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Antibacterial Activity of Different Vinegars Against Selected Pathogenic Bacteria

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Abstract:

This study aimed to evaluate the effectiveness of grape vinegar, apple cider vinegar, and organic apple cider vinegar against five types of pathogenic bacteria strains: two Gram-positive (*Staphylococcus* and *Streptococcus*) and three Gram negative (*Klebsiella*, *Escherichia coli*, and *Salmonella*). The well diffusion method was used to test different concentrations of each vinegar (25%, 50%, 75%, and 100%). Apple cider vinegar exhibited effectiveness against four of the tested bacterial strains, while organic apple cider vinegar was effective against three strains. Grape vinegar showed weak activity only against *Streptococcus*, whereas *Staphylococcus* showed complete resistance to all tested vinegars. Overall, most vinegar samples demonstrated variable antibacterial activity, except against *Staphylococcus*.

Keywords: Antibacterial, Vinegar, Pathogenic bacteria, Well diffusion.

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النشاط المضاد للبكتيريا لأنواع مختلفة من الخل ضد بكتيريا الممرضة

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الملخص

هدفت هذه الدراسة إلى تقييم فعالية خل العنب، وخل التفاح، وخل التفاح العضوي ضد خمسة أنواع من البكتيريا الممرضة؛ نوعان موجبا صبغة غرام (المكورات العنقودية والمكورات العقدية)، وثلاثة أنواع سالبة صبغة غرام (الكليبسيلة، والإشريكية القولونية، والسالمونيلا). استُخدمت طريقة انتشار الأقراص لاختبار تراكيز مختلفة من كل نوع من أنواع الخل (25%، 50%، 75%، و100%). أظهر خل التفاح فعالية ضد أربعة أنواع من البكتيريا المختبرة، بينما كان خل التفاح العضوي فعالاً ضد ثلاثة أنواع منها. اقتصرت فعالية خل العنب على بكتيريا المكورات العقدية فحسب، في حين أظهرت بكتيريا المكورات العنقودية مقاومة لجميع أنواع الخل المختبرة. وبشكل عام، أظهرت معظم عينات الخل نشاطاً مضاداً للبكتيريا بدرجات متفاوتة.

الكلمات المفتاحية: مضاد للبكتيريا، خل، بكتيريا ممرضة، اختبار الانتشار القرصي.

1. Introduction

Fruit vinegar, a product of acetic acid fermentation by bacteria, is a significant component of the daily human diet (Kelebek *et al.*, 2017). It has also been used in various pharmaceutical and nutritional industries (Singh and Garg, 2022). Historically, vinegar emerged with the dawn of agriculture, following the discovery of how to ferment fruits, grains, and vegetables to produce alcohol (Solieri and Giudici, 2009). Although vinegar is considered one of the lowest quality fermented foods, it is still widely used as a condiment and preservative, and in some countries as a nutritious

http://www.doi.org/10.62341/istj-vol38-2-ah66

beverage (Jingyi *et al*., 2009). Food poisoning is a foodborne illness, a critical global issue affecting both developed and developing nations, with significant implications for public health and economic development (Health and Threats, 2006). Food spoilage is known to be caused by a variety of pathogenic and saprophytic microorganisms, and is therefore responsible for foodborne illnesses (Malhotra *et al*., 2015).

An efficient sanitation process is the only way to produce food that is dependable and clean (Troller, 2012). There are various approaches that are frequently employed in food sanitation, and one of the most popular techniques for doing so at home is using vinegar. Vinegar is used not just for food sanitation but also in the making salad dressings and mayonnaise to lend its distinctive flavor to the food and keep it fresh for a long period. (Türker, 1963, Tan, 2005). Several previous studies have shown the composition and vinegar's health advantages (Budak *et al*., 2014). Although apples are used to make juices and sauces, it can be processed into products such as apple juice and apple cider vinegar, potentially representing a new product suitable for small scale production and a valuable dietary supplement. Apple cider vinegar has recently become popular due to its health benefits compared to the limited local alternatives.

In addition to its numerous culinary uses, vinegar can sometimes result from failed cider fermentation or unsuitable storage conditions, although its commercial production is a sophisticated process (Heikefelt, 2011). Both vinegar and cider are produced through fermentation; in the case of alcoholic fermentation, yeasts are used, and an additional aerobic fermentation step is required, where bacteria convert the ethanol in the cider to acetic acid. The microorganisms used in the fermentation processes and the chosen processing methods significantly influence the sensory properties of vinegar and cider (Downing, 1989; Lea, 1989). Cider also contains vitamins and polyphenols (vitamin C, folic acid, biotin, and B-

http://www.doi.org/10.62341/istj-vol38-2-ah66

complex vitamins (B1, B2, B6, and pantothenic acid), as well as trace amounts of minerals (such as magnesium, sodium, phosphorus, and potassium) (Tripathi and Mazumder, 2020).
Apple cider vinegar contains catechin, epicatechin, gallic acid, caffeic acid, coumaric acid, and chlorogenic acid (Budak *et al*., 2011). Grapes, on the other hand, contain water (74%), 25% sugars such as glucose and fructose, 0.8% organic acids such as malic and tartaric acids, 0.5% potassium, phenolic compounds, flavonoids, aromatic compounds, and 0.2% nitrogenous substances, making them a rich source of nutrients (Kocher and Gill, 2016). Grape vinegar also contains the same acids, in addition to fructolic acid, caffeic acid, and syringic acid (Budak and Guzel_Seydim, 2010). Vinegar possesses numerous medicinal properties, including antibacterial, antihypertensive, antioxidant, and cardioprotective effects, thanks to its bioactive compounds (Dávalos *et al*., 2005; Sugiyama *et al*., 2003). Apple cider vinegar is used to treat eczema (Lee *et al*., 2016). Its use as a complementary therapy is gaining popularity due to its antibacterial properties, which inhibit the growth and biofilm formation of various human skin pathogens, including *Staphylococcus aureus,* in vivo (Fraise *et al*., 2013). Although there is limited high-quality evidence to support the use of diluted apple cider vinegar baths for atopic dermatitis, one study reported that vinegar baths combined with topical treatment, alleviated the condition (Pham and Eberting, 2016). However, diluted apple cider vinegar compresses did not reduce the *Staphylococcus aureus* bacterial load on eczema-affected skin (Lim *et al*., 2019b).

2. Aims of study

This study was designed to evaluate and examine the antibacterial properties of apple cider vinegar, organic apple cider vinegar, and grape vinegar against several harmful, including gram positive

http://www.doi.org/10.62341/istj-vol38-2-ah66

bacteria and three types of gram negative bacterial species.

3. Materials and Methods

- **Vinegar Samples**

Natural, additive free apple cider vinegar and grape vinegar samples were purchased from a local store. A freeze dryer (Crest, Germany) was used to lyophilize 500 mL of each vinegar sample of each vinegar sample at -82 °C and 0.12 atm. Samples were obtained after freeze-drying. A stock solution (1 mg/mL) was prepared using distilled water for each sample, and these basic solutions were sterilized by filtration through a 0.45 µm pore-size membrane filter.

- **Microorganisms**

Five bacteria served as the test microorganisms for the antimicrobial activity screening.: Two Gram positive (*Streptococcus, Staphylococcus*), and three Gram-negative (*Escherichia coli, Salmonella, Klebsiella*).

- **Agar Well Diffusion Assay**

Antibacterial activity was assessed using the diffusion well method. A sterile cork borer was used to create wells in each of the nutrient agar (NA) plates inoculated with the bacteria used in the study. A sterile syringe was used to add approximately 100 µL of the different extracts to each well. After incubating the plates at 37°C for 18–24 hours, the inhibition zones were measured in millimeters using a Vernier caliper as described in (Mahmoudi *et al*. 2011).

4. Results

The outcomes of diffusion-based antibacterial action against some pathogenic bacteria shown that organic apple cider vinegar exhibited the largest inhibition zone (19 mm) against *E. coli* at a concentration of 100%As shown in the Figure (1) while grape vinegar showed the smallest inhibition zone (4 mm) against *Streptococcus* as shown in the fig. (8).

http://www.doi.org/10.62341/istj-vol38-2-ah66

whereas *Staphylococcus* bacteria were completely resistant to all vinegar varieties utilized in this investigation.

In addition, grape vinegar showed efficacy against *Streptococcus* bacteria only at a 100% concentration, with a zone of inhibition of 4 mm. As for apple cider vinegar, the largest zone of zone of inhibition was observed against *Escherichia coli* (15 mm), as shown in Fig. (1), and the smallest zone (7 mm) was recorded against *Klebsiella* Fig. (3). In contrast, *Staphylococcal* bacteria were resistant to this type of vinegar. When testing organic apple cider vinegar, the highest inhibition zone was found in *E. coli* bacteria (19 mm), and the lowest inhibition zone was found in *Klebsiella* and *Streptococcus* bacteria (6mm), fig. (3,7) whereas *Staphylococcus* bacteria showed resistance to this type of vinegar.

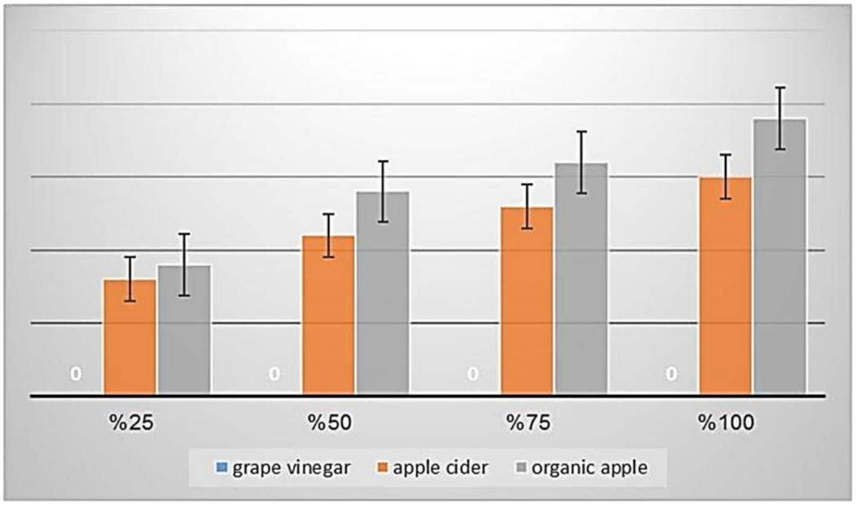

Fig. 1 Inhibition zones of grape vinegar, apple cider vinegar, and organic apple cider vinegar against *E. coli* at 100% concentration

http://www.doi.org/10.62341/istj-vol38-2-ah66

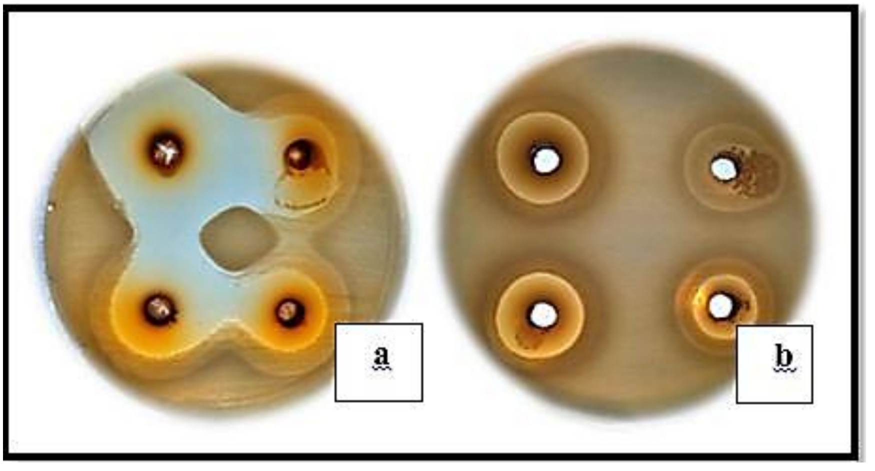

Fig.2 Inhibition zones of (a) organic apple cider vinegar and (b) apple cider vinegar against *E. coli* at 100% concentration.

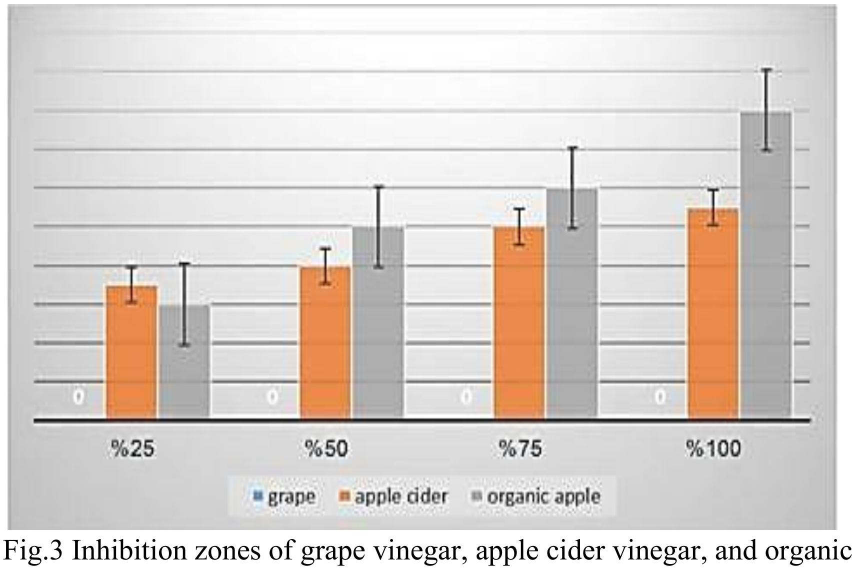

Fig.3 Inhibition zones of grape vinegar, apple cider vinegar, and organic apple cider vinegar against Klebsiella at 100% concentration shows the effect of the three types of vinegar on Klebsiella.

http://www.doi.org/10.62341/istj-vol38-2-ah66

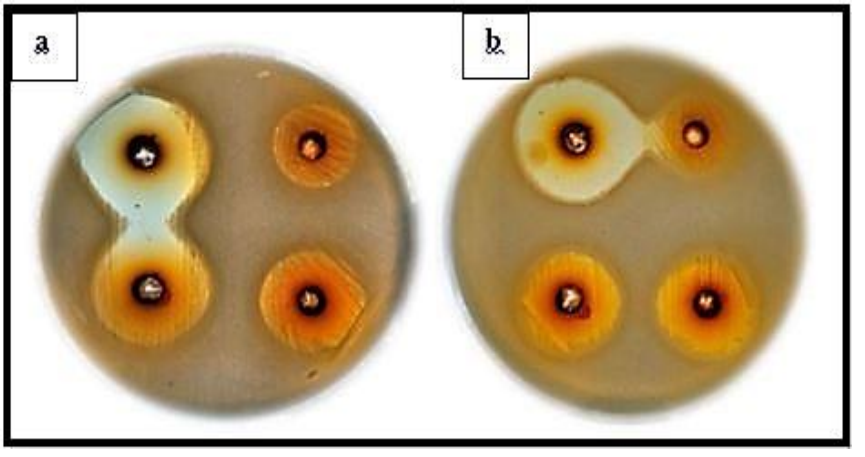

Fig. 4: Inhibition zones of (a) organic apple cider vinegar and (b) apple cider vinegar against Klebsiella at 100% concentration.

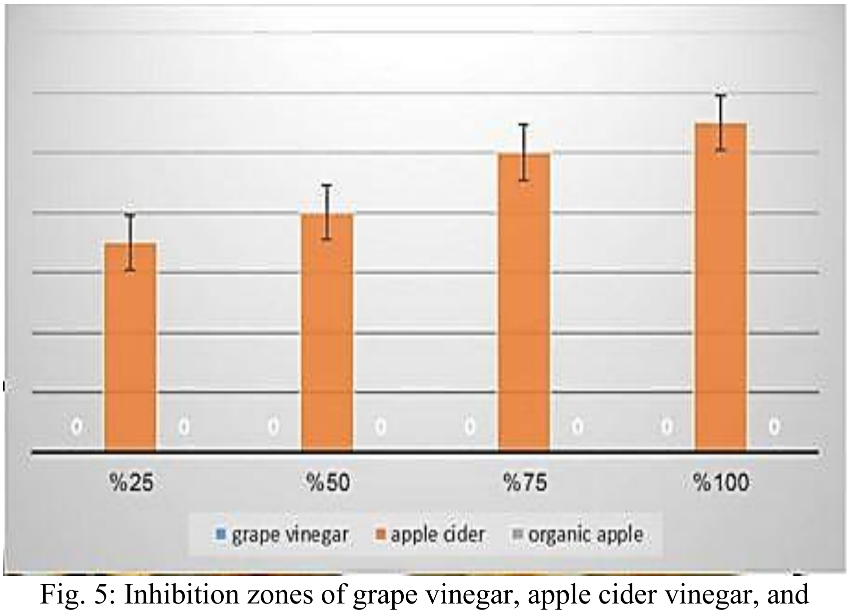

Fig. 5: Inhibition zones of grape vinegar, apple cider vinegar, and organic apple cider vinegar against Salmonella at 100% concentration.

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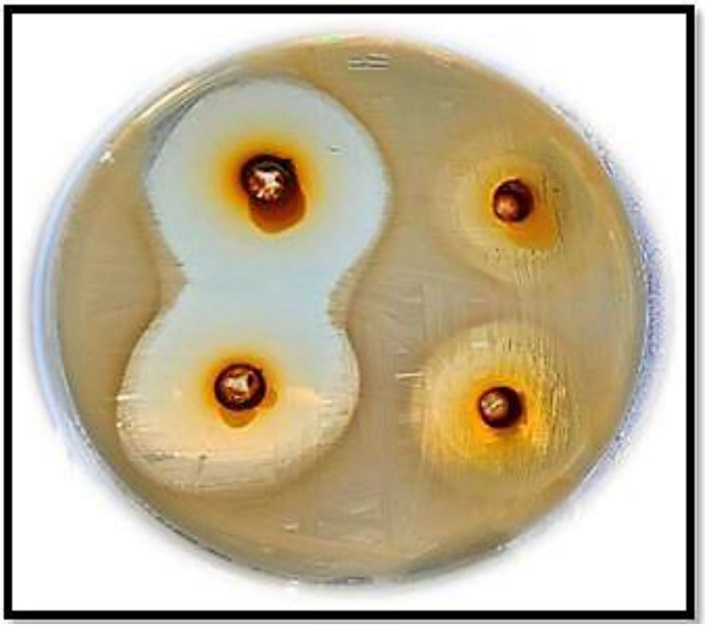

Fig. 6: Inhibition zones of grape vinegar, apple cider vinegar, and organic apple cider vinegar against Salmonella at 100% concentration.

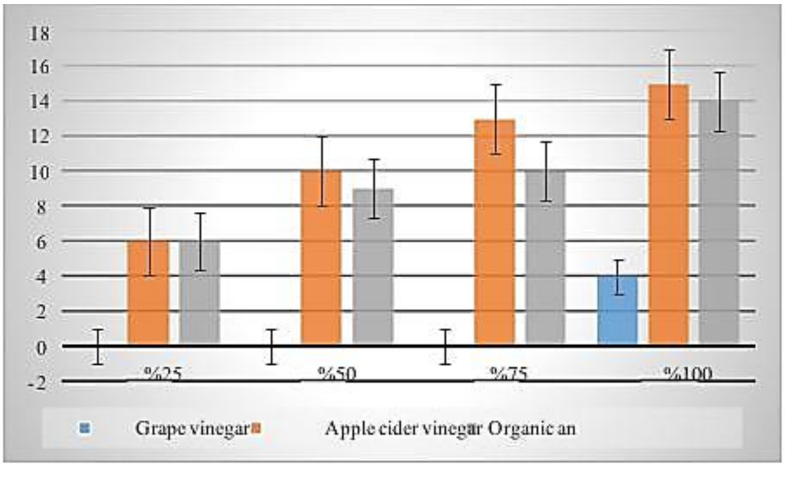

Fig. 7: Comparative inhibition zones (mm) of grape vinegar, apple cider vinegar, and organic apple cider vinegar against Streptococcus at different concentrations (25%, 50%, 75%, and 100%).

http://www.doi.org/10.62341/istj-vol38-2-ah66

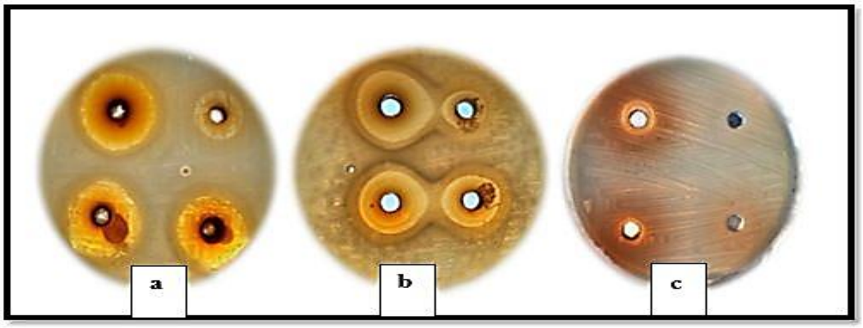

Fig. 8: Inhibition zones of (a) organic apple cider vinegar, (b) apple cider vinegar, and (c) grape vinegar against *Streptococcus* at 100% concentration.

5. Statistical analysis

revealed a significant effect of the treatment type (vinegar species). The calculated F-value was F = 50.538, which markedly exceeded the critical value (F_{crit} = 5.143). Furthermore, the probability value (P = 0.000176) was well below the significance threshold (α = 0.05), confirming significant variations among the tested groups. Regarding the concentrations, the calculated F-value was F = 3.662, which is lower than the critical value (F_{crit} = 4.757). The corresponding P-value was 0.082, suggesting that the different concentration levels didn't exert a statistically substantial impact on the measured parameters. Using the Least Significant Difference (LSD) test showed that both Organic and apple extracts significantly outperformed the grape extract. The Organic extract recorded the highest mean value (14.50), followed by the apple extract (11.75). The difference between the Organic and apple extracts (2.75) was less than the LSD value of 3.74, indicating no significant difference between these two treatments. In contrast, the grape extract yielded no measurable zones of inhibition, resulting in a statistically significant difference compared to the other treatments.

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http://www.doi.org/10.62341/istj-vol38-2-ah66

6. Discussion

The past few years have seen increasing intrigue with the research and development of antimicrobial agents. Because of the misuse of antimicrobial, bacteria have evolved complex opposition mechanisms to avoid elimination. Apple cider vinegar has gained popularity, becoming a staple in commercial health drinks and comprehensive treatment for many ailments (Singh and Garg, 2022). This study showed good results for the types of vinegar used against bacteria and the highest inhibition zone was achieved by organic apple cider vinegar in *E. coli* bacteria, this study agreed with (Yagnik *et al*., 2018) because vinegar contains antimicrobial and antioxidant compounds.

Although *Staphylococcus aureus* bacteria were resistant to all types of vinegar used in this study, this finding is consistent with those of Kavanaugh and Ribbeck (2012), as the peptidoglycan envelopes surrounding Gram-positive bacteria are several times thicker than those surrounding Gram-negative bacteria. While the other vinegar samples inhibited the growth of all the dangerous bacteria tested, grape vinegar was only successful in stopping the growth of *Streptococcus*. With the exception of *Staphylococcus aureus*, which showed resistance to all types of vinegar, this finding is consistent with those of Medina *et al*. (2007).

As for apple cider vinegar, the highest inhibition zone was in *E.coli,* fig.(1), the lowest inhibition zone was in *Klebsiella* bacteria fig.(3), while *Staphylococcus* and *Streptococcus* bacteria were resistant to this type of vinegar this study agreed with (Lim *et al*., 2019a). The current findings are in line with previous reports on the antibacterial action of vinegar (Ousaaid *et al*., 2021; Patole *et al*., 2022).

7. Conclusion

The tested bacteria (*Streptococcus*, *Klebsiella*, *E. coli*,

http://www.doi.org/10.62341/istj-vol38-2-ah66

and *Salmonella*) were variably affected by different concentrations of the vinegars, indicating that vinegar possesses selective antibacterial activity against certain Gram-negative and Gram-positive bacteria.

8. Recommendations

Vinegar may be recommended as a natural disinfectant and a potential alternative to chemical agents like sodium hypochlorite. It can also be used for surface sterilization and cleaning of vegetables, fruits, and meats. Vinegar is associated with numerous health benefits, including improved digestion, cardiovascular health, blood sugar regulation, and weight management.

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