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ANTIBACTERIAL ACTIVITY OF NATURAL (DATES AND APPLE CIDER) VINEGARS AGAINST FOODBORNE BACTERIAL PATHOGENS

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ABSTRACT

Local food samples investigated for the presence of pathogenic bacteria. Hash meat sample was used to isolate *Escherichia coli* and chicken meat (poultry) was used to isolate *Salmonella typhi*. Biochemical tests and API20E system used in order to identify these isolates. Two natural vinegar samples (dates & apple cider) were used in order to study its antibacterial activity against the two tested bacteria. Disc diffusion method was used, the results showed that two vinegar samples have antibacterial activity against the two tested bacteria. Date vinegar showed inhibition zone 19mm against *E. coli* & 9mm against *S. typhi*, while Apple cider vinegar showed diameter of inhibition zone 20mm against *S. typhi* and 13mm against *E. coli*, also the results showed that control (water) have no antibacterial activity against the two tested bacteria.

Keywords: Antibacterial activity, dates and apple cider vinegars, food borne bacterial pathogens.

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الفعالية الضد بكتيرية لخل التمر وخل التفاح الطبيعي ضد الممرضات البكتيرية المنقولة بالأغذية

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الخلاصة

تم التحقق من وجود البكتريا الممرضة في عينات الاغذية المحلية، اذ استخدمت عينات اللحم المفروم لعزل بكتريا القولون ولحم الدجاج (الدواجن) لعزل بكتريا السالمونيلا، واستخدمت الاختبارات الكيموحيوية ونظام التشخيص للعائلة المعوية API20E لغرض تشخيص هذه العزلات واستخدمت عينتين من الخل الطبيعي (خل التمر وخل التفاح) لدراسة فعاليتها الضد بكتيرية ضد كلتا البكتريا المختبرة، اذ استخدمت طريقة الانتشار من القرص واظهرت النتائج ان كلا النوعين من الخل يمتلك فعالية ضد بكتيرية ضد كلتا البكتريا المختبرة، واظهر خل التمر منطقة تثبيط بلغت 19ملم ضد بكتريا القولون و9ملم ضد السالمونيلا، بينما اظهر خل التفاح قطر منطقة تثبيط 20ملم ضد السالمونيلا و13ملم ضد بكتريا القولون، كذلك اظهرت النتائج ان أنموذج السيطرة (الماء) لا يمتلك فعالية ضد كلتا البكتريا المختبرة.

الكلمات المفتاحية: الفعالية الضد بكتيرية، خل التمر وخل التفاح، الممرضات البكتيرية المنقولة بالأغذية.

INTRODUCTION

There is an increasing importance in applying natural antimicrobial compounds in the food production. Consumers are increasingly avoiding the ingesting of foods preserved with

chemicals. Natural alternatives are required to accomplish a high level of security with respect to food borne pathogenic microorganisms **(Rauha *et al.*, 2000)**. Among the natural products, vinegar, also known as acetic acid, contains sterilizing properties. Vinegar is an acidic liquid that is made from the fermentation of an alcoholic beverage mainly wine **(Nascimento *et al.*, 2003)**. Vinegar is a liquid containing of about 5-20% acetic acid (CH3 -COOH), water and different trace chemicals, which can include flavorings. The acetic acid is manufactured by the fermentation process of ethanol by microorganisms. Based on their raw materials, vinegars can mainly be considered grain vinegars, which contain sorghum, rice, wheat, or other grains as the raw materials, or fruit vinegars, which are based on fruits such as grapes ,apples and dried dates as raw materials. In addition, vinegars can also be fermented from sugar and alcohol. Both grain vinegars and fruit vinegars, which are fermented by traditional methods, possess a variety of physiological functions, such as antibacterial, anti-infection, antioxidation, blood glucose control, lipid metabolism regulation, weight loss, and nticancer activities. The antibacterial and anti-infection abilities of vinegars are mainly due to the presence of organic acids, polyphenols, and melanoidins **(Chen *et al.*, 2016)**.

Many studies have shown that fruit vinegars containing 0.1% acetic acid efficiently inhibit the growth of food-borne pathogens *in vitro*, including those by *Escherichia coli* O157:H7, *Salmonella enteritidis, S. typhimurium, Vibrio parahaemolyticus ,Staphylococcus aureus, Aeromonas hydrophila, and Bacillus cereus* **(Entani *et al.*, 1998)**. Apple vinegar powerfully inhibits the growth of pathogenic bacterias such as *Staphylococcus epidermidis, Pseudomonas aeruginosa, Proteus mirabilis,* and *Klebsiella pneumoniae*. High amounts of acetic acid in vinegar made it very effective in preventing bacterial food poisoning **(Medina *et al.*, 2007**). This study was aimed to isolate pathogenic bacteria from local food samples and using natural vinegar samples (dried dates & Apple cider) to prevention and control the growth of this bacteria in vitro.

MATERIALS AND METHODS

Samples collection and Isolation of Microorganisms

Five sample of hash meat and 5 sample of chicken meat (poultry) were collected from local markets in sterile containers in order to isolate pathogenic bacteria, five grams of each food sample suspended in 45mL of peptone water then use rinse and washing method, then inoculate 0.1mL of dilute solution in sterile Petri dish and put MacConkey agar and *Salmonela shigella* agar (SS agar) separately by pouring plate method then incubate at 37°C for 24hr.

Identification of the isolates

Identification of isolates depends on microscopic, morphologic characteristics and using biochemical tests for diagnosis: Gram stain used to stain the isolates of the bacteria that obtained, as well as study the morphological characteristics on MacConkey agar and SS agar. API20E used to diagnose the bacteria that isolated from food samples.

Vinegar samples

Two types of natural vinegar (home made) were used Dates vinegar & Apple cider vinegar. pH was measured for vinegars using pH-paper.

Antibacterial activity of vinegar samples against food borne bacterial pathogens

Disc diffusion method **(Burt, 2004)** with modification was used to study the effect of antibacterial activity of the natural vinegar (dates & apple) samples against the isolated bacteria from food, as follows:-

1. The discs were made of filter paper and sterilized.

2. The isolated bacteria from the food samples were activated in Brain Heart infusion broth and incubated at 37°C for 18hr.
3. Muller Hinton agar medium prepared and autoclaved and poured in sterile Petri plates.
4. The activated bacteria were diluted with saline solution after comparing them with McFarland tubes standard 0.5 (1×10^8 CFU/mL).
5. The diluted bacteria were implanted by swabbing on Muller Hinton agar plates.
6. 0.2mL of vinegars (dates & apple) used to submerged discs, dried & then put it on the surface of cultured Muller Hinton agar plates aseptically and incubated at 37°C for 18hr.
7. Discs soaked with sterile distilled water were used as control.

The diameter of inhibition zones was measured in (mm) by using the ruler. All examinations were made two times, and the outcomes were the mean estimation of the two records.

RESULTS & DISCUSSION

Isolation and Identification of the isolates

E. coli (one isolate) was isolated from hash meat samples and *Salmonella typhi* (one isolate) from chicken meat (poultry) after diagnosis of the isolates by biochemical tests in API20E system. Each isolate confirmed by Gram stain technique which appeared short bacilli Gram negative red color under light microscope. *E. coli* was appeared on MacConkey agar pink colonies due to lactose fermentation as show in (Figure1).

Figure (1): *Escherichia coli* on MacConkey agar that isolated from hash meat samples.

Salmonella typhi isolated from poultry samples and appeared in colonies with black center on SS agar due to precepitation of FeS as shown in (Figure 2).

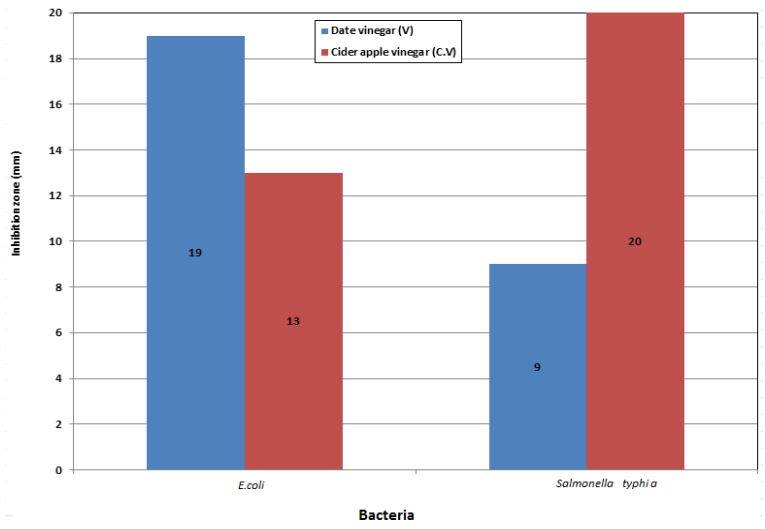
Figure (2): *Salmonella typhi* on SS agar that isolated from chicken meat sample.

pH measurement of vinegar samples

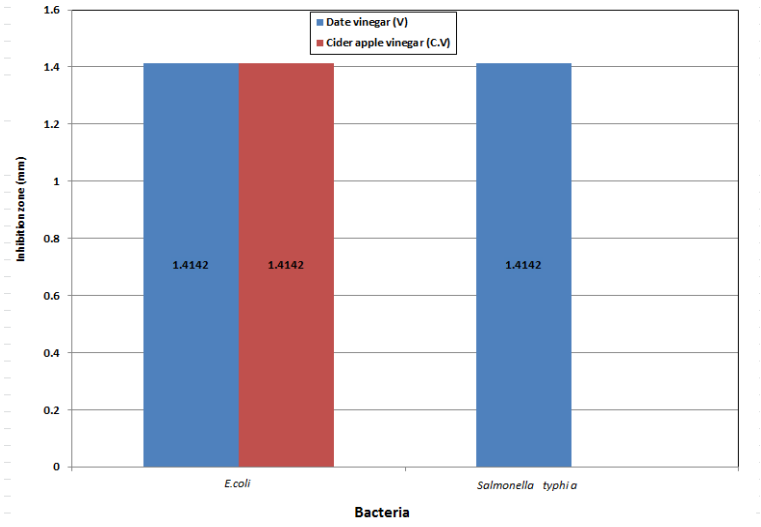
The results of pH measurement for vinegar samples were 3.5 for Dates vinegar and 3.2 for Apple cider vinegar sample.

Antibacterial activity of Dates and Apple cider Vinegar Against *E. coli* & *S. typhi*

(Figure, 3 and 4) showed the results of antibacterial activity of vinegar against food isolated bacteria, Disc diffusion method was used. The diameters of date vinegar inhibition zones were 19mm against *E. coli* & 9mm against *S. typhi*, while apple cider vinegar showed diameter of inhibition zone 20mm against *S. typhi* & 13mm against *E. coli*.



Figure(3): Anti-bacterial efficacy (mean) of vinegar samples (dates and cider apples) against food isolated bacteria.



Figure(4): Anti-bacterial efficacy(standard deviation) of vinegar samples (dates and cider apples) against food isolated bacteria.

Vinegar samples are affected against both types of bacteria isolated from food, but the effect ratio varies from one type of vinegar to another and from one type of bacteria to another. The effect of dates vinegar on *E. coli* was more than its effect on *S. typhi* while the effect of cider apple vinegar on *S. typhi* was more than its effect on *E. coli.* The control (water) had no antibacterial activity against the two tested bacteria. The antibacterial effect of vinegars may be attributed to their low PH values and their acidity.

The presence of *E. coli* in samples of minced meat evidence of the presence of fecal contamination as well as the presence of *S. typhi* (must be zero) based on the Iraqi standard specifications, the presence of these bacteria is dangerous because it is a pathogenic bacteria and danger causes of food poisoning, also the presence of *E. coli* that mean minced meat sample contaminated with the feces of animals. Our results were in agreement with other research since they showed that, vinegar, olive oil and tea extracts exhibited bactericidal activity against the food borne pathogens tested. Among them, vinegar indicated the strongest bactericidal effect, followed by the aqueous extract of virgin olive oil, wines and tea extracts. Vinegar reduced the counts of inoculated *Listeria monocytogenes*, *Salmonella enteritidis*, *Shigella sonnei*, and *Yersinia* sp. to levels below the detection limit and killed most of the *E. coli* and *S. aureus* cells. The strong bactericidal activity of vinegar is well recognized **(Entani *et al.*, 1998; Wu *et al.*, 2000)** due of its high acetic acid content, and our data confirmed these previous results. **Medina *et al.* (2007)** showed the vinegar reduced that count of *Salmonella enteritidis* & *E. coli*, that mean our results were in agreement with previous research.

Results of research reported by **Shah *et al.* (2013)** indicated that acetic acid in vinegar and phenolic compounds in olive oil had strong antimicrobial activity against *Salmonella* and *E. coli*. However, vinegar showed stronger antimicrobial activity as compared to virgin olive oil. The existence of acetic acid (CH3-COOH) in vinegar may make it more strong antimicrobial compound as compared to olive oil that contains phenols and poly phenols. **Niaz & Aziz (2017)** showed that vinegar has revealed effective antimicrobial activity against bacteria including *E. coli*, *Klebsiella*, *Salmonella* & *Shigella* & also observed that virgin olive

oil was also inhibit microbial growth. **Medina *et al.* (2007)** showed bactericidal activity of vinegar (5% acetic acid) against *Staphylococcus aureus, Listeria monocytogenes, Salmonella enteritidis, E. coli 0157:H7, Shigella sonnei, and Yersinia sp.* which was attributed to its acidity.

CONCLUSION

Local food samples like minced meat and chicken meat (poultry) were contaminated with pathogenic bacteria like *E. coli* and *S. typhi*. The presence of these bacteria in local food samples represent a potential threat to consumer health, Natural vinegar samples (dates & cider apple) shows antibacterial activity against the two tested bacteria, vinegar may be use for preserving foods such as meat products, fish, fruit and vegetables (pickles) in addition to its use as food flavoring agent.

REFERENCES

I. Burt, S. (2004). Essential oils: their antibacterial properties and potential applications in foods--a review. *International Journal of Food Microbiology,* 94, 223-253.

II. Chen, H., Tao, C., Paolo, G. & Fusheng, C. (2016). Vinegar functions on health: constituents, sources, and formation mechanisms. *Comprehensive Reviews in Food Science and Food Safety,* 15, 1124-1138.

III. Entani, E., Asai, M., Tsujihata, S., Tsukamoto, Y. & Ohta, M. (1998). Antibacterial action of vinegar against food-borne pathogenic bacteria including *Escherichia coli* O157:H7. *Journal of Food Protection,* 61, 953-959.

IV. Medina, E., Romero, C., Brenes, M. & De Castro, A. (2007). Antimicrobial activity of olive oil, vinegar, and various beverages against food borne Pathogens. *Journal of Food Protection*, 70(5), 1194-1199.

V. Nascimento, M. S., Silva, N., Catanozi, M. P. & Silva, K. C. (2003). Effects of different disinfection treatment on the natural microflora of lettuce. *Journal of Food Protection,* 66, 1697-1700.

VI. Niaz, K. & Aziz, F. (2017). Screening antibacterial activity of vinegar & olive oil on enteric bacteria. *RADS Journal of Biological Research and Applied Sciences,* 8(1), 14-17.

VII. Rauha, P., Remes, S., Heinonen, M., Hopia, A., Kähkönen, M., Kujala, T., Pihlaja, K., Vuorela, H. & Vuorela, P. J. P. (2000). Antimicrobial effect of finnish plant extracts containing flavonoids and other phenolic compounds. *International Journal of Food Microbiology,* 56, 3-12.

VIII. Shah, Q. A., Bibi, F. & Shah, A. H. (2013). Anti-Microbial Effects of Olive Oil and Vinegar against *Salmonella* and *Escherichia coli*. *The Pacific Journal of Science and Technology,* 14(2), 479-486.

IX. Wu, F. M., Doyle, M. P., Beuchat, L. R., Wells, J. G., Mintz,E. D. & Swaminathan, B. (2000). Fate of *Shigella sonnei* on parsley and methods of disinfection. *Journal of Food Protection,* 63, 568-572.