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ANTIBACTERIAL AND ANTI-BIOFILM ACTIVITIES OF GRAPE SEED OIL AND BLACK SEED OIL AGAINST UROPATHOGENIC *ESCHERICHIA COLI* ISOLATED FROM WOMEN WITH URINARY TRACT INFECTIONS

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ABSTRACT

This study was conducted to assess the antibacterial and anti-biofilm activity of grape seed and black seed oils against clinical isolates of *E. coli* which obtained from women suffer from urinary tract infections. Different concentrations of both oils were tested against twelve isolates of *E. coli*. Biofilm inhibition was measured at doses between 6.25 and 200 µg/mL to determine the anti-biofilm activity. The anti-biofilm capacity of both oils was concentration-dependent, and the inhibitory effect enhanced with oil concentration. Black seed oil demonstrated 89.37% biofilm inhibition at 200 µg/mL, whereas grapeseed oil demonstrated a maximum inhibition of 95.44%. Significant variations between the measured concentrations for each oil were shown by statistical analysis. In conclusion, both oils demonstrated a supportive in vitro anti-biofilm action against clinical isolates of *E. coli*, with grapeseed oil exhibiting a greater percentage of inhibition than black seed oil.

Keywords: Antibacterial, Anti-biofilm, *E. coli*, Grape seed oil (GSO), Black seed oil (BSO).

1 INTRODUCTION

Approximately 80% of the world's population, who reside in underdeveloped nations, use natural product therapies for a range of illnesses [1]. Natural food products offer a variety of herbal remedies for UTIs that meet the criteria of efficacy, palatability, convenience of use, and consistency in availability [2]. Therapeutic botanicals are plants and their derivatives with potential medical uses [3].

Numerous bioactive compounds found in these natural products serve as the basis for innovative medications. There are numerous advantages to using medicinal botanicals, such as reduced costs, more patient acceptance, less side effects, and natural replenishment [3].

Millions of individuals worldwide suffer from urinary tract infections (UTIs), which are common bacterial infections that frequently require antibiotic therapy [4].

The term "UTIs" refers to infections of the kidney (pyelonephritis), bladder (cystitis), or urethra (urethritis) [5].

It is among the most frequently occurring illnesses worldwide, with a high risk of death, morbidity, and financial difficulties [6].

In light of anatomical and physiological characteristics, women are more exposed than men to urinary tract infections, which occur more frequently [7].

Urinary tract infections (UTIs) are more common as people age, possibly exceeding 20% in women over 65 [8] due to weakening immune systems [9] and declining estrogen levels [10]. Men might suffer from prostatitis, cystitis, epididymitis, urethritis, and other lower-tract disorders [11].

UTIs can be brought on by both Gram-positive and Gram-negative bacteria along with some fungi [5]. Up to 95% of community-acquired UTIs and 50% of nosocomial UTIs are caused by Uropathogenic *Escherichia coli* (UPEC), which is the most common cause of both simple and complex UTIs [6, 12].

According to Memar et al. [13], plant extracts seem to be the most promising replacement to synthetic or chemical antioxidants and antimicrobials.

The bioactive substances found in grape seeds, such as polyphenols with antioxidant and antibacterial qualities, have attracted interest. According to studies, such characteristics may help with a number of health issues and enhance food preservation methods [14-16].

The general mechanisms of phenols are responsible for the antibacterial effects of grape seed extract (GSE), which is rich in flavan-3-ol polymers like catechin and epicatechin. It has been demonstrated that grape phenolic chemicals prevent bacteria from growing. According to Furiga et al. [17], the antibacterial activity of the grape plant was found in the following sequence: flesh, entire fruit, grape extracts, fermented marc, skin, leaves, and seeds.

When it comes to pathogens like *Staphylococcus aureus* and *Escherichia coli*, GSEs show strong antibacterial action. Different grape types do, however, differ from one another [18-24].

With its wide range of bioactive chemicals, *Nigella sativa* also referred to as black seed or cumin provides possible medicinal properties that may help reduce such health issues. Uzbekistan, Egypt, Southern Europe, Southwest Asia, North Africa, India, and Pakistan are the species' main habitats. It has a long history of traditional use, is highly significant in Greek medicine and Islamic culture, and is renowned for its broad-spectrum pharmacological properties [25, 26].

The majority of *Nigella seeds* have more than 30% oil, and some have as much as 40% [27].

Organic antioxidants with antidiabetic, anti-inflammatory, anti-cancer, and wound-healing characteristics, as well as antibacterial and antifungal activity, are among the numerous natural constituents with in vitro therapeutic potential found in *N. sativa*. also, *N. sativa* has long been used traditionally to treat bacterial infections [28, 29].

Alkaloids, proteins, saponins, and both volatile and fixed essential oils are abundant in *Nigella sativa* seeds. In particular, a significant amount of the biological activity of the seed extracts is attributed to thymoquinone (TQ), the primary component of the essential oil. O-cymene is the primary component of the fixed oil [30][34]. In the meantime, important fatty acids and sterols like linoleic and oleic acids, b-sitosterol, and stigma sterol are found in the lipid section of black seed. According to reports, *N. sativa* proteins and peptides have a range of bioactive properties, including antibacterial, anti-cancer, and anti-inflammatory properties [26].

This study was conducted to assessment the activity of these oils extract on the uropathogenic isolates of *E. coli*.

2 MATERIALS AND METHODS

2.1 Preparation of plants oil extract

2.1.1 Grape seed oil extraction

Grapes were obtained from local markets in Baghdad, Iraq. They were thoroughly washed to remove dust and impurities, then pressed to extract the seeds. The seeds were collected, washed, and dried, and the oil was subsequently

extracted using a cold-pressing method according to [31-33]; a process that involves neither heat nor organic solvents, thereby preserving the active chemical compounds.

2.1.2 Black seed oil extraction

Black seed were purchased from the local market in Baghdad, Iraq, washed and dried at room temperature then grinded to fine powder. These powder then used in preparation of the oil extract according to the method of [34, 35].

2.2 Preparation of bacterial isolates and inoculum

Twelve clinical *E. coli* isolates related to a UTIs that had already been identified were reactivated on the proper culture medium and incubated in suitable aerobic conditions. From each isolate, a new bacterial colony was chosen and suspended in sterile saline. Before being used in the anti-biofilm test, the turbidity of the bacterial suspension was corrected to a 0.5 McFarland standard. The standardized antibiotic susceptibility testing protocols advised by CLSI and EUCAST were followed in the creation of the inoculum [36].

2.3 Minimum Inhibitory Concentration (MIC) assay

Grape seed oil and black seed oil are the test compounds, while the test microorganism was *E. coli* isolated from women were infected with urinary tract infections. 96-well microplates were used for this. First, 100 µl of MSA broth growth media was added to each well. The first well was then filled with 10 µl of supernatant, homogenized with a sampler, and peptonized. Lastly, 100 µl of the well was mixed with the two solutions.

One hundred microliters were pipetted into the second well, then the third, and finally the fourth. Up until the sixth well, this procedure was repeated; after that, 100 µl was thrown away. Consequently, the serial dilution approach was used to create dilutions of 200, 100, 50, 25, 12.5, and 6.25 µg/ml. These six dilutions were therefore created. Finally, the half-McFarland microbial suspension, that was made to produce 10⁶ CFU/ml, was diluted to a hundredth of its initial volume using a nutrient broth culture medium. This produced a final volume of 200 µl, which was subsequently added to each well.

Microbial suspension (control) and culture media were present in Well 7. Before the results were evaluated, the microplates were incubated for eighteen hours at 37 °C in an aerobic atmosphere after three replications [37]. It was discovered that the first well with no turbidity or growth had the lowest concentration of growth-inhibiting microorganisms. In order to prevent optical errors in the OD test, all wells were further inspected using ELISAR equipment at a wavelength of 620 nm. This test was performed three times for each strain.

The following formula was used to determine percentages [38]:

Percentage % = no. of isolates at a given final MIC / total no. of isolates x 100

2.4 Biofilm assay

Trypton soy broth mixed with 1% (w/v) glucose was used to dilute the cultures (1:100) overnight. A 96-well polystyrene microtiter plate was filled with 200µl aliquots of the culture, which were then incubated at 37°C for the entire night. After the biofilms were incubated, they were carefully rinsed twice with ordinary saline, the supernatants were removed from each well, and they were dried and fixed for an hour at 65°C.

After staining with 0.1% (w/v) crystal violet for 10 minutes, the plates were gently washed twice before 200 µl of 95% ethanol was added to perform a quantitative analysis of biofilm formation. Finally, the absorbance of the methylene blue in the ethanol de-staining solution at 630 nm was measured using a microplate reader.

The following equation was used to determine the percentage of biofilm inhibition [39]:

Biofilm inhibition % = (OD of control – OD of the treatment)/OD of control x 100

2.5 Statistical analysis

Microsoft Excel was used for statistical analysis. Every experiment was carried out in triplicate. To determine the statistical significance of variations between the measured oil concentrations, data was analyzed using one-way analysis of variance (ANOVA) without replication. Statistical significance was defined as a p-value < 0.05.

3 RESULTS

3.1 Minimum Inhibitory Concentration (MIC) assay:

Table (1) indicates that MIC values were expressed as n (%) of the total tested isolates.

Table 1: Comparative distribution of MIC values for grape seed and black seed oils against 12 Uropathogenic isolates of *E. coli*

Final MIC (µg/ml)	No. of isolates treated with Grape seed oil	Percentage %	No. of isolates treated with Black seed oil	Percentage %
200	0		0	
100	0		0	
50	0		8	66.6%
25	3	25%	4	33.3%
12.5	9	75%	0	
6.25	0		0	

3.2 Biofilm inhibition assay

The results in table (2) show the activity of grape seed oil on *E. coli* biofilm inhibition.

Table 2: The activity of grape seed oil against *E. coli* biofilm formation

Isolates no.	OD Control	Concentration of Grape seed oil (µg/ml)					
		Mean OD ± SD 200	Mean OD ± SD 100	Mean OD ± SD 50	Mean OD ± SD 25	Mean OD ± SD 12.5	Mean OD ± SD 6.25
1	0.409 ± 0.0006	0.012 ± 0.0006	0.099 ± 0.0006	0.112 ± 0.001	0.214 ± 0.001	0.322 ± 0.0006	0.357 ± 0.0015
2	0.409 ± 0.0006	0.015 ± 0.001	0.0913 ± 0.0006	0.123 ± 0.0012	0.256 ± 0.001	0.314 ± 0.001	0.372 ± 0.0006
3	0.409 ± 0.0006	0.0137 ± 0.002	0.089 ± 0.001	0.103 ± 0.002	0.215 ± 0.001	0.332 ± 0.002	0.356 ± 0.002
4	0.409 ± 0.0006	0.0131 ± 0.004	0.089 ± 0.002	0.125 ± 0.002	0.226 ± 0.001	0.302 ± 0.001	0.336 ± 0.001
5	0.409 ± 0.0006	0.012 ± 0.002	0.086 ± 0.001	0.125 ± 0.002	0.222 ± 0.002	0.300 ± 0.001	0.372 ± 0.001
6	0.409 ± 0.0006	0.017 ± 0.001	0.097 ± 0.002	0.124 ± 0.001	0.216 ± 0.001	0.345 ± 0.003	0.382 ± 0.002
7	0.409 ± 0.0006	0.018 ± 0.001	0.018 ± 0.002	0.162 ± 0.001	0.222 ± 0.001	0.352 ± 0.001	0.378 ± 0.001
8	0.409 ± 0.0006	0.024 ± 0.001	0.086 ± 0.001	0.104 ± 0.001	0.208 ± 0.001	0.327 ± 0.002	0.367 ± 0.002
9	0.409 ± 0.0006	0.027 ± 0.001	0.084 ± 0.001	0.197 ± 0.002	0.207 ± 0.002	0.311 ± 0.001	0.348 ± 0.001
10	0.409 ± 0.0006	0.0177 ± 0.001	0.088 ± 0.001	0.186 ± 0.001	0.218 ± 0.001	0.314 ± 0.001	0.374 ± 0.001

	0.0006	0.002	0.001	0.002	0.001	0.001	0.002
11	0.409 ± 0.0006	0.022 ± 0.001	0.0807 ± 0.002	0.188 ± 0.001	0.208 ± 0.002	0.316 ± 0.001	0.336 ± 0.001
12	0.409 ± 0.0006	0.0327 ± 0.002	0.094 ± 0.002	0.214 ± 0.002	0.252 ± 0.002	0.311 ± 0.001	0.354 ± 0.002

P < 0.001

Table (3) shows the activity of black seed oil against *E. coli* biofilm formation.

Table 3: The activity of black seed oil against *E. coli* biofilm formation

Isolates no.	OD Control	Concentration of Black seed oil (µg/ml)					
		Mean OD ± SD 200	Mean OD ± SD 100	Mean OD ± SD 50	Mean OD ± SD 25	Mean OD ± SD 12.5	Mean OD ± SD 6.25
1	0.409 ± 0.0006	0.053 ± 0.001	0.092 ± 0.001	0.235 ± 0.002	0.288 ± 0.001	0.348 ± 0.001	0.387 ± 0.002
2	0.409 ± 0.0006	0.043 ± 0.001	0.176 ± 0.002	0.244 ± 0.002	0.273 ± 0.002	0.336 ± 0.001	0.381 ± 0.002
3	0.409 ± 0.0006	0.026 ± 0.001	0.097± 0.002	0.196 ± 0.001	0.259 ± 0.002	0.346 ± 0.004	0.377± 0.001
4	0.409 ± 0.0006	0.045 ± 0.002	0.086± 0.001	0.234 ± 0.003	0.272 ± 0.001	0.363± 0.002	0.386 ± 0.002
5	0.409 ± 0.0006	0.063 ± 0.0006	0.092 ± 0.001	0.198 ± 0.002	0.276± 0.002	0.344 ± 0.002	0.373 ± 0.002
6	0.409 ± 0.0006	0.044 ± 0.001	0.084 ± 0.0006	0.186 ± 0.002	0.264 ± 0.002	0.327 ± 0.001	0.368 ± 0.001
7	0.409 ± 0.0006	0.036 ± 0.001	0.092 ± 0.001	0.208 ± 0.002	0.258 ± 0.001	0.343 ± 0.002	0.372 ± 0.002
8	0.409 ± 0.0006	0.044 ± 0.002	0.088 ± 0.002	0.199 ± 0.002	0.256 ± 0.001	0.327 ± 0.002	0.379 ± 0.002
9	0.409 ± 0.0006	0.034 ± 0.002	0.093 ± 0.0006	0.206 ± 0.002	0.287 ± 0.002	0.352 ± 0.002	0.378 ± 0.002
10	0.409 ± 0.0006	0.054 ± 0.002	0.086 ± 0.0006	0.217 ± 0.002	0.265 ± 0.002	0.347 ± 0.002	0.362 ± 0.0006
11	0.409 ± 0.0006	0.032 ± 0.001	0.091 ± 0.0006	0.199 ± 0.002	0.238 ± 0.001	0.315 ± 0.002	0.376 ± 0.002
12	0.409 ± 0.0006	0.048 ± 0.001	0.086 ± 0.002	0.156 ± 0.002	0.228 ± 0.001	0.316 ± 0.002	0.366 ± 0.002

P < 0.001

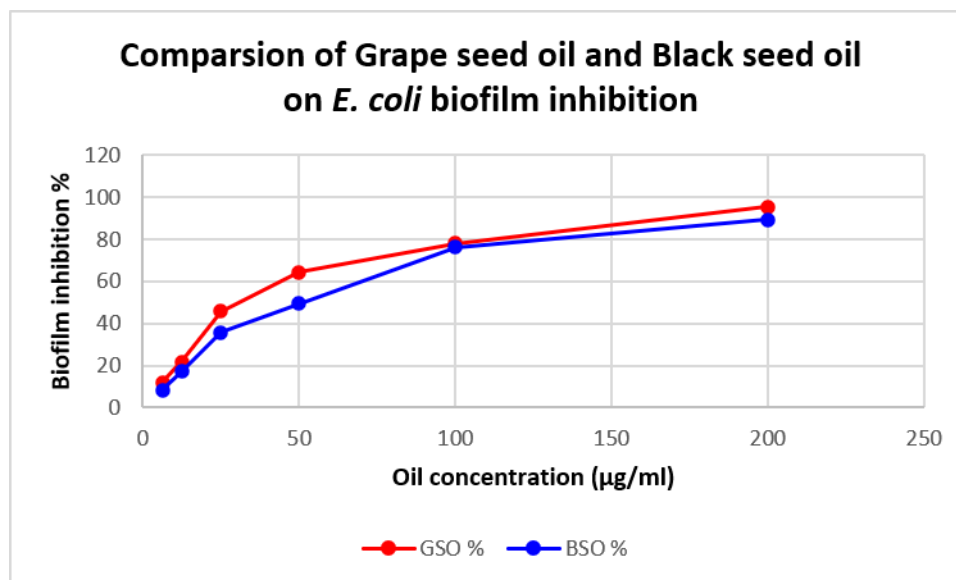


Figure 1: Comparison of Grape seed oil and Black seed oil on *E. coli* biofilm inhibition.

4 DISCUSSION

The results in table 1 showed that the MIC distribution among 12 isolates of *E. coli* of the activity of GSO and BSO. The MIC values of GSO were ranged from 12.5 to 25 (µg/ml) with the highest percentage of inhibition was 75% at 12.5 (µg/ml), while the MIC values for BSO were ranged from 25 to 50 (µg/ml) with the highest percentage of inhibition was 66% at 50 (µg/ml). this result revealed strong antibacterial activity of grape seed oil than black seed oil.

Table 2 revealed the activity of GSO against biofilm formation of *E. coli* isolates. The highest reduction in OD reading was 0.012 ± 0.0006 at 200 (µg/ml) comparing to control which was 0.409 ± 0.0006 , these results indicated that the highest reduction in biofilm formation of *E. coli* was occur at 200 (µg/ml), in other words, the higher the concentration of grape seed oil, the greater the inhibition of biofilm formation.

Also, 95.4% of isolates were inhibited by GSO at 200 (µg/ml) as shown in figure 1.

On the other hand, the results in table 3 demonstrated the activity of BSO against biofilm formation of *E. coli* isolates. The highest decreasing in OD reading was 0.026 ± 0.001 at 200 (µg/ml) in comparison with control was 0.409 ± 0.0006 , these findings showed that the highest reduction of Biofilm formation of *E. coli* caused by BSO was occur at 200 (µg/ml). so the inhibition in the biofilm formation of *E. coli* isolates increases with the increase of BSO concentration.

However, about 89.3% of isolates were inhibited by the effect of BSO at 200 (µg/ml) as shown in figure 1.

Statistical analysis showed a significant effect of these oil on biofilm formation ($P < 0.001$).

The current findings are in agreement with the study of [40] which found that the grape seed extract affected on *E. coli* by suppressing quorum sensing with reduction in motility, flagella protein expression and Shiga toxin production.

In the study on White Grape Juice extract (WGJe)[41] found that the extract inhibited the formation of biofilm in *E. coli* and *P. aeruginosa* with a dose-dependent effect, so its show a bactericidal and bacteriostatic activity in vitro.

Algon et al. [42] found that the methanolic extract of grape seed has the inhibition effect on biofilm formation may be due to the presence of the chemical constituents as catechin esters of galic acid.

The findings of Mahdi et al. [43] demonstrated that the MIC of black seed oil ranged from 64-502 µg/ml, also found that the oil of black seed has inhibitory activity against *E. coli* and its Shiga toxin and can inhibit virulence in *E. coli*.

Anti-inflammatory, cardioprotective, antibacterial, and anticancer characteristics are among the health benefits of grape seed oil that are mostly identified by in vitro research. It can also be associated with cellular and molecular pathways. The components of grape seed oil, including tocopherol, linolenic acid, resveratrol, quercetin, procyanidins, carotenoids, and phytosterols, have been linked to these effects [44].

According to Yang et al. [45] the yield and the compositions of grape seed oil is differ depending on the cultivar of the chosen grape, origin and extraction methods [46].

One of the main ingredients of black cumin seed oil, thymoquinone (TQ), has many health benefits. Black cumin seed oil and its main constituent, TQ, have been thoroughly investigated by researchers. Numerous pharmacological characteristics, such as anticancer, immunomodulatory, analgesic, antibacterial, antidiabetic, and anti-inflammatory actions, have been demonstrated by these investigations [47].

On the other hand, Chaieb et al. [48] found that the presence of thymoquinone in the oil of black seed prevent the formation of biofilm which consider the active chemical compound in BSO.

The antimicrobial activity of several of these phytoconstituents of black seed oil in relation to their functional groups is noteworthy here. They also account for other medicinal properties like anticancer, hepatoprotective, antioxidant, and anti-inflammatory activities. The detected functional groups suggest that BSO extracted with 100% ethanol contains numerous therapeutically active components, such as thymoquinone, or significant phytochemicals, such as phenolic compounds, flavonoids, and terpenoids [49-52].

The antibacterial mechanism of black seed oil is due to the presence of TQ, which can cause significant biocidal effects, increase membrane permeability, block efflux pumps, cause oxidative stress, and cause cell death (by generating reactive oxygen species) [53].

In summary, both oils showed encouraging antibiofilm action against clinical *E. coli* isolates that was concentration-dependent.

5 CONCLUSION

The current investigation showed that black seed and grapeseed oils had concentration-dependent anti-biofilm action against clinical *E. coli* isolates linked to UTIs. The greatest inhibition, 95.44% for grapeseed oil and 89.37% for black seed oil, was seen at 200 µg/mL. These results indicate both oils' potential as natural anti-biofilm agents and recommend for additional research.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

Disclosure of conflict of interest

The author declares no conflicts of interest regarding this manuscript.

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