove aqueous and ethanolic extract resulted in inhibition zones of 12.2–25.2 mm against many pathogens, such as *E. coli*, *Vibrio parahaemolyticus*, *P. aeruginosa*, *Salmonella enteritidis*, *Bacillus cereus*, *S. aureus* and *Candida albicans* [23]. Thus, here we confirm the potency of clove as antibacterial agent against MDR-UPEC.

In this study, we did not analyze the phytochemical composition of clove extract to identify possible active compounds, but we present a theoretical concept based on a similar work. In their phytochemical analyses, Rosarior et al. [26] revealed that the major constituents of clove ethanolic extract were phenolic compounds, mainly eugenol, kaempferol, gallic acid and catechin. As per studies, phenolic compounds are well known for their antibacterial activity against a wide range of bacterial pathogens [27–29]. Moreover, it is proposed that phenolic compounds, especially kaempferol, possess a synergistic effect with eugenol, which results in enhanced antibacterial activity of extracts containing this combination [26]. Hence, the antibacterial activity we observed for clove extract could be attributed mainly to the rich phenolic content of this plant.

Table 1

Diameters of inhibition zones (in mm) for clove extract against *E. coli* strains

Strains	Clove extract (mg/mL)			
	25	50	100	200
UPEC 1	0 ± 0.0	0 ± 0.0	7 ± 0.8	9 ± 0.0
UPEC 2	0 ± 0.0	0 ± 0.0	7 ± 0.0	8.5 ± 0.5
UPEC 3	0 ± 0.0	0 ± 0.0	7 ± 0.6	10 ± 0.0
E. coli ATCC 25922	10 ± 0.6	11 ± 0.8	12 ± 0.0	15 ± 0.3

Note: UPEC — uropathogenic *E. coli*.

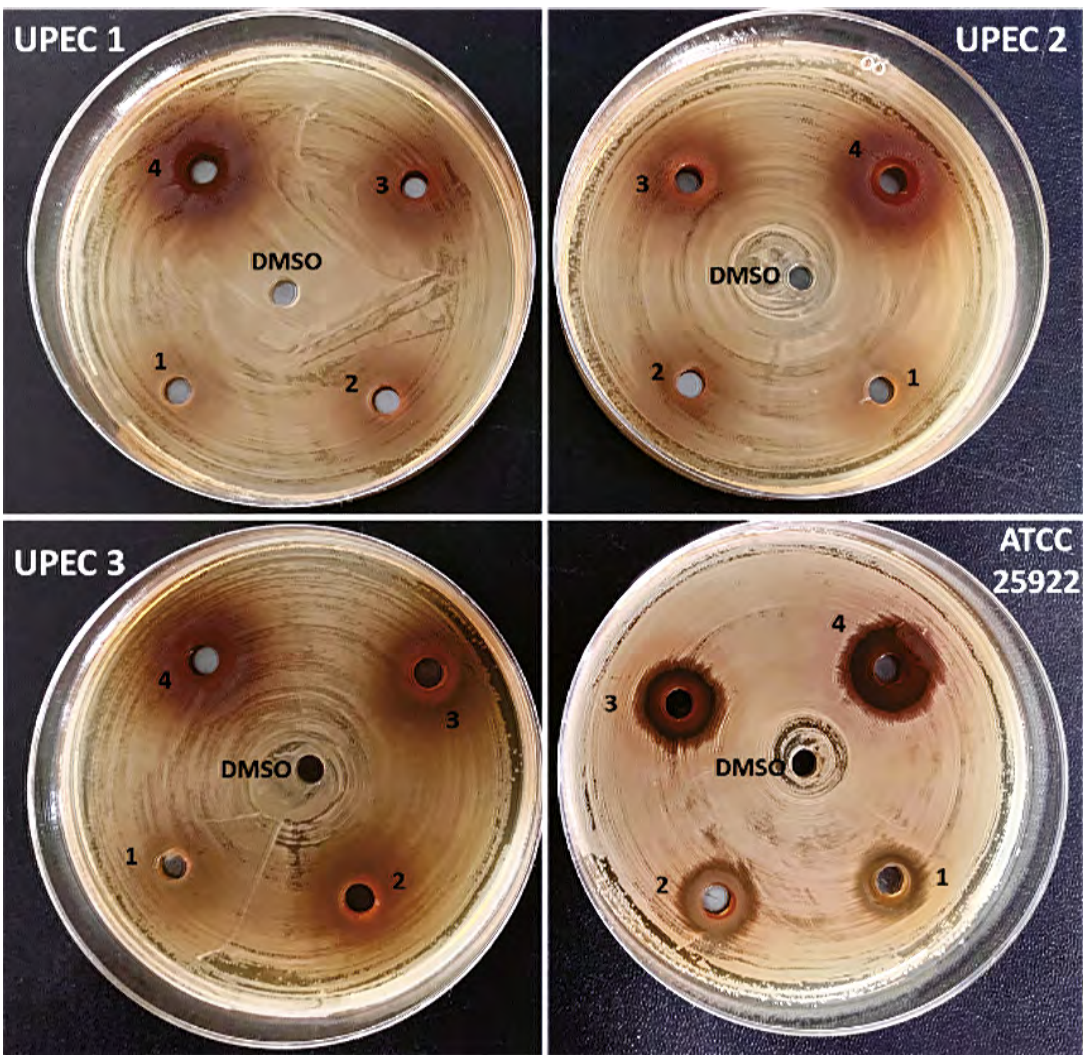

Fig. 2. Inhibition zones of clove extract against E. coli strains. Extract concentrations: 25 (1), 50 (2), 100 (3) and 200 mg/mL (4). DMSO 10 %: negative control. UPEC: uropathogenic *E. coli*

Quantitative antibacterial assay

Antibacterial activity of clove extract was assessed quantitatively by determining MICs and MBCs (Table 2). MIC of clove extract against UPECs was 25 mg/mL and the same concentration resulted in no growth on agar plates which indicates the MBC to be also 25 mg/mL. The extract showed a lower MIC against *E. coli* ATCC 25922 (6.25 mg/mL), while the MBC for this strain was 25 mg/mL. These results are in correlation with those of well diffusion method, as UPECs showed quite similar sensitivity to the extract, while the reference train was the most sensitive to all tested concentrations. Similar MIC (25 mg/mL) was reported for clove ethanolic extract against *E. coli*, while the aqueous extract had a MIC of 50 mg/mL [23]. However, it is well known that many factors affect the content of antibacterial compounds in plant extracts, this includes the extraction method and the type of solvent, which in turn results in such differences between various extracts of the same plant [30,31]. MBC/MIC ratio is a commonly used indicator of the antibacterial nature as it gives an idea whether the agent tends to be bacteriostatic or bactericidal [20]. Here, clove extract has been shown to be bactericidal agent against all tested strains, with a ratio of 1 for UPECs and 4 for the reference strain.

Table 2

MICs and MBCs of clove extract against *E. coli* strains

Strains	MIC (mg/mL)	MBC (mg/mL)	MBC/MIC ratio
UPEC 1	25 ± 0.0	25 ± 0.0	1
UPEC 2	25 ± 0.0	25 ± 0.0	1
UPEC 3	25 ± 0.0	25 ± 0.0	1
E. coli ATCC 25922	6.25 ± 0.0	25 ± 0.0	4

Note: MIC — minimum inhibitory concentration, MBC — minimum bactericidal concentration, UPEC — uropathogenic E. coli.

Morphological changes in bacteria after exposure to clove

The capacity of clove extract to cause morphological changes in UPEC was investigated (Fig. 3). All tested UPEC isolates underwent morphological change to spherical L-form in percentages of 96–99 %. However, the 1st generations restored their walled rod-shaped state. For control samples with DMSO, no abnormalities were observed as they all contained normal rods. Phytochemicals have been shown to cause morphological changes in bacterial cells, such as shortness [32, 33] or filamentation [34], probably depending on tested bacteria and chemical nature of these compounds. The cell wall is an essential protective structure in a bacterial cell, giving it its shape and maintaining the integrity of the cell. Cell wall mainly consists of peptidoglycan (PG) which is synthesized via a well-conserved biochemical pathway that begins in the cytosol with the synthesis of the precursor lipid- II which is then transported out of the cell membrane where cell wall is finally assembled by specialized proteins [35]. The cell wall is one of the most important targets of antibiotics such as β-lactams. Despite the great importance of the cell wall, some bacteria can transform into L-form; a wall-deficient cell that possesses spherical or pleomorphic shape [36]. This transformation is usually induced by cell wall-targeting antimicrobials or innate immune effectors such as lysozyme, thus considered as a resistance mechanism [37]. L-form bacteria can be stable or unstable, i.e., remain L-form or revert back to original shape after withdrawal of the inducing agent. By switching to L-form, bacteria can resist β-lactams, lytic bacteriophages and probably innate immune response [36, 37]. Precise molecular mechanism underlying L-form formation and its role in human infections remains undefined and controversial [36, 38]. However, using genome-wide transcriptome analysis of unstable L-form *E. coli*, Glover et al. [39] reported an up-regulation of

many genes with unknown function and stress pathways, which are also found in persister cells and biofilms, have been also over-expressed. In addition, it has been suggested that a rigid outer membrane is essential for L-form *E. coli* to survive [40].

Here, we showed that clove extract at MIC/2 was able to cause UPEC to convert to unstable L-form. This transformation is likely due to targeting the bacterial cell wall by certain substances in the extract. Thus, bacteria have turned into L-form as a defense mechanism to protect themselves from phytochemicals. These observations highlight the importance of using the extract at concentration of MIC or higher to avoid creating resistant forms of bacteria that are difficult to target and destroy later, especially with antibiotics that act on the cell wall such as β-lactams. In addition, outer membrane inhibitors can be useful in this case if companied with the extract to prevent L-form cells from dividing and surviving. Thus, clove extract may represent a simple and affordable way to obtain L-form bacteria in laboratory for further characterization and studies.

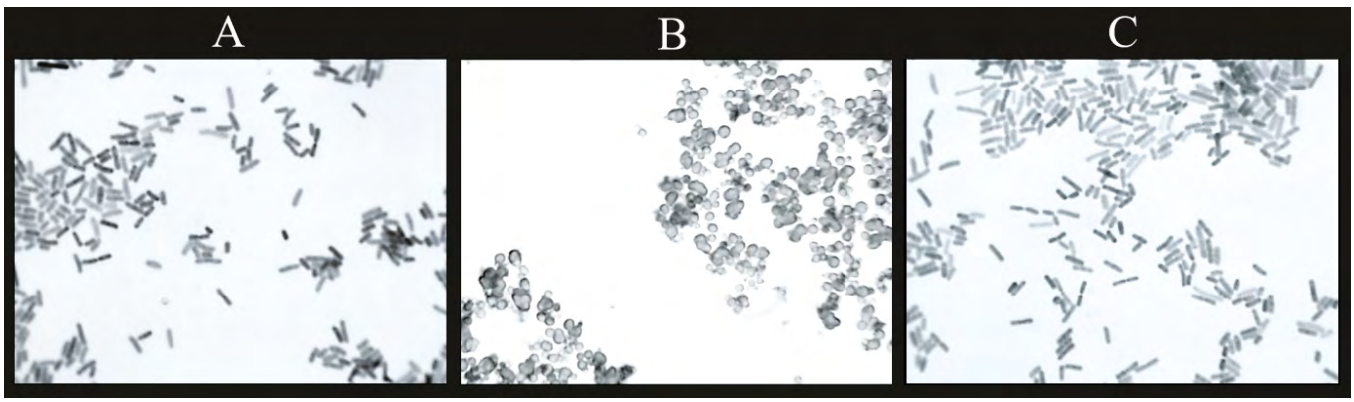

Fig. 3. Morphological changes in uropathogenic E. coli (UPEC) after exposure to clove extract. A: control (normal rods). B: with extract (L-form spherical cells). C: 1st generation after extract withdrawal (normal rods). Magnification ×1,000

Synergy test

Besides the keen interest in developing plant-based antibacterial compounds, using medicinal plants as resistance modifying agents is another promising approach that emerges noticeably, as synergism between plant extracts and conventional antibiotics can enhance the antibiotheraby and, to some extent, restore the sensitivity or prevent the emergence of resistance. Moreover, this approach would bring back to use old and cheap antibiotics that are relatively no longer effective [16, 41]. Indeed, synergism between clove and antibiotics is already reported in literature. For instance, water and ethanolic clove extracts exhibited a synergistic effect with different antibiotics against *S. aureus* and *K. pneumoniae* [42]. Eugenol, which is the major constituent of clove essential oil, has been shown to work synergistically with antibiotics such as vancomycin, ampicillin, and gentamicin [4]. Here we evaluated the synergistic effect between clove extract and two antibiotics from different classes of antimicrobial agents; nitrofurantoin and ciprofloxacin, which are commonly used to treat UTIs [43, 44]. Results are presented in Table 3. MICs of antibiotics alone were of 8, 64 µg/mL for nitrofurantoin and 0.5, 1024 µg/mL for ciprofloxacin. Loewe additivity-based model revealed that the combination of the antibiotics and clove extract resulted in no interaction against all tested strains, with FICI of 0.63, 0.75 for clove and nitrofurantoin and 0.63, 0.75, 1 for clove and ciprofloxacin. Regardless of FICI interpretation, in was observed that all the MICs of antimicrobial agents in combinations decreased in different degrees i.e., MICs decreased by 2, 4, 8 folds for clove and ciprofloxacin and by 2 folds for nitrofurantoin. Thus, to some extent we can conclude that clove can

potentiate the efficacy of these antibiotics if used in combination. While analyzing the available literature on synergy effects between antimicrobials, we found an inconsistency in term of interpretation criteria of FICI values and the agreement in the interpretation of the FICI and other evaluation models. For example, some studies considered a FICI of 0,5–1 as additive effect [19, 45, 46] while others considered it as indifference [47, 48]. Moreover, high variability was observed between the interpretation of FICI and the response surface approach (Bliss model) [22]. In general, our results are an impetus to study the synergistic effect of clove with antibiotics against UPEC and more investigations with different models should be performed to assess the agreement in the interpretation of the FICI.

Table 3

MICs of clove extract (mg/mL) and antibiotics (µg/mL), alone (A) and in combination (B), and FICI values. (SD ± 0.0 for the three trials)

Strains	NIT+ Clove					CIP + Clove				
	NIT		Clove		FICI	CIP		Clove		FICI
	A	B	A	B		A	B	A	B	
UPEC 1	8	4	25	6.25	0.75	0.5	0.125	25	12.5	0.75
UPEC 2	8	4	25	6.25	0.75	1024	128	25	12.5	0.63
UPEC 3	64	32	25	3.125	0.63	1024	512	25	12.5	1

Note: MIC — minimum inhibitory concentration, FICI — fractional inhibitory concentration index, NIT — nitrofurantoin, CIP — ciprofloxacin, UPEC — uropathogenic *E. coli*

Conclusions

UTIs pose a significant health concern globally, with antibiotics resistance becoming a growing problem. As traditional antibiotics face increasing challenges in effectively treating UTIs, the exploration of alternative treatments has become crucial. Medicinal plants hold great promise as potential alternatives, with their diverse bioactive compounds and historical use in traditional medicine. Harnessing the therapeutic potential of medicinal plants may provide new avenues for combating UTIs while reducing the risk of antibiotic resistance. The results of this study highlight the promising applications of clove extract in combating MDR-UPEC. The significant antibacterial effects observed, as evidenced by concentration-dependent inhibition zone diameter and minimum inhibitory and bactericidal concentrations, indicate the effectiveness of clove extract in inhibiting the growth of these bacteria. Moreover, the combination of clove extract with commonly used antibiotics demonstrated a potential synergistic effect, as evidenced by a decrease in their minimum inhibitory concentrations. Additionally, the incubation with clove extract resulted in the transformation of uropathogenic strains into unstable spherical L-forms. Further research is needed to investigate the mechanism behind this transformation and evaluate the implications for potential therapeutic uses.

In conclusion, this *in vitro* study serves as a foundation for more comprehensive research aimed at identifying the active antibacterial compounds present in clove and exploring its potential synergistic effects with other antibiotics, which in turn will offer valuable insights into the potential utilization of clove as a potent antibacterial agent in clinical practice.

Библиографический список/References

1. Cortés-Rojas DF, de Souza CRF, Oliveira WP. Clove (Syzygium aromaticum): a precious spice. *Asian Pac J Trop Biomed*. 2014;4(2):90–96. doi:10.1016/S2221-1691(14)60215-X.

2. Pérez-Jiménez J, Neveu V, Vos F, Scalbert A. Identification of the 100 richest dietary sources of polyphenols: An application of the Phenol-Explorer database. *Eur J Clin Nutr*. 2010;64(3):112–120. doi:10.1038/ejcn.2010.221.

3. El-Saber Batiha G, Alkazmi LM, Wasef LG, Beshbishy AM, Nadwa EH, Rashwan EK. *Syzygium aromaticum* L. (Myrtaceae): Traditional Uses, Bioactive Chemical Constituents, Pharmacological and Toxicological Activities. *Biomolecules*. 2020;10(2):202. doi:10.3390/biom10020202.

4. Kamatou GP, Vermaak I, Viljoen AM. Eugenol — From the remote Maluku Islands to the international market place: A review of a remarkable and versatile molecule. *Molecules*. 2012;17(6):6953–6981. doi:10.3390/molecules17066953.

5. Vicidomini C, Roviello V, Roviello GN. Molecular Basis of the Therapeutical Potential of Clove (Syzygium aromaticum L.) and Clues to Its Anti-COVID-19 Utility. *Molecules*. 2021;26(7):1880. doi:10.3390/molecules26071880.

6. Han X, Parker TL. Anti-inflammatory activity of clove (Eugenia caryophyllata) essential oil in human dermal fibroblasts. *Pharm Biol*. 2017;55(1):1619–1622. doi:10.1080/13880209.2017.1314513.

7. Reichling J, Schnitzler P, Suschke U, Saller R. Essential oils of aromatic plants with antibacterial, antifungal, antiviral, and cytotoxic properties — An overview. *Forsch Komplementarmed*. 2009;16(2):79–90. doi:10.1159/000207196.

8. Fu YJ, Zu YG, Chen LY, Shi XG, Wang Z, Sun S, Efferth T. Antimicrobial activity of clove and rosemary essential oils alone and in combination. *Phytotherapy Research*. 2007;21(10):989–994. doi:10.1002/ptr.2179.

9. Santoro GF, Cardoso MG, Guimarães LGL, Mendonça LZ, Soares MJ. Trypanosoma cruzi: Activity of essential oils from Achillea millefolium L., Syzygium aromaticum L. and Ocimum basilicum L. on epimastigotes and trypomastigotes. *Exp Parasitol*. 2007;116(3):283–290. doi:10.1016/j.exppara.2007.01.018.

10. Machado M, Dinis AM, Salgueiro L, Custódio JBA, Cavaleiro C, Sousa MC. Anti-Giardia activity of Syzygium aromaticum essential oil and eugenol: Effects on growth, viability, adherence and ultrastructure. *Exp Parasitol*. 2011;127(4):732–739. doi:10.1016/j.exppara.2011.01.011.

11. El-kady AM, Ahmad AA, Hassan TM, El-Deek HEM, Fouad SS, Althagfan SS. Eugenol, a potential schistosomicidal agent with anti-inflammatory and antifibrotic effects against Schistosoma mansoni, induced liver pathology. *Infect Drug Resist*. 2019;12:709–719. doi:10.2147/IDR.S196544.

12. Kumar K, Yadav A, Srivastava S, Paswan S. Recent Trends in Indian Traditional Herbs Syzygium Aromaticum and its Health Benefits. *J Pharmacogn Phytochem*. 2012;1:13–22.

13. Fazly Bazzaz BS, Darvishi Fork S, Ahmadi R, Khameneh B. Deep insights into urinary tract infections and effective natural remedies. *African Journal of Urology*. 2021;27(1):6. doi:10.1186/s12301-020-00111-z.

14. Abushaheen MA, Muzaheed, Fatani AJ, Alosaimi M, Mansy W, George M, Acharya S, Rathod S, Divakar DD, Jhugroo C, Vellappally S, Khan AA, Shaik J, Jhugroo P. Antimicrobial resistance, mechanisms and its clinical significance. *Disease-a-Month*. 2020;66(6):100971. doi:10.1016/j.disamonth.2020.100971.

15. Rai MK, Deshmukh SD, Ingle AP, Gade AK. Silver nanoparticles: The powerful nanoweapon against multidrug-resistant bacteria. *J Appl Microbiol*. 2012;112(5):841–852. doi:10.1111/j.1365-2672.2012.05253.x.

16. Anand U, Jacobo-Herrera N, Altemimi A, Lakhssassi N. A comprehensive review on medicinal plants as antimicrobial therapeutics: Potential avenues of biocompatible drug discovery. *Metabolites*. 2019;9(11):258. doi:10.3390/metabo9110258.

17. Najumudin K, Ayubu J, M. Elnazeer A. Antigiardial Activity of Some Plant Extracts Used in Traditional Medicine in Sudan in Comparison with Metronidazole. *Microbiology: Current Research*. 2018;02(04):75–82. doi:10.35841/2591-8036.18-1025.

18. Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity: A review. *J Pharm Anal*. 2016;6(2):71–79. doi:10.1016/j.jpha.2015.11.005.

19. 19. Arsène MMJ, Podoprigora I V., Davares AKL, Razan M, Das MS, Senyagin AN. Antibacterial activity of grapefruit peel extracts and green-synthesized silver nanoparticles. *Vet World*. 2021;14(5):1330–1341. doi:10.14202/vetworld.2021.1330-1341.

20. Mouafo HT, Tchuenchieu ADK, Nguedjo MW, Edoun FLE, Tchuente BRT, Medoua GN. In vitro antimicrobial activity of Millettia laurentii De Wild and Lophira alata Banks ex C.F. Gaertn on selected foodborne pathogens associated to gastroenteritis. *Heliyon*. 2021;7(4):e06830. doi:10.1016/j.heliyon.2021.e06830

21. Bellio P, Fagnani L, Nazzicone L, Celenza G. New and simplified method for drug combination studies by checkerboard assay. *Methods* X. 2021;8:101543. doi:10.1016/J.MEX.2021.101543.

22. Celenza G, Segatore B, Setacci D, Bellio P, Brisdelli F, Piovano M, Garbarino JA, Nicoletti M, Perilli M, Amicosante G. In vitro antimicrobial activity of pannarin alone and in combination with antibiotics against methicillin-resistant Staphylococcus aureus clinical isolates. *Phytomedicine*. 2012;19(7):596–602. doi:10.1016/J.PHYMED.2012.02.010.

23. Gonelimali FD, Lin J, Miao W, Xuan J, Charles F, Chen M, Hatab SR. Antimicrobial Properties and Mechanism of Action of Some Plant Extracts Against Food Pathogens and Spoilage Microorganisms. *Front Microbiol*. 2018;9:1639. doi:10.3389/fmicb.2018.01639.

24. Oulkheir S, Aghrouch M, El Mourabit F, Dalha F, Graich H, Amouch F, Ouzaid K, Moukale A, Chadli S, Chadli Antibacterial S. Antibacterial Activity of Essential Oils Extracts from Cinnamon, Thyme, Clove and Geranium Against a Gram Negative and Gram Positive Pathogenic Bacteria. *Journal of Diseases and Medicinal Plants Special Issue: New Vistas of Research in Ayurveda System of Medicine*. 2017;3(1):1–5. doi:10.11648/j.jdmp.s.2017030201.11.

25. Revati S, Bipin C, Chitra PB, Minakshi B. In vitro antibacterial activity of seven Indian spices against high level gentamicin resistant strains of enterococci. *Archives of Medical Science*. 2015;11(4):863–868. doi:10.5114/aoms.2015.53307

26. Rosarior VL, Lim PS, Wong WK, Yue CS, Yam HC, Tan SA. Antioxidant-rich Clove Extract, A Strong Antimicrobial Agent against Urinary Tract Infections-causing Bacteria in vitro. *Trop Life Sci Res*. 2021;32(2):45–63. doi:10.21315/tlsr2021.32.2.4.

27. SM M, RO D, OL F. Phenolic Compounds in Antimicrobial Therapy. *J Med Food*. 2017;20(10):1031–1038. doi:10.1089/JMF.2017.0017.

28. Mak KK, Kamal M, Ayuba S, Sakirolla R, Kang YB, Mohandas K, Balijepalli M, Ahmad S, Pichika M. A comprehensive review on eugenol's antimicrobial properties and industry applications: A transformation from ethnomedicine to industry. *Pharmacogn Rev*. 2019;13(25):1. doi:10.4103/phrev.phrev_46_18.

29. Bouarab-Chibane L, Forquet V, Lantéri P, Clément Y, Léonard-Akkari L, Oulahal N, Degraeve P, Bordes C. Antibacterial Properties of Polyphenols: Characterization and QSAR (Quantitative Structure–Activity Relationship) Models. *Front Microbiol*. 2019;10(APR):829. doi:10.3389/FMICB.2019.00829

30. 30. Kothari V, Gupta A, Naraniwal M. Comparative study of various methods for extraction of antioxidant and antibacterial compounds from plant seeds. *Journal of Natural Remedies*. 2012;12/2:162–173. doi:10.18311/jnr/2012/271.

31. Pham H, Nguyen V, Vuong Q, Bowyer M, Scarlett C. Effect of Extraction Solvents and Drying Methods on the Physicochemical and Antioxidant Properties of Helicteres hirsuta Lour. Leaves. *Technologies (Basel)*. 2015;3(4):285–301. doi:10.3390/technologies3040285.

32. Szakiel A, Ruszkowski D, Grudniak A, Kurek A, Wolska KI, Doligalska M, Janiszowska W. Antibacterial and antiparasitic activity of oleanolic acid and its glycosides isolated from marigold

(Calendula officinalis). *Planta Med*. 2008;74(14):1709–1715. doi:10.1055/s-0028-1088315.

33. Kurek A, Grudniak AM, Szwed M, Klicka A, Samluk L, Wolska KI, Janiszowska W, Popowska M. Oleanolic acid and ursolic acid affect peptidoglycan metabolism in Listeria monocytogenes. *Antonie van Leeuwenhoek, International Journal of General and Molecular Microbiology*. 2010;97(1):61–68. doi:10.1007/s10482-009-9388-6.

34. Dorota W, Marta K, Dorota TG. Effect of asiatic and ursolic acids on morphology, hydrophobicity, and adhesion of UPECs to uroepithelial cells. *Folia Microbiol (Praha)*. 2013;58(3):245–252. doi:10.1007/s12223-012-0205-7.

35. Dörr T, Moynihan PJ, Mayer C. Bacterial Cell Wall Structure and Dynamics. *Front Microbiol*. 2019;10:2051. doi:10.3389/fmicb.2019.02051.

36. Sykes JE. Cell wall-deficient bacterial infections. In: *Canine and Feline Infectious Diseases*. W.B. Saunders; 2013:380–381. doi:10.1016/B978-1-4377-0795-3.00039-9.

37. Fabijan A, Kamruzzaman M, Martinez-Martin D, Venturini C, Mickiewicz K, Flores-Rodriguez N, Errington J, Iredell JR. L-form switching confers antibiotic, phage and stress tolerance in pathogenic Escherichia coli. *bioRxiv*. 2021;(10):2021.06.21.449206. doi:10.1101/2021.06.21.449206.

38. Kawai Y, Mickiewicz K, Errington J. Lysozyme Counteracts β-Lactam Antibiotics by Promoting the Emergence of L-Form Bacteria. *Cell*. 2018;172(5):1038–1049.e10. doi:10.1016/J.CELL.2018.01.021.

39. Glover WA, Yang Y, Zhang Y. Insights into the Molecular Basis of L-Form Formation and Survival in Escherichia coli. *PLoS One*. 2009;4(10):7316. doi:10.1371/JOURNAL.PONE.0007316.

40. Chikada T, Kanai T, Hayashi M, Kasai T, Oshima T, Shiomi D. Direct Observation of Conversion From Walled Cells to Wall-Deficient L-Form and Vice Versa in Escherichia coli Indicates the Essentiality of the Outer Membrane for Proliferation of L-Form Cells. *Front Microbiol*. 2021;12:537. doi:10.3389/FMICB.2021.645965/BIBTEX.

41. Sibanda T, Okoh A. The challenges of overcoming antibiotic resistance: Plant extracts as potential sources of antimicrobial and resistance modifying agents. *Afr J Biotechnol*. 2010;6(25):2886–2896. doi:10.4314/ajb.v6i25.58241.

42. Atteia HG, Hussein E. In vitro antibacterial and synergistic effects of some plant extracts against Staphylococcus aureus and Klebsiella pneumoniae. *Journal of Antimicrobials*. 2014;129:338–346.

43. Chu CM, Lowder JL. Diagnosis and treatment of urinary tract infections across age groups. *Am J Obstet Gynecol*. 2018;219(1):40–51. doi:10.1016/J.AJOG.2017.12.231.

44. Uppala A, King EA, Patel D. Cefazolin versus fluoroquinolones for the treatment of community-acquired urinary tract infections in hospitalized patients. *European Journal of Clinical Microbiology and Infectious Diseases*. 2019;38(8):1533–1538. doi:10.1007/S10096-019-03582-3.

45. Faleiro ML, Miguel MG. Use of Essential Oils and Their Components against Multidrug-Resistant Bacteria. *Fighting Multidrug Resistance with Herbal Extracts, Essential Oils and their Components*. 2013:65–94. doi:10.1016/B978-0-12-398539-2.00006-9.

46. Scorzoni L, Sangalli-Leite F, de Lacorte Singulani J, de Paula e Silva ACA, Costa-Orlandi CB, Fusco-Almeida AM, Mendes-Giannini MJS. Searching new antifungals: The use of in vitro and in vivo methods for evaluation of natural compounds. *J Microbiol Methods*. 2016;123:68–78. doi:10.1016/J.MIMET.2016.02.005.

47. Segatore B, Bellio P, Setacci D, Brisdelli F, Piovano M, Garbarino JA, Nicoletti M, Amicosante G, Perilli M, Celenza G. In vitro interaction of usnic acid in combination with antimicrobial agents against methicillin-resistant Staphylococcus aureus clinical isolates determined by FICI and ΔE model methods. *Phytomedicine*. 2012;19(3–4):341–347. doi:10.1016/J.PHYMED.2011.10.012.

48. Xu X, Xu L, Yuan G, Wang Y, Qu Y, Zhou M. Synergistic combination of two antimicrobial agents closing each other's mutant selection windows to prevent antimicrobial resistance. *Sci Rep*. 2018;8(1):7237. doi:10.1038/s41598-018-25714-z.

Антибактериальная активность экстракта гвоздики *Syzygium aromaticum* L. и синергизм его действия с антибиотиками по отношению к уропатогенным кишечным палочкам с множественной лекарственной устойчивостью

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Аннотация. *Актуальность.* Инфекции мочевыводящих путей представляют растущую угрозу человечеству из-за роста устойчивости уропатогенов к антибиотикам. Изучение природных источников для альтернативных методов лечения стало важным подходом. *Цель* исследования заключалась в изучении антибактериального действия гвоздики (*Syzygium aromaticum* L.) по отношению к уропатогенным *E. coli*. *Материалы и методы.* Исследование проведено на трех клинических изолятах уропатогенных *E. coli* с множественной лекарственной устойчивостью и *E. coli* ATCC 25922. Водно-спиртовой экстракт гвоздики получен методом холодной мацерации. Для оценки антибактериального действия экстракта применяли диффузионный метод в агаровых лунках. Минимальную ингибирующую и минимальную бакте-

рицидную концентрации экстракта определяли методом микроразбавления бульона. С помощью световой микроскопии исследовали морфологические изменения уропатогенных *E. coli* после воздействия экстракта гвоздики. Для оценки синергизма между экстрактом гвоздики и антибиотиками использовали метод шахматной доски. Все полученные данные были статистически обработаны. *Результаты и обсуждение.* При использовании диффузионного метода в агаровых лунках действие экстракта на бактерии зависело от его концентрации при диаметре зоны задержки роста 7–10/10–15 мм для уропатогенных штаммов и *E. coli* ATCC 25922, соответственно. Минимальную ингибирующую и минимальную бактерицидную концентрации экстракта гвоздики по отношению к уропатогенным штаммам составляла 25 мг/мл. Экстракт показал более низкую минимальную ингибирующую концентрацию на *E. coli* ATCC 25922 (6.25 мг/мл) при минимальной бактерицидной концентрации, составляющей 25 мг/мл. Соотношение минимальной ингибирующей и минимальной бактерицидной концентраций показало, что экстракт гвоздики обладает бактерицидным действием. Тест на синергизм показал, что комбинация экстракта гвоздики и нитрофурантоина или ципрофлоксацина не приводила к взаимодействию. Однако минимальные ингибирующие концентрации всех протестированных агентов в комбинациях снижались в разной степени. Инкубирование уропатогенных штаммов с экстрактом трансформировало их в нестабильную сферическую L-форму в процентном соотношении 96–99 %. *Выводы.* Это исследование подчеркивает гвоздику как потенциальное природное антибактериальное средство по отношению к уропатогенным *E. coli* с множественной лекарственной устойчивостью, что требует дальнейшего изучения ее антибактериальных свойств.

Ключевые слова: гвоздика, Syzygium aromaticum, инфекции мочевыводящих путей, уропатогенные кишечные палочки, фитохимические вещества, антибактериальное средство, устойчивость к антибиотикам

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