

Inhibitory Effect of Verjuice Against Some Dental Decay Bacterial Species

Hiba ALhamed ALduihi^{1,*}, Mahmoud Rawal¹, Chadi Khatib²

¹Department of Biology, Faculty of Science, Aleppo University, SYRIA.

²Faculty of pharmacy, Manara University, SYRIA.

*Correspondent contact: hiba1973@hotmail.com

Article Info

Received
05/03/2022

Accepted
25/10/2022

Published
30/03/2023

ABSTRACT

People in most countries suffer from tooth decay due to various reasons, and the germs responsible for tooth decay show an increase in resistance towards standard antibiotics used in treatment because of the inappropriate and indiscriminate use of these antibiotics. This study aimed to search for natural products that have fewer side effects and are more effective for treatment. Natural verjuice was used to study its effectiveness against some bacterial species including *Escherichia coli*, *Streptococcus mutans*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* that cause caries, which were obtained from the Microbiology Research Laboratory in a previous study. The results showed the superiority of the effectiveness of verjuice over the effectiveness of most of the selected antibiotics against the studied bacteria, which confirms its effectiveness as a natural product without the need to extract the active substances with organic solvents.

KEYWORDS: Verjuice; teeth decay; antibiotics; bacterial species.

الخلاصة

تعاني الناس في معظم دول العالم من تسوس الاسنان لأسباب مختلفة إذ ان الميكروبات المسببة لتسوس الاسنان تبدي مقاومة للكثير من المضادات الحيوية القياسية المستخدمة في العلاج كنتيجة للاستعمال الخاطئ والعشوائي لها. لذا هدفت الدراسة البحث عن منتج طبيعي أقل آثارا جانبية وأكثر فاعلية لذا استخدم عصير الحصرم الطبيعي لدراسة فعاليته تجاه الجراثيم المسببة لتسوس الاسنان *Escherichia coli*, *Streptococcus mutans*, *Staphylococcus aureus* *Pseudomonas aeruginosa* التي تم الحصول عليها من مختبر أبحاث الأحياء الدقيقة في دراسة سابقة. أظهرت النتائج تفوق فعالية عصير الحصرم على فعالية معظم المضادات الحيوية المنتخبة تجاه الجراثيم المدروسة مما يؤكد فعاليته كمنتج طبيعي دون الحاجة لاستخلاص المواد الفعالة بالمذيبات العضوية.

INTRODUCTION

Tooth decay

Dental caries is defined as diseases that affect hard tissues as a result of the consumption of fermentable sugary materials by bacteria, leading to the disintegration of the organic structure of dental tissues [1]. The caries-causing germs exist in the form of a layer known as the bacterial plaque, which consists of a large group of bacteria that exist symbiotically inside the mouth, and their accumulation over time leads to the formation of a white sticky layer that increases in thickness and complexity of composition with the increase in its survival period without removal [2]. *Streptococcus mutans* (*S. mutans* is) one of the species most closely associated with dental caries [3], where

germs of this type are found in the bacterial plaque, and adhere to the surfaces of the teeth to produce a large portfolio of glucan, which depends on the carbon source to develop it [4]. Teeth decay is a common health problem, and according to the definition of the World Health Organization (WHO), it is a localized infection caused by some types of pathogenic bacteria including: *S. mutans*, *Lactobacillus spp.*, *Actinomyces viscusus* as Oral flora. A suitable environment assists these bacteria to multiply rapidly and intensively and then attack the tooth tissues, starting with the enamel layer [5].

Grape plant

Grapes are one of the richest fruits and the most useful and profitable, and it has an effective role in building the body, straightening, restoring its

tissues and treating many diseases and ailments, as well as the eating it all year round in a fresh or dried form [6]. Grapes are at the forefront of fruit trees in terms of production and cultivated area and constitute one third of the world's fruit production [7]. The high nutritional value of grapes is because its fruits contain 15-25% sugars and 0.3-1.5% organic acids, in which tartaric acid prevails, in addition to containing a small amount of protein and some vitamins and mineral elements such as potassium, magnesium and calcium. [8,9]. Some studies have shown that the grape plant has a wide physiological and pharmacological effect, as it is used to treat many common diseases, and that it can inhibit or kill many pathogenic germs. In addition to being safe to use and its side effects are less harmful compared to commercial antibiotics [10].

MATERIALS AND METHODS

Preparation method:

Plant material

The plant material (fresh sour grapes) was obtained from grape trees in the city of Aleppo. Figure 1 represents the vine trees from which the verjuice was obtained.



Figure 1. Verjuice.

Extracts preparation

The botanical extract of verjuice was prepared as follows [11]:

- 1- verjuice was prepared by taking 100 g of fresh fruits after washing them with physiological serum, weighing them using a sensitive electronic scale, then mashing them well using a mortar.
- 2- The resulting juice was filtered to get rid of plant residues using filter paper.

- 3- The juice was filtered using microbial pore filters with a diameter of 0.45 micrometers to obtain sterile juice without exposing it to high temperatures that may lead to damage to the active substance.
- 4- The yield and its percentage were calculated, and its acidity was measured.

Study of the inhibitory activity of verjuice against the tested bacteria using the Agar well diffusion method:

The effectiveness of the extracts against the tested bacteria was tested using the pellet method according to [11]:

- 1- Muller-Hinton Agar was used to test for susceptibility to germs after being sterilized by autoclave and poured into sterile Petri dishes.
- 2- The bacterial suspension was prepared using a sterilized physiological solution (NaCl 0.9%) at a density of 0.5 McFarland ($10^8 \times \text{CFU/mL} 1.5$) and the dishes were inoculated with microorganisms using a sterile cotton swab homogeneously.
- 3- Drills were made in the agar using a sterile steel drill with a diameter of 8 mm.
- 4- Extracts were added to the agaric pits of different sizes (50-100) micro liters, noting that three replicates were prepared for each extract.
- 5- The plates were left for half an hour at room temperature and then incubated at 37°C for 24 hours
- 6- The inhibition zones were measured using a ruler and the arithmetic mean of the three replicates was taken.

Tested bacterial species

Bacterial isolates were obtained from the Microbiology Research Laboratory at the Faculty of Science, Aleppo University. Table 1 shows bacterial species that tested:

Table 1. Tested bacteria.

<i>Pseudomonas aeruginosa</i> (<i>P. aeruginosa</i>)
<i>Escherichia coli</i> (<i>E. coli</i>)
<i>Streptococcus mutans</i> (<i>S. mutans</i>)
<i>Staphylococcus aureus</i> (<i>S. aureus</i>)

A study of the sensitivity of the tested bacterial species to antibiotics under study

The sensitivity of the tested bacterial isolates to several antibiotics was studied using the disc diffusion method on Mueller-Hinton Agar medium. Standard antibiotic tablets were used for this purpose.

EXPERIMENT RESULTS

Calculation of the yield of plant extract

100 g of the plant material (verjuice) was weighed, and after mashing, squeezing, and filtering the juice, we got 54 g of juice, so the yield = 54% of the original weight.

Antagonistic activity of verjuice against the tested bacteria

The results showed the effectiveness of sour grapes juice against all tested Gram-positive and Gram-negative bacteria, as it showed the greatest inhibition against *S. aureus* with a diameter of 40 mm, followed by *E. coli* with a diameter of 28.33 mm. Table 2 shows the diameters of the inhibition zone of different extracts on gram-positive and gram-negative bacteria in mm. The effectiveness of extracts on positive bacteria more than negative bacteria can be explained by the difference in the composition of the cell wall, as the cell wall of

negative bacteria consists of several layers of complex structure, which is an obstacle to the entry of the extract. Figure 2 shows the effectiveness of the extracts on some tested bacteria.

Table 2. The effectiveness of the verjuice extracts on the bacterial species under Study.

Average diameter of the inhibition zone in mm				volume μ L
<i>S. mutans</i>	<i>S. aureus</i>	<i>P. Aeruginosa</i>	<i>E. coli</i>	
20	36.67	13	19	50
24.33	40	17.33	28.67	100

Sensitivity of the tested bacteria to standard antibiotics and their comparison with the effectiveness of verjuice:

The results showed that the most effective antibiotic on gram-positive and gram-negative bacteria was IPM, followed by CIP, while all bacterial isolates were resistant to both AMP and AMC antibiotics. Table 3 shows the results of the study of sensitivity of the tested bacteria to antibiotic.



(a) *Pseudomonas aeruginosa*.

(b) *Streptococcus mutans*

(c) *Escherichia coli*

Figure 2. Antibacterial Activity.

Table 3. Sensitivity of the tested bacteria against antibiotics and Verjuice extract.

	Antibiotic	Mic/ mcg	<i>S. aureus</i>	<i>S. mutans</i>	<i>E. coli</i>	<i>P. aeruginosa</i>
VA	Vancomycin	30	0	25	0	0
IMP	Imipenem	10	32	30	28	31
CAZ	Ceftazidime	30	0	0	16	0
CN	Gentamicin	10	18	15	18	17
AMC	Amoxicillin/Clavulanic acid	30	0	0	0	0
COT	Cotrimoxazole	25	0	0	0	0
CIP	Ciprofloxacin	5	31	24	11	0
AZM	Azithromycin	15	0	22	12	0
AMP	Ampicillin	10	0	0	0	0
LEV	Levofloxacin	5	25	27	18	10
Verjuice extract			40	24.33	28.67	17.33

CONCLUSION

By comparing the results of sensing the bacterial species to antibiotics with the results of their sensitivity to verjuice, it was found that the efficacy of sour grapes was superior to all the tested antibiotics against *S.aureus*, while its effectiveness was equal with that of IMP against *E. coli* and superior to the effectiveness of the rest of the antibiotics in the study. The effectiveness of verjuice was also equal to that of gentamicin and was superior to all studied antibiotics except for IMP against *P. aeruginosa*. The activity of sour grapes was equal with that of CIP, and it was close to that of AZM, LEV, and VA, and it was superior to the rest of the study antibiotics except for IMP against *S.mutans.*, and this may be explained by the different bacterial strains and their resistance in addition to the difference in the composition and quantity of active substances in conclusion considering that many nosocomial infections are caused by *Pseudomonas aeruginosa*, washing hands with Verjuice juice will reduce the spread of nosocomial infections and provide a reasonable alternative to alcohol-based hand washes [11].

Disclosure and conflict of interest: The authors declare that they have no conflicts of interest.

REFERENCES

- [1] B. Islam, S.N. Khan, & A. U. Khan, Dental caries: from infection to prevention. *Medical Science Monitor*, 2007, 13(11), pp RA196-RA203.
- [2] P. D. Marsh, Dental plaque as a biofilm: the significance of pH in health and caries. *Compendium of continuing education in dentistry* (Jamesburg, NJ: 1995), 2009, 30(2), 76.
- [3] J. M. Tanzer, J. Livingston, & A.M. Thompson, The microbiology of primary dental caries in humans. *Journal of dental education*, 2001,65(10), pp.1028-1037.
<https://doi.org/10.1002/j.0022-0337.2001.65.10.tb03446.x>
- [4] L. Samaranayake, "Essential Microbiology for Dentistry". 3rd ed. n Churchill. Livingstone. Elsevier, (2006).
- [5] A.H. Rogers. *Molecular Oral Microbiology*. Caister Academic Press. ISBN 978-1-904455-24-0 (2008).
- [6] V. Georgiev, A. Ananga, & V.Tsolova, Recent advances and uses of grape flavonoids as nutraceuticals. *Nutrients*, 2014, 6(1), pp.391-415.
<https://doi.org/10.3390/nu6010391>
- [7] S., Al-Majeed, J. Al-Nahafi, & Daoud D. Abdullah. The effect of adding different levels of foaming sulfur on some chemical soil properties in grape orchards. *Iraqi Journal of Agricultural Sciences*, 2005,36 (6), pp 23-29.
- [8] A. C. Hulme, *The Biochemistry of Fruit and Their Products*. N. Y. Academic Press. USA, pp 788,1971.
- [9] R. A., Kadhum, Effect of Pruning Severity on The Growth and Yield Quantity and Quality of Two Grape Cultivars. *Iraqi Journal of Agricultural Sciences*, 2009, 40(4), pp. 1-8.
- [10] L., Jahodar, Plants with hypoglycemic effects. *Gesk farm*, 1993, 42(6):pp. 251-259.
- [11] M. I, Okeke, A.S. Okoli, E.N. Eze, G. C Ekwume, E.U. Okosa, E& C.U. Iroegbu, Antibacterial activity of Citrus limonum fruit juice extract. *Pak Journal Pharm Sci*, 2015, 28(5), pp 1567-1571.
- [12] Al-Hamid, Al-Duwaihi Heba, The antibacterial activity of *Bacillus subtilis* isolated from the soil of the city of Aleppo against some pathogenic bacteria. Master's thesis, Faculty of Science, Aleppo University, 2016, Syria.

How to Cite

H. AlhamedAlduihi, M. . Rawal, and C. Khatib, "Inhibitory Effect of Verjuice Against Some Dental Decay Bacterial Species", *Al-Mustansiriyah Journal of Science*, vol. 34, no. 1, pp. 6–9, Mar. 2023.