

The activity of table vinegar in reducing and eradicating the biofilm producing *Escherichia coli*SM.Nachammai^{1*}, Dr.Karthika Jayaumar², Vinithra suresh³, Kousalya.M⁴^{1*}Department of Microbiology, SSSMCRI, Sri Balaji Vidhayapeeth University, Chennai²Department of Microbiology, SSSMCRI, Sri Balaji Vidhayapeeth University, Chennai³Department of Microbiology, SRKDC Dr.MGR University, Coimbatore⁴Department of Microbiology, SRKDC, Dr.MGR University, Coimbatore**Corresponding Author:** SM. Nachammai, Department of Microbiology, SSSMCRI, Sri Balaji Vidhayapeeth University, Chennai**Type of Publication:** Original Research Paper**Conflicts of Interest:** Nil**Abstract**

Urinary tract infection caused by *E. coli* is responsible for recurrences and relapses of infection due to its ability to form biofilm. Many studies had trailed using silver nanoparticles the natural products like green tea extracts, essential oils, clove extracts, eugenol as an antibiofilm agent. This study has been carried out to assess the action of table vinegars (Date, Apple cider, Grape cider) to prevent the biofilm formation using microtitre plate assay. Out of 212 UPEC isolates, 128 isolates produced biofilm of which date vinegar has eradicated 100% of biofilm in all the isolates, 95.3% and 94.5% of the biofilm were eradicated with reduced optical density values indicating the less biofilm production in other remaining biofilm producers by apple cider and grape vinegar respectively. Better results of biofilm prevention in using date vinegar compared to other type of vinegars depend on the varying concentrations of amino acid, acetic acid and other chemical components. More studies are required to prove the action of these vinegars against the biofilm production scientifically and also for its health benefits.

Keywords: UTI, Biofilm, *E. coli*, Table vinegars, antibiofilm.**Introduction**

Urinary tract infection (UTI) is the second most common human infection, predominantly caused by *Escherichia coli* (50% - 90%). Recurrence and chronic infection of the urinary tract occurs most likely due to biofilm formation, where it encompasses the sessile bacteria than planktonic state. Inside the urinary tract, *E. coli* with its long filamentous structure interbind together leading to the formation of Intracellular Bacterial Communities (IBC). Some of the *E. coli* produces exopolymer enzymes resulting in the formation of extracellular matrix called Biofilm, which protects the bacteria from host defenses as well as promotes the bacterial growth¹. Many products like plant extracts, green tea extract, plant essential oils and eugenols, silver nanoparticles are assessed for its antibiofilm action against biofilm formation by *E. coli* and also other bacteria². Vinegar has been used as a traditional medicine in ancient times for treating fever, cystitis, liver and abdominal troubles, wound infections, to get relieve from inflammation etc.

Vinegar, a fermentation product contains 4 to 5% acidic content mainly acetic acid are called as Consumable vinegar (Table vinegar) which has more health benefits as the Journal of Food Science says³. Many types of vinegar

from the fermentation process of various fruits and vegetables are available in the industrial production used for different purposes. Date vinegar from date fruit was more popular in Middle Eastern countries, Apple cider vinegar, Grape cider vinegar are the fermentation products of apple and grape which are industrially produced in India. The general property of three table vinegars are antimicrobial potential, antioxidant, antimutagenic properties, antiglycemic affect, maintains the acid-alkaline base of the body, increases the blood flow in the colon by maintaining its consistency, reduces the oxidative stress, lowers the cholesterol level, regulates digestion and renal function. The apple vinegar was used especially for cleaning and disinfection, to treat nail fungus, warts, ear infections and dandruff. This study is aimed to assess the action of three table vinegars -Date, Apple cider and Grape Cider Vinegar to reduce or eradicate the biofilm produced by *E. coli*_{4,5,6}.

Methodology

Bacterial isolates:The total of 212 *E. coli* strains isolated from urine samples collected from clinically suspected UTI patients were identified and confirmed by standard conventional biochemical method.

Biofilm determination by quantitative method₈:

Microtitre plate assay was used in accordance with Stepanovic et al (10), with some modifications. Three to five *E. coli* colonies were suspended in 5 ml of Trypticase Soy Broth (TSB) and incubated for 18h at 37°C. After incubation, the media was diluted to 1:100 in TSB with 0.25% glucose, and 200 µl of this suspension was transferred to 96-well plates and incubated for 18h at 37°C. Media with suspended bacteria was then discarded; the plates were carefully washed four times with PBS (Phosphate buffer saline), air dried and fixed with 99% methanol. Then 200 µl of 1% crystal violet dye was added and kept for 20 minutes. The dye solution was then

removed and washed with PBS, 200µl of 95% ethanol was added to solubilize the attached dye. The optical density of the adherent biofilm was determined thrice with a filter of 450/630nm in a microtitre plate reader (). Background absorbance (Negative control) was calculated using TSB with 0.25% glucose alone. All isolates were tested in triplicate. The interpretation of biofilm production was based on the criteria, depending on standard calculations – weak biofilm producer if the $OD_{450/630} \leq OD_{control}$, moderate biofilm producer $2OD_{control} < OD_{450/630} \leq 4OD_{control}$ and strong biofilm producer $OD_{control} < OD_{450/630} \leq 2OD_{control}$. An optical density (OD) of 0.0601 was chosen as a guideline OD value to distinguish biofilm producers from those that did not form biofilm.

Assessment of Vinegar for its antibiofilm activity₈:

Three types of vinegar (Date, Apple cider, Grape) - 10µL (1:10) of the raw material was added to prewashed biofilm of standardized bacterial suspension a in microtiter plate for each study isolate, and incubated for 18 hrs at 37°C with gentle agitation and the remaining steps as followed in quantitative biofilm assay. The optical density (OD) of each well was measured at the same previous wavelengths by ELISA reader. Similarly the optical density reading interpretations was depended on the biofilm criteria.

Statistical analysis:Data were analyzed using SPSS statistical program (Statistical Package for the Social Science) version 20.0, LSD test for dependable samples and multiple comparisons. Statistical significance differences were taken with P- value <0.01.

Results: One hundred and twenty eight isolates (60.4%) produced biofilm out of 212 *E. coli* isolates, of which 4 (3.1%) isolates were strong biofilm, 14 (11%) were moderate and 110 (85.9%) were weak biofilm producers as in figure 1.

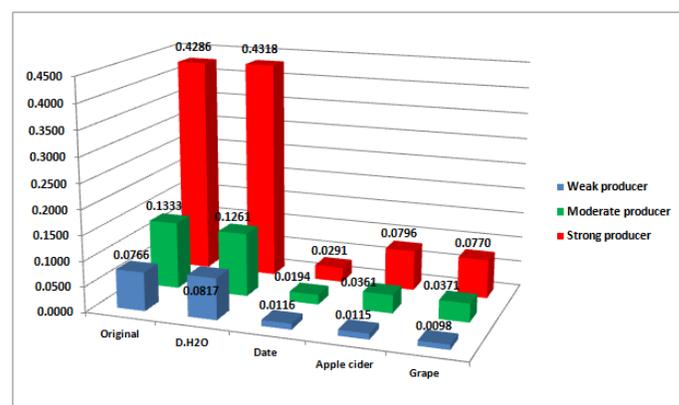
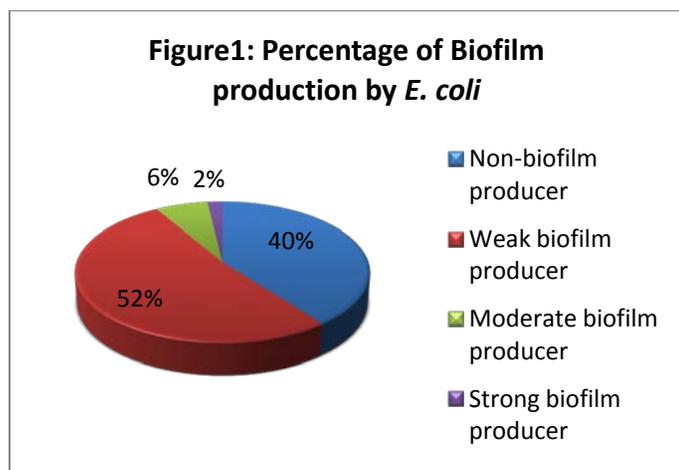


Figure 2: Antibiofilm activity of three types of vinegar in compared with control and original OD values according to the biofilm categories

The statistical significant difference was high before and after using of these three types of vinegar (P-value=0.000 <0.01). The negative control with distilled water showed no biofilm eradication for the study isolates. There was no significant difference (P-value was high 0.418>0.01).

Among the three types of vinegar there was no statistical significance according to P-values, the biofilm prevention ability of the three types of vinegar and distilled water was different among the three biofilm categories (Strong, moderate, and weak).

Discussion

Biofilm makes the *E. coli* more adherent to the uroepithelial cells mainly via type 1 fimbriae which forms interbacterial communities. It makes the bacteria more resistant to antibiotics due to its low penetration, horizontal transfer of antimicrobial resistance genes and alteration in the growth rate as it maintains the bacteria in a sessile state. This longer persistence of the bacteria contributes the recurrent and chronic urinary tract infection.

This Date, Apple cider and grape vinegar possess antimicrobial potential and also had the antibiofilm property as shown in a previous study⁹. The same results

The impact of three different types of vinegars as an antibiofilm under in vitro conditions showed efficient eradication of biofilm in polystyrene microtitre plate assay. Date vinegar eradicated biofilm formation in 128 (100%) biofilm producer isolates comparing to the OD values with the standard criteria of this study. In Apple cider vinegar (AV), 122 (95.3%) isolates were eradicated except 6 isolates of which 4 strong and 2 moderate biofilm producers. In this case AV has reduced the biofilm formation as the optical density (OD) value is lesser when compared to the OD value of original and control with distilled water. In grape vinegar (GV), 121 (94.5%) isolates eradicated from biofilm formation except 7 isolates of which 4 were strong and 3 were moderate biofilm producers. This represents that high amino acid concentration in DV essential to completely eradicate the biofilm but AV, GV reduced the biofilm rate of the isolates with strong and the few moderate biofilm producers with high OD values as in figure 2.

had been noted in this study that these vinegars had the capability to reduce the biofilm formation.

The chemical components present in Date vinegar (DV) were acetic acid which is a secondary metabolite. The sugars in these fruits were fermented to ethanol and further converted to acetic acid after undergoing secondary fermentation. Other than acetic acid, this date vinegar contains carbohydrates, alkaloids, tannins, saponins anthocyanins, flavanoids and vitamin A, B1, B2, high potassium followed by calcium, low sodium and polyphenols. The high amino acid content of date vinegar prevents the initial colonization of bacteria to form biofilm.

Apple cider vinegar (AV) fermented from crushed apple contains vitamins B1, B2, and B6; biotin; folic acid; niacin; pantothenic acid; and vitamin C. Also contains less minerals, antioxidants, anthocyanins, cyanins, flavonols, few amino acids and polyphenolic compounds reduces the exopolymer producing enzymes. Grape vinegar has low calories along with polyphenols with various concentrations which influences the eradication of biofilm formation₈.

The previous study conducted by Narjis.F et al tried these three vinegars against the biofilm produced by *Streptococcus pyogenes* from recurrent tonsillitis, in vitro₈. The study had been concluded that date vinegar prevented 100% biofilm formation compared to other vinegars though not statistically significant.

In this study date vinegar has completely eradicated the biofilm compared to AV, GV types. Though there is no statistical significance among these three vinegars in prevention of biofilm, the variation in optical density values implied the different concentration of these chemical components.

Conclusion

The table vinegars – date, apple cider, grape showed good results in its antibiofilm property under in vitro condition in this study. The usage of this regularly for health beneficial as in ancient times needs more research studies to prove scientifically.

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