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Synergistic Impact of Basil Extract (Ocimum basilicum) and Levofloxacin on the Proliferation and Biofilm Development of Escherichia coli Isolated from Clinical Specimens

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Abstract

This work intended to investigate on the effectiveness of total flavonoids as well as crude basil extract on the biofilm formation inhibition and also to determine the interaction of these compounds with Levofloxacin (Lfx) against biofilms developed by *E.coli* isolates Total flavonoids extractions was carried out using 75% methanol while crude basil extract using 100% methanol. One hundred nineteen clinical stool and urine Specimens collected from patients attending medical city hospital, Baghdad, Iraq. Out of 76 isolates, that hit in the Vitik 2 assay as *E.coli*, only 46 of these isolates formed biofilm when cultured in microtitre plates. Molecular detection was performed to determine whether the bacterial isolates harbour genes that predict the formation of biofilms. The bacterial strains were selected based on their ability to form biofilm and sundry other factors including, antibiotic resistance. The objectives showed that 21 (70%) of the isolates contain the fimH gene, while 17 (56.6%) out of the isolates had the pap gene for E.coli. The research showed that the crude extract had a higher antibiofilm effect than flavonoids with a concentration of 512 µg/mL. The results which were obtained showed that co-administration of Levofloxacin (8 µg/ml) and the crude extract (128µg/ml) offered considerable inhibition of biofilm formation of E. coli with an inhibition percentage of 88%. The flavonoids tested against E. coli in combination with Levofloxacin had inhibition concentrations of 128 µg/ml while Levofloxacin was 8 µg/ml giving inhibition ratio of 81.1%. This proved to exhibit additional synergistic action against bacterial biofilm formation at sub-to-micromolar concentrations. These consequences imply that in future this medication can be used to treat E. coli biofilm infection and act as antibacterial drugs. The findings of current study clarified the Synergistic Impact of Basil Extract and Levofloxacin on the Proliferation

and Biofilm Development of *E. coli* .And prove that it restricted this development.

Keywords: E. coli, Bacterial infection, Flavonoids, Plant extract, Antibiotics, Levofloxacin.

Introduction

E.coli leading bacterial class of urinal infection, the navel moving from urinal system to bladder, cause irritation and inflammation of kidneys, bladder, urethra, and ureters,; renal inflammations is more serious than cystitis. These infections known as the second most common bacterial diseases among healthcare workers (1,2). According to WHO, 150 million sexually active women suffering from urinary tract infections which E.Coli causes most f them globally each year (3,4). This structure is present in about 60% of human diseases and is considered to be quite resistant to both biocides and antibiotic. Recent studies revealed marked increases in resistance of biofilm cell to mortality compared with planktonic cells *in vitro* (5).The following benefits related to bacteria behavior can be described: the biofilms adapt for the bacteria, facilitate efficient consumption of nutrients and hinder the penetration of white blood cells, antibodies, and antimicrobial agents (6). They contain many enzymes to inactivate drugs, for instance beta-lactamases which neutralise antibiotics. This has positioned them as a central point where antimicrobial resistance (AMR) revolves around (7). Antigen 43, type 1 fimbriae (fim-H) and other genes such as cytotoxic, hemolysin aerobactin nd necrotizing factor are associated with uropathogenic E. coli (UPEC) strains. By all the above reasoning, therefore, it is possible to arrive at (8).Many of these characteristics assist the organism in residing within, evading, or overcoming the host's defense mechanisms, damaging or penetrating host cells, and provoking inflammation that results in clinical illness (9). One of the factors that indicate pathogen’s fitness is biofilm formation that acts like a virulence factor. Despite the fact that the bacteria within a biofilm are microorganisms, they are much more resistant than the bacteria that are loose in the host body and the drugs that are used to treat the infection. The concept of combining plant extracts has also shown the potential to stop bacterial biofilm formation, movement, attachment and signaling (10). Those plant extracts could also diminish the synthesis of the main virulence factors and thus prevent bacterial toxins synthesis (11). Several works has demonstrated that adding plant extracts to antibiotics increases the effectiveness of these agents while decreasing the necessary amounts, and subsequently – side effects. Interactions of outline positivity are

being researched in the prioritization of bacterial resistance technique. The next sections will reveal the interactive effects of plant extracts and antibiotics (11).

Combination therapy is a strategy utilized to augment the efficacy of antimicrobial treatment for difficult infections, particularly those linked to biofilms, owing to the limited availability of novel antimicrobial agents with unique mechanisms and the rise in infections caused by resistant pathogens (12).

This technique highlights the use of natural enhancers to boost the antimicrobial agent's antibiofilm effectiveness at concentrations where the antibiotic previously exhibited no antibiofilm activity. Consequently, researchers have concentrated on the interactions between phytocompounds and antibiotics or chemotherapeutic antimicrobial therapies. Numerous studies suggest that these phytocompounds exhibit beneficial synergistic effects when combined with these medications, offering advantages such as enhanced efficacy, improved safety and tolerability, reduced toxicity, and decreased resistance to antimicrobial agents (13).

Materials and methods

Isolation of bacterial specimens

110 isolates were obtained from urine and stool swab specimens of individuals suffering from urinary tract and digestive system infections who had attended the different hospitals in Baghdad during this study from July 21 to September 15, 2024 at Al-Kindy Hospital, Baghdad Educational hospital , and Al-Kadhmiya Educational Hospital. All specimens were cultured on Eosin Methyl Blue (EMB) agar and MacConkey agar. then all plates were incubated at 37°C for 24h .

Methodology on preparations of crude (*Ocimum basilicum extract*)

Preparations of crude (basil extracts) Fresh basil leaves purchased from the Baghdad University gardens, Iraq, during April-June 2024 as referenced by (14). Basil methanolic extract was prepared.A combinations of 250 g of basil plant leaf powder were extracted with 1.5 L of 80% methanol at 65° C for seventy two hours using soxhlet apparatus.The extract solution was freeze dried under vacuum and themethanolic extract was frozen at negative twenty degree Celsius until preparation of the desired concentrations.

Flavonoids of plants, their classification and separations

Mthanolic extract of basil done (14). Soxhlet made it possible to extract 60 grams of plant leaf powder using 600 ml of 100 percent methanol in 72 hours at 60 °C. known, the extract solution was concentrated by rotary

evaporator to the required status for freezing at -20 °C for the preparation of the right concentrations.

High-performance liquid chromatography (HPLC)

Due to flavonoids is effective component, the content of flavonids were determined under various conditions by using (HPLC). The HPLC (Shimadzu 10 AV-LC) chromatogram of flavonoids which was used for identification of and quantification of flavonoids in the samples is presented here. A C18-DB end-capped 3 µm packing material in a 25 mm x 1.0 mm I.D. separation column.The detector's wavelength was determined to be 285 nm. The mobile phase gradient commenced with 0.06% trifluoroacetic acid in deionized water, whereas solvent B consisted of 0.06% trifluoroacetic acid in methanol at a pH of 2.8, with a flow rate of 1.1 mL/min. The flavonoid extracted was mixed with methanol in order to get the test concentration of 50g/L.

Table (1): Making stock solutions of plant active compounds

Plant active compound	Dose	solvent Amount	Primary concentrations
Crude	250 mg	5 ml of D.W	25 mg .ml^{-1} = 25000 µg.ml^{-1}
Flavonoid	250 mg	5 ml of D.W	25 mg .ml^{-1} = 25000 µg.ml^{-1}

Antibiotics compositions

Preparation of stock. solutions (15). The antibiotic stock solution prepared as described in the Table 2.

Table 2: Preparations of Antibiotic Stock. Solution

Antibiotics	Dose	solvent Quantity	Primary Concentration
Levofloxacin	250 mg	5 ml of D.W	25 mg. ml $^{-1}$= 25000 µg .ml^{-1}

Assess bacterial capacity to develop biofilm and slime layers.

The capacity of E. coli to generate slime layers and establish biofilms was assessed through two methodologies: The microbiological tools representative of the current study are Congo red agar and microtiter plates (MTP). Studies were done to determine slime layer production by 50 isolates of *E. coli*.

Congo red agar [CRA]

selectively used in the isolation of specific species of bacteria and fungi. The medium was prepared(NACL (15 g/L), Brain Heart agar (37 g/L), Red Congo (0.8g/L) and Sucrose (38g/L)).

All mediums were dissolved and mixed in a total of 1L of distilled water ,then autoclaved each aliquot . The Congo red solution prepared by dissolving Congo red stain in 100 ml of distilled water and was separately sterilized by autoclaving at 121°C and at a pressure of 1.5 pounds for 15 minutes after which, it was added to the other parts of the agar and mixed well when the agar had reached 55°C then pour the mixture in to sterile Petri dishes.

Assessment of biofilm formation capability

Measurement of biofilms was done using modifications to the Crystal Violet (CV) test done in a semi-quantitative manner. The Crystal Violet assay depend on the ability of dye to penetrate the biofilm, bind to negatively charged extracellular structures, including cell surface appendages and the polysaccharan matrix of mature biofilms. This gives information about the total counts of cells which are attached to the biofilm. In column 1-11 a total of 180µL of tryptic soy broth and 20µL bacterial suspension was added. In the column 12 basic TSB broth was added and it was considered as a negative control. Place the microtiter plate in incubator at 37°C for a period of 24 hours. After incubation the media were tipped off onto wells and sterile micro test tubes washed by sterile distilled water or phosphate buffered saline (PBS) to get rid of any contaminations . Attached cells were stained with 150 µl of (CV) solution for the period of 15 minutes. Two percent The residual dye was washed off using PBS, the microtiter plates were then aerated at 40°C for 20 minutes. The biofilm was afterwards quantified by the addition of 150 µl of 75% acetic acid to each well and then read by ELISA reader at 595 nm OD. The experiment was repeated for three times and the mean of (OD) value was used (16).

Molecular Test

This part means in a technique employed in a laboratory to identify biomarkers in the genomic and transcribed sequences. It is used in the diagnosis of diseases, tracking health states, and management of care plans. By using a commercial extraction kit (PrestoTM Mini gDNA Bacteria Kit Quick Protocol, Geneaid) DNA extracted from E. coli isolate , adhering to the manufacturer guidelines for DNA purification from both gram-positive and gram-negative bacteria as detailed below:

primers selecting in the study

In current study, the reverse and forward primer sequence for the pap and fimH genes in E. coli were chosen, and the PCR products obtained are shown in Table 3 below.

Table 3: Primer Sequences Utilized in Escherichia coli

Name of primers	Primer sequenc	Size products
Pap–F	GCAACAGCAACGCTGGTTGCATCAT	336
Pap–R	AGAGAGAGCCACTCTTATACGGAC A	336
fimH–F	GAGAAGAGGTTTGATTTAACTTATT G	560
fimH–R	AGAGCCGCTGTAGAACTGAGG	560

PCR amplification

Polymerase chain reaction is a technique of copying selected segments of DNA of interest in order to enable analysis of the DNA.

The PCR tubes placed in thermo-cycler and DNA was amplified according to conditions mentioned in table .4 for the multiplex PCR of each gene. Through gradient PCR, the appropriate temperature and the duration of the PCR program were determined.

Table (4) conditions of gene multiplex PCR amplification.

Genes	Steps	cycle number	Temperatures	Time
FimH	Initial denaturations	1	94 ºC	4 min
	Denaturations	35	94 ºC	30 seconds
	Annealings		58 ºC	1 min
	Extensions		72ºC	1 min
	Final extensions	1	72ºC	5 min
Pap	Initial denaturations	1	94 ºC	5 min
	Denaturations	35	94 ºC	30 seconds
	Annealings		60ºC	1 min
	Extensions		72ºC	1 min
	Final extensions	1	72ºC	5 min

Screening of antibiotics for its ability to prevent biofilm formation

The assay was done as described in(17). The antibiotics employed in this research was Levofloxacin. Three to five colonies are then inoculated into a tube with 5 ml of normal saline (NaCl 0.85%) before dispensing 100 µl of MHB to the microtiter plate wells. Five ml of bacterial suspension is then appropriately diluted and a 20µl volume of the diluted bacterial suspension is added to each well. After that, 100µl of the Levofloxacin antibiotic was given. The negative control wells consist of broth, which is 200 µl of MHB, and were incubated at 37 degree Celsius for 24 hours. Thereafter, the wells were washed with 300ml of phosphat buffer twice. Stain was rinsed with phosphate buffered saline or distilled water then resuspended in each wells with 150 µl of 75% acetic acid. Adherent bacteria densities were determined by Micro ELISA reader at 595 nm wavelength in triplicate from three independent experiments (15).

Methodology for determining biofilm inhibition through the use of active plant extract

This method was performed based on (18), a change in the media used was employed. E. coli from fresh agars was grown in 100 well U bottom tissue culture plates with 100 µl of MHB and 25 µl of bacterial suspension. After that, inoculate an additional 100 µl of plant extract for the process of dilution. The negative control wells comprised only of broth (250 µl of MHB) and the all negative control wells were incubated for 24 hours. After incubation, the wells were washed two times with 300 ml phosphate buffered saline. After that the stain was washed with distilled water or PBS and resuspended in each well with 100µl of 75% acetic acid. The amount of stained adherent bacteria was determined using a micro-ELISA reader at a test wavelength of 595 nm (18).

Assessing interaction between plant extract and antimicrobial agents by the checkerboard microtiter technique.

This technique was used to determine the synergistic effect between Levofloxacin and crude extracts as well as between flavonoid compounds and Levofloxacin against E. coli (19). The first step carried out involves the addition of 100µl of sterile Muller Hinton broth to every well of the agar plates. , followed by four sets of plant extracts at the next lower concentrations of 64, 32, and 16 µg and four antibiotic concentrations of 1, 2, 4, and 8 µg. The procedure involves multiplication of the antibiotics with the concentration that was applied to the 1st plant, second antibiotic with the concentration applied to the 2nd plant, third antibiotic with the concentration

applied to the 3rd plant as well as the third antibiotic with the concentration applied to the fourth plant. Column 11 consisted only of bacterial inoculum and broth, which acted as a positive control, while in column 12 only media was used and it was the negative control. In order to establish the baseline, a 2 mL bacterial inoculum containing 1 x 10^6 cfu/ml was added and mixed in all wells except for column 12. All plates were incubated in an aerobic incubator at 37 °C for one night. After incubation, the relative growth in the wells of the plates was determined by scanning the plates at 595 nm using ELISA reader(18). The combined effects of active plant compounds with antimicrobial agents were determined from such computations; FIC = MIC of the antibiotic either in conjunction with or on its own. The FIC of an active plant compound is responded by the MBC of the active plant extract in combination or the MBC of the plant extract. FICI is obtained from the addition of the FIC of an antibiotic and the FIC of plant extract. If FICI > 4 means antagonism, if FICI ≤0.5 means synergy, if FICI 1–4 means indifference. And if FICI > 0.5–1 means additive.

Statistical Analysis

In the present study, the software used to analyze the collected data Statistically by (SPSS) version 23. The Chi-square ($\chi 2$) test is used in testing for independence and goodness of fit. Statistically significant P-value is equal to or less than 0.01 while highly statistically significance is equal to or less than 0.01.

Results

Detection of *E.coli*

Typing and phenotyping of E. coli isolates were done based on morphological characters from culture media and biochemical test in addition to the VITEK 2 compact system.

Cultures of *E. coli*

From the morphological examination and application of culture media, 119 samples were confirmed. The collected samples were being cultured on MacConkey agar and Eosin Methyl Blue (EMB) agar for the purpose of morphological study. The results discovered revealed that 76 (63.8%) favoured the growth of gram-negative bacteria at the same time as suppressing the growth of gram-positive bacteria (1,7). Such as, as could be seen in figures 1-A and 1-B.

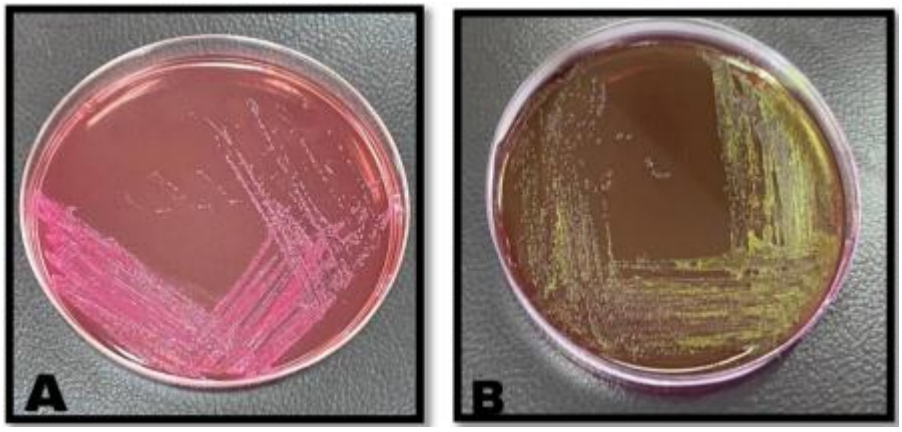

Figure 1: E. coli isolates on (A) MacConkey agar exhibiting lactose fermentation, resulting in pink colonies, and (B) EMB agar displaying a greenish metallic sheen.

Antibiotics susceptibility tests (ASTs)

This was done on 60 E. coli isolates using the disc diffusion method in compliance with the CLSI 2020 guidelines. The level of resistance shown in the results indicated in Table 5 is somewhat diverse. The result derived show a highly significant difference t-test at 0.01 degree of significance value.

Table 5: Results of Antibiotic Susceptibility Testing for E. coli Isolates

Antibiotics	Resistances		Intermediate		Sensitivities		P-value
	No.	%	No.	%	No.	%	
Ertapenem	9	15	3	5	48	80	0.0019
Nitrofurantoin	33	55	3	5	24	40	0.004
Trimethoprime Sulfanethaxole	44	73.3	4	6.7	12	20	0.0078
Norfloxacin	38	63.3	3	5	19	31.7	0.0053
PiperacillnTazobactam	16	26.4	2	3.3	42	70	0.0022
Gentamicin	30	50	4	6.7	26	43.3	0.0095
Levofloxacin	21	39	1	1.7	32	56.1	0.0097
Amoxicillin	23	38	3	5	34	50.7	0.0071
Ciprofloxacin	24	40	1	1.7	35	58.3	0.0064
Ceftriaxone	35	58.4	1	17	24	40	0.0031

Data is presented as Chi-square (χ 2) goodness of fit. ** Significant at $P \leq 0.01$.

Out of the 60 E. coli isolates, 11 (55%) produced resistance to Nitrofurantoin, its resistance to Norfloxacin was found in 13 (63.3%), and 14 (73.3%) were

resistant to trimethoprim-sulfamethoxazole. The rates of resistance of these isolates to different antibiotics are as follows: Ciprofloxacin 40% Piperacillin-tazobactam 26.7 % Gentamicin 50 % , Amoxicillin 38.3% , Levofloxacin 39% and Ceftriaxone 58.4% (It was identified that nine isolates (15%) exhibited reduced resistance to Ertapene to other antibiotics under study. The highest level of resistance observed in this study was 19 mm on Ceftriaxone and the lowest was nil on trimethoprim sulfamethoxazole.

Biofilm development generated by E. coli on Congo red agar

The ability of E. coli to form biofilms was determined by the Congo red agar method, where formation of biofilms was predicted from black colored colonies.

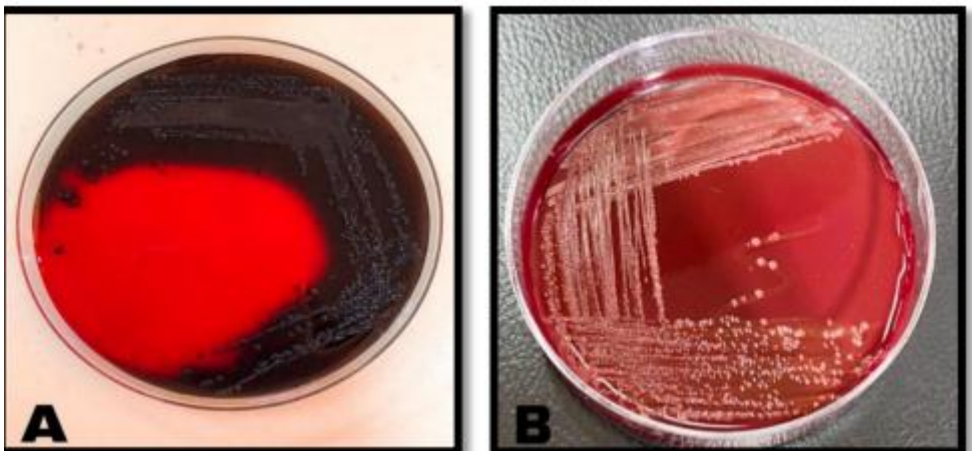

Figure 2: Biofilm formation on Congo red agar. Figure A illustrates black colonies indicative of biofilm formation, whereas Figure B presents red colonies that do not exhibit biofilm formation.

Microtiter plate

The microtiter plate method, utilized for assessing biofilm formation, yielded the following results for E. coli: These are as follows: weak = 15 (30%), moderate = 4 (8%), strong = 21 (42%) and non biofilm = 10 (20%).

Isolation and determination of the amount and purity of the DNA in *E.coli*

DNA was successfully isolated from 30 isolates. All thirty E. coli samples were successfully extracted using presto TM Mini gDNA Bacteria Kit Quick protocol Geneaid. E. coli DNA concentration obtained from the method of DNA isolation of microbial origin is ranged from 34.6 to 180 ng/µl DNA of

specimen and the purity of DNA is ranged from 1.75 to 2.02. In figure (3) it is shown that the extracted DNA from E. coli shows a single band of extracted DNA which confirmed that the technique used for DNA extraction by transilluminator was quite effective.

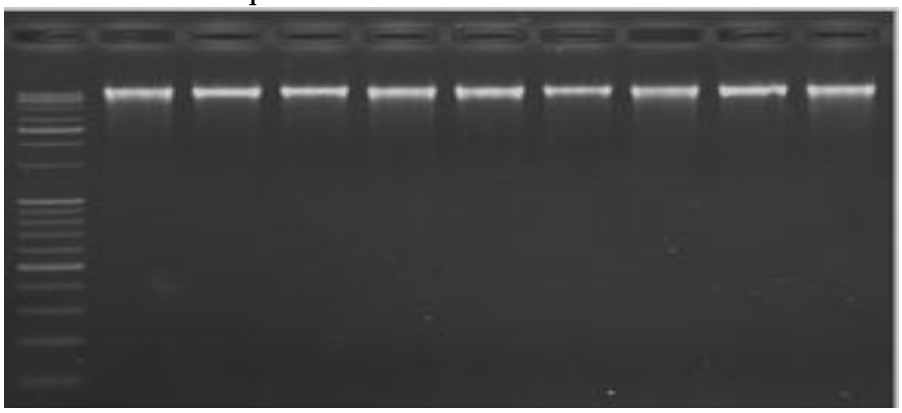

Figure (3): Gel electrophoresis of the whole genomic DNA of E. coli. Agarose gel at 1%, subjected to 70 V for 1 hour, imaged using a UV transilluminator.

Molecular characterization of biofilms in *Escherichia coli*

13 isolates of antibiotic-resistant Escherichia coli , which shows a strong, moderate and weak biofilms forming capabilities, were used to establish the presence of the biofilm associated genes; pap and fimH which are essential mediators of biofilm formation in Escherichia coli isolate. Through controlling the production of Curli (fimbriae) and further biofilms (6). Based on gel electrophoresis and PCR technology to cut DNA and Primers to ascertain the presence of fimH and pap genes in the study bacterial isolates. The result provided showed that 21 affiliated bacterial isolates of E.coli has the fimH gene out of which 17 has the pap gene as depicted in figures 4 & 5.

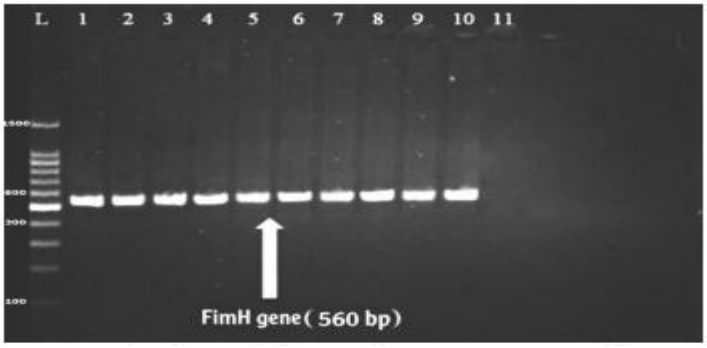

Figure 4: PCR analysis of E. coli fimH locus adapted and performed by conventional PCR to amplify 560 bp of the DNA fragment. To practice

the protocol, agarose gel at 1.5% concentration was prepared, place the gel under the electric field of 70 V/cm for 90 minutes, the gel was then stained with ethidium bromide then visualize under ultraviolet transilluminator It is observed the 100 base pairs. DNA laddering Lane (L). Lane 11 is the negative control lane.

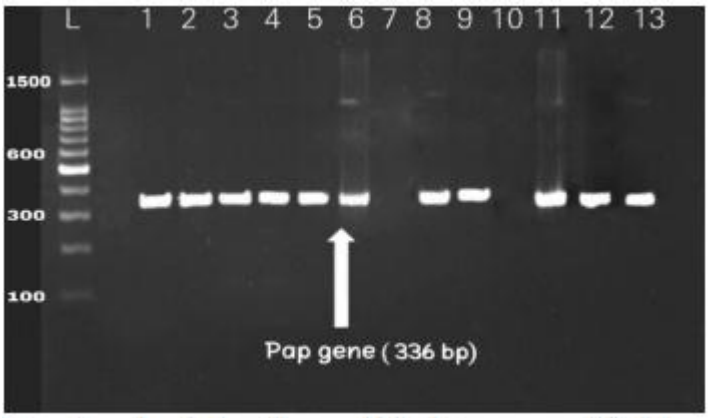

Figure 5: Conventional PCR product of E. coli fimH gene size is 560bp Gel electrophoresis analysis of E. coli fimH. A 1.5% agarose gel was made, run at 70 V/cm for 90 minutes and then stained with ethidium bromide and the bands visualized with an ultraviolet transilluminator and the photograph captured is a records 100 base pairs. DNA ladder is describing molecular weight that used in gel electrophoresis to measure DNA fragments. It contains a combination of fragments of DNAs of specific sizes from which sample sizes can be estimated based on the similarity. Lane (L). Lane 11 is the control sample.

Isolation of total flavonoids from the extract of basil plant (*Ocimum basilicum)*

Methanol was used in the extraction process in the Soxhlet system since it exhibited high extraction capacity and since its polarity is more favorable as indicated also for 80% methanol extraction. The polarity of water is considerable ; but it cannot dissolve and extract all plant materials. Hence, 80% of methanol comprises of methanol and water, thereby encouraging high extraction capability. Crude and total flavonoids extraction yields in basil consisted of 250g and 60 g, respectively with extract weight yield of 28 g and 12 g, respectively. Concentration of the crude extract and flavonoid extract was determined to have values of 11.2% and 20%. To estimate the ratio, the following equation was used: ER Which is the extraction ratio that was established according to reference (20). Utilizing the subsequent equation:

ER = (extract concentration (yield) (g)/fresh plant initial weight (g)) x 100.

Total flavonoids identification in basil using high performance liquid chromatography.

The isolated flavonoids from plans were characterized using high-performance liquid chromatography (HPLC) to compare with respective standards as described in refs (21, 22). In figure (6) characterization where the flavonoid separated basil sample is represented, five peaks were noted. The first, second, third fourth and fifth peaks of the sample were identified at 5.79, 18.02, 24.15, 30.08 and 35.58 minutes respectively. The observed fifth peaks in partially purified flavonoids support the adequate separation of the flavonoids. The peaks of lower intensities indicate that slight variations were accompanied by the synthesis of other additional compounds extracted from the main flavonoids. This observation is also a testimony to the efficiency of the extraction method used more so when tested against other methods of extracting basil (23).

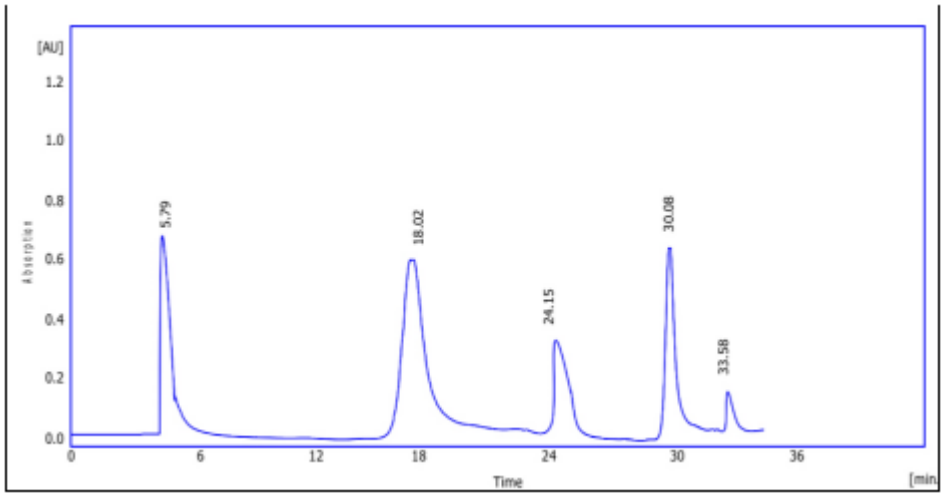

Figure 6: High-Performance Liquid Chromatography (HPLC) analysis of total flavonoid extract from basil.

Effect of antibiotics in biofilm development

Among the two methods used only the microtiter plate method that was employed to determine antibiotics' effect exclusively addresses biofilm development. The effect of the antibiotic tested alone on biofilm formation in E.coli is shown in figure 7. The percentage inhibition of biofilm formation at CLSI of Levofloxacin at a concentration 128 µg/mL was 83.3%.

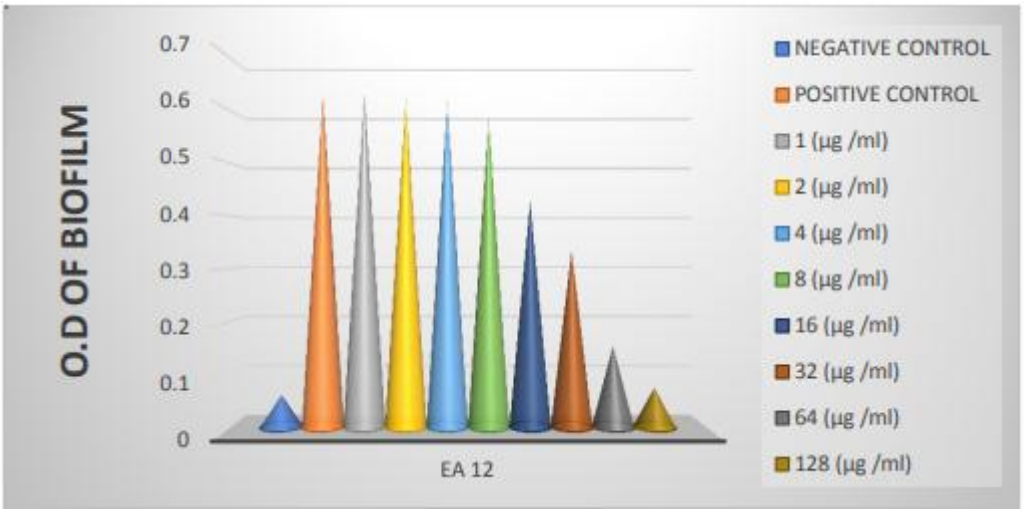

Figure 7: Levofloxacin's efficacy in inhibiting E. coli biofilm formation.

Effect of plant extracts on the mechanisms of microbial adhesion

Biofilm formation by E. coli in the presence of minimum inhibition concentration of crude extract and flavonoids at the microtiter plate assay using crystal violet staining was also determined in this study. The responses were quantified by the absorbance of stained biofilms at 590 nm using ELISA reader and subsequently expressed as % of control(18). According to the results presented herein, crude extract and flavonoid compounds show promise in the ability to inhibit the biofilm formation of Escherichia coli. Concentrated crude extract at 512 µg/mL of its solution showed that the inhibition of biofilm formation in E.coli was 90.6%. The ability of a flavonoid compound to reduce biofilm formation for Escherichia coli bacteria was 80.6% at a concentration of 512 µg/mL. The effect of plant extract alone on biofilm formation is illustrated in fig 8 and fig 9.

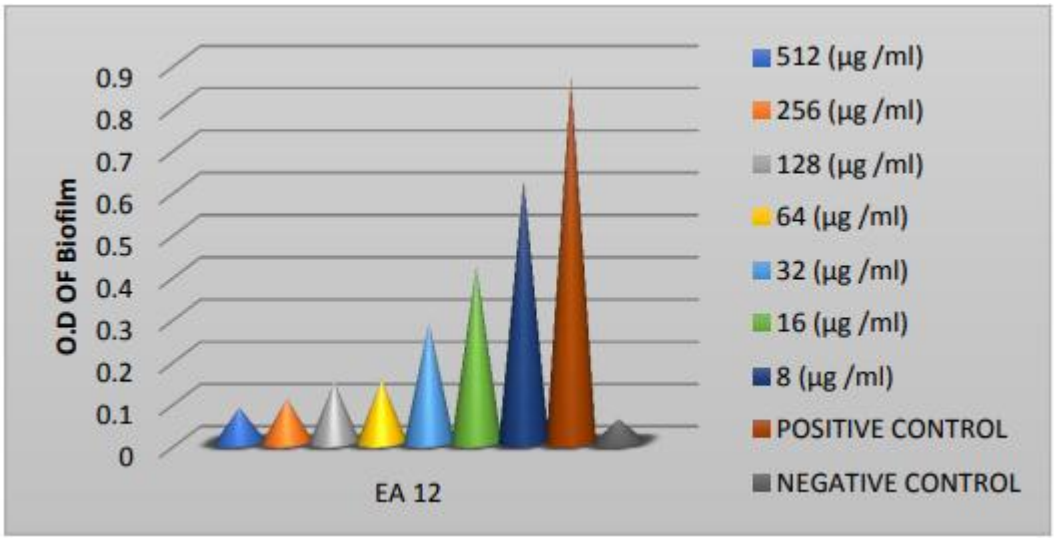

Figure 8: Crude extract's effect on E. coli biofilm formation

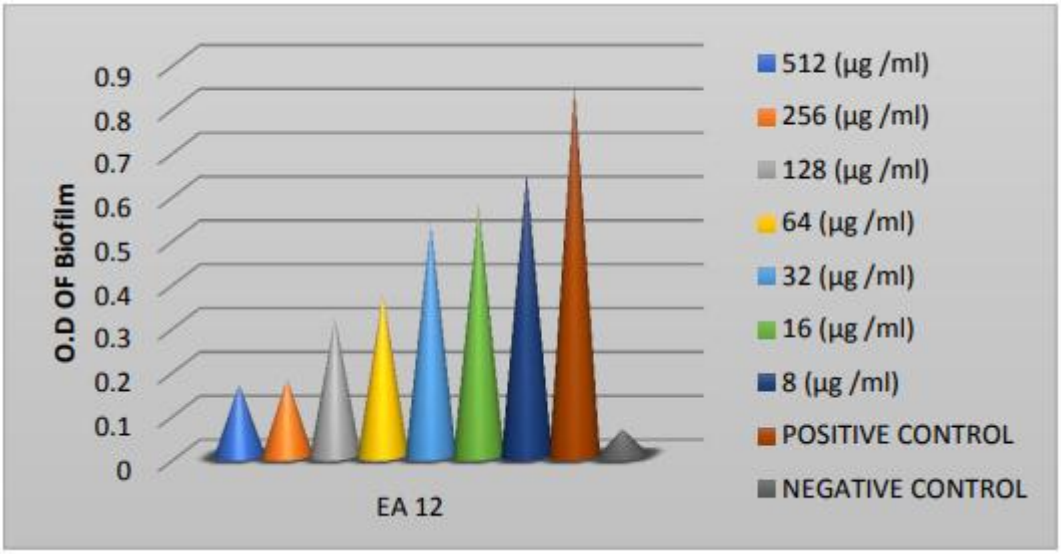

Figure 9: The effect of flavonoid compounds on E. coli biofilm formation.

Contribution by antibiotics and plant extracts on biofilms formulation The microtiter plate technique was used to determine the effects of plant extract compositions on biofilms during the treatment with antibiotics. Figures (10) and (11) show the findings related to the effect of the antibiotic Lfx in combination with crude extract and flavonoid compound in the case of E. coli.

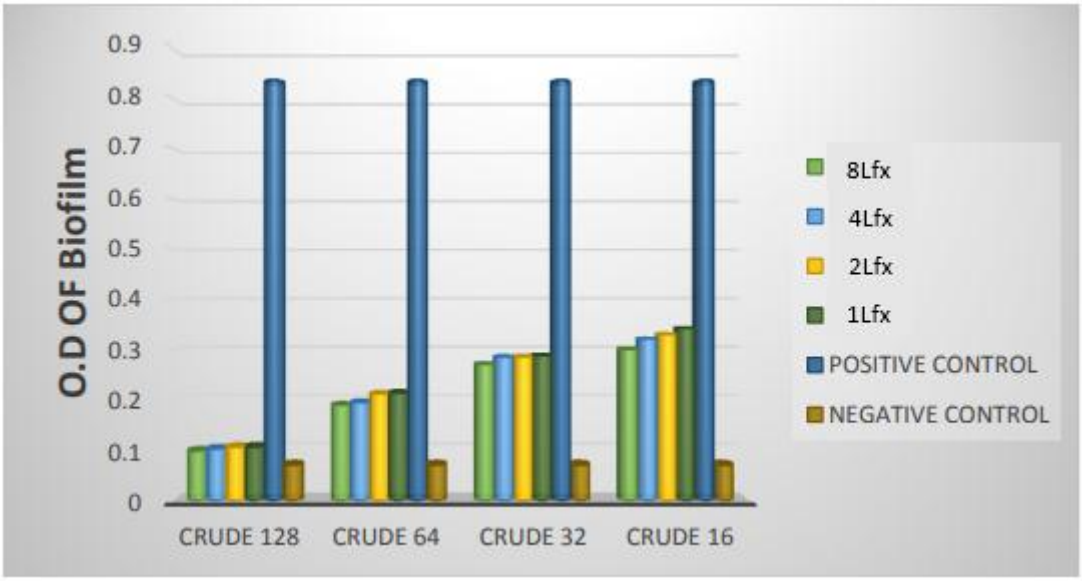

Figure 10: Effect of Levofloxacin combined with crude extract on biofilm formation in E. coli.

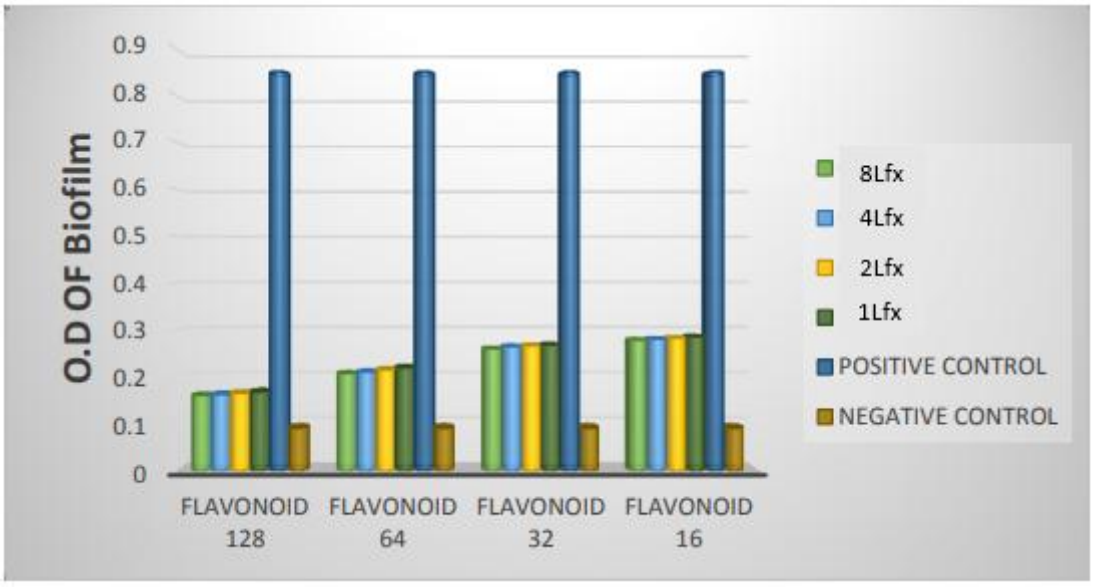

Figure 11: The effect of Levofloxacin in combination with a flavonoid compound on the biofilm formation of E. coli.

Discussions

The combination of plant extract and antibiotics gives a promise hope to prevent microbial infections and makes biofilm-associated infection a big challenge to clinicians because of the ability of biofilms to develop a multidrug resistance. Lfx is known to have an impact on biofilm breakdown as well as biofilm eradication, modification in outer membrane of bacteria and suppression of virulence factors (24). Lfx should block the interaction between PBPs and bacteria cell wall synthesis. The outer membrane of E.coli pass through porin to confirm selectivity in the drug transport with basic amino acids. The findings suggest combination therapy with Lfx and flavonoid and crude extract compounds in the stool and urine infection by E.coli, showing an obvious reduction in biofilm formation .

Shinde and colleagues (25) examined the impact of epigallocatechin gallate (EGCG), a flavonoid/catechin found in green tea. EGCG's chemical structure is characterized by many hydroxyl groups and a gallate moiety. EGCG is predominantly present in green tea leaves, constituting a substantial fraction of their flavonoid composition. EGCG demonstrates antioxidant, anti-inflammatory, anti-tumor, and antibacterial properties against several Gram-positive and Gram-negative bacteria (25). EGCG has antimicrobial actions through many methods, including cell membrane damage, enzyme inhibition, and disruption of fatty acid production. EGCG undermines cell wall integrity by disrupting the polysaccharides constituting the glycocalyx and binding peptidoglycan, hence influencing biofilm adhesion to surfaces. Recent results suggest that the synergistic interaction between EGCG and certain antibacterial agents may disrupt bacterial biofilm by degrading extracellular matrix components and enhancing the efficacy of antibiotics [26]. Shinde and coworkers demonstrated that a modified lipid-soluble EGCG, epigallocatechin-3-gallate-stearate (EGCG-S), in conjunction with tetracycline, may suppress 94% of E. coli ATCC 140 biofilm development [25]. Numerous investigations have shown that EGCG disrupts the assembly of phenol-soluble modulins (PSMs), inhibits Bap production, and compromises cell wall integrity, suggesting that this flavonoid may affect cell adherence to surfaces. The efficacy of EGCG as a synergistic agent with antibiotics may stem from its ability to disrupt extracellular matrix components, hence facilitating antibiotic penetration and enhancing their effectiveness against biofilms (25,26).

A further study indicated that Rutin, a flavonoid extracted from the peels of leafy plants with antibacterial capabilities, has been investigated for its antibiofilm efficacy against E. coli, in conjunction with the standard antibiotic gentamicin (27). The findings indicated that rutin demonstrated a minimum inhibitory concentration (MIC) of 800 µg/mL against E. coli. The treatment of the bacterium with rutin (200 µg/mL) and gentamicin (2.5 µg/mL) enhanced the inhibition of biofilm formation synergistically (27). The creation of EPS was linked to adherence and biofilm formation, which was significantly decreased in the tested strain, suggesting a possible synergistic effect when combined with gentamicin. Rutin may serve as an adhesion inhibitor for treating E. coli infections, exhibiting improved antibiofilm efficacy when taken in conjunction with gentamicin. Although the mechanism by which rutin inhibits biofilm formation remains ambiguous, the study suggests its potential as an effective antibiofilm agent against E. coli, as it promotes ROS generation in bacteria, resulting in oxidative stress and bacterial mortality (27).

The majority of chronic diseases linked to biofilms are as a result of polymicrobial biofilms, a crucial aspect never considered in both conventional and advanced anti-biofilm treatment approaches. Therefore, there is no perfect method for the total debridement of biofilm; however, the most crucial approach is the application of agents that employ complementary mechanisms that degrade biofilm matrix and kill bacteria. The insights into anti-biofilm tendencies must involve the usage of the computational methods to assess the approach. These compounds may be used to study the effectiveness and possible effects on formation of novel resistant microorganisms of anti-biofilm action(28). The authors of this research postulated that the synergy between flavonoids and crude extracts with the commercially available antimicrobial agents Lfx was positive. Moreover, it is clearly seen that using Lfx in conjunction with biofilm treatment developed from E. coli is more profitable than using antibiotics only. To our knowledge, this study proposes for the first time to investigate the interactions between Lfx and the crude extract and between Lfx and flavonoid compound in vitro.

The data obtained with Lfx, crude, and flavonoid *in vitro* investigations suggest the possibility of choosing efficient combination treatments. The Chequerboard assay for the planktonic cells also revealed synergism when Lfx at 8 µg/mL was used with crude flavonoids at 128 µg/mL showing an

FICI < 0.5. The inhibition concentration for Lfx alone was 16 µg/mL The for Lfx with flavonoids improved to 1, 2, 4 and 8 µg/mL. This also appears to increase the potential for the combination at inhibitory concentrations to prevent biofilm formation after 24 hours.

Conclusions

Microbial pathogens residing in biofilms exhibit significantly greater resistance to drugs and immune system eradication, hence necessitating the exploration of novel techniques for managing biofilm infections. Numerous studies have integrated plant-derived molecules exhibiting antibacterial properties with antibiotics or chemotherapeutics. Promising outcomes were noted with Flavonoids in conjunction with the Lfx antibacterial agent against E. coli. many combinations are intriguing and generate significant expectations; however, it is essential to recognize that the mechanisms of action for many substances remain unidentified, and many medications interact with one another, complicating pharmacokinetics. Standardization of activities is essential to facilitate the comparison of results, which is frequently challenging. Continuation of these investigations is essential to guarantee the appropriate application of combinations and facilitate systemic administration.

This study and other investigations proving influence of crude extract, the flavonoid compounds, and Lfx in counteracting antibacterial activities lead to this conclusion. It is also notable that concurrent clinical use of crude extract and flavonoid compounds with Lfx is favorable. Hence, the earlier observation that crude extract and flavonoid compounds increase efficacy of Lfx when used in conjunction with it in the treatment of biofilms can be built upon. This work was useful in understanding the antithetical impacts of Lfx alone and in conjunction with the crude extract and flavonoid compounds on Escherichia coli isolates in vitro.

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التأثير التآزري لمستخلص نبات الريحان والليفوفلوكساسين على تكاثر وتطور الأغشية الحيوية لبكتيريا الإشريكية القولونية المعزولة سريريا

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