Research Article

Antifungal and Antibacterial Activities of Apple Vinegar of Different Cultivars

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This study was designed to assess the antimicrobial potencies of apple vinegar against pathogenic microbes. The acidity and total phenolic content were carried out by titration with NaOH 0.1 N and the Folin–Ciocalteu method, respectively, while the spread plate method, agar well diffusion, and MIC assays were used to determine the antimicrobial activities of different vinegar samples. Acidity and phenolic content were dependent on the variety, where the highest values were observed in S2 with 4.02 ± 0.04% and 1.98 ± 0.05 mg GAE/mL for acidity and total phenolic content, respectively. The spread plate method revealed that samples S1 and S2 obtained from the Red delicious variety and Golden delicious variety, respectively, inhibit the growth of all tested strains, while S3 obtained from different varieties and S4 obtained from the Gala royal variety inhibit only two microbes (Escherichia coli and Vibrio cholerae). Sample S1 presented moderate antimicrobial effect against all examined strains with a diameter of inhibition ranging from 11 ± 0.7 to 19 ± 0.5 mm and with MIC values ranging between 1/2 and 1/100. The findings of the current study confirm the usefulness of apple vinegar as a natural sanitizer that inhibits the growth of pathogenic microbes.

1. Introduction

The development of different methods used to produce food products is closely related to the reduction of infections caused by microorganisms. Moreover, food-borne epidemics continue to be a major public health problem. The profuse use of chemical antibacterial agents is harmful to human health and enhances the incidences of drug-resistant pathogens [1]. Natural products are healthy and safe products that offer antimicrobial effects and antioxidant properties simultaneously [2, 3].

Apple vinegar provides several pharmacological effects, for instance, antidiabetic effect [4–6], anti-Alzheimer effect [7], and antioxidant properties [2]. In addition, the administration of apple vinegar controls body weight gain and enhances glucose tolerance [8]. In experimental trials, the ability of apple vinegar as a natural product has been proved against human pathogens [9]. Thereafter, the sanitizing properties of vinegar have been reviewed in several studies. They reported that apple vinegar has an inhibitory effect against different bacterial strains such as Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus pyogenes, Enterococcus faecalis, Streptococcus pneumoniae, Pseudomonas aeruginosa, Pseudomonas fluorescens, Escherichia coli, Salmonella typhi, Enterobacter aerogenes, Klebsiella pneumoniae, Proteus mirabilis, Proteus vulgaris, and Acinetobacter [10, 11]. The remedial properties of apple vinegar are ascribed to its organic acids and its bioactive substances. It has been shown that organic acids pass into bacterial membranes which increases the synthesis of antimicrobial peptides, increases internal osmotic pressure, stimulates the consumption of energy, and sabotages macromolecular synthesis [12]. In addition to organic acids, apple vinegar contains other bioactive compounds that proved their antimicrobial potencies such as phenolic acids and flavonoids [3, 13–17].

In this vision, the present study was designed to determine the acidity and the phenolic content of different
vaccine samples as well as their possible antimicrobial action against three bacterial pathogens and two fungal strains.

2. Materials and Methods

2.1. The Sampling of Apple Vinegar. The chosen samples of vinegar were produced by the artisanal process using three varieties of apples as presented in Table 1.

2.2. Bacterial and Fungal Strains. A total of five microbial strains, three bacterial strains, and two yeast isolates were used to examine the antimicrobial ability of different vinegar samples. The bacterial strains were represented by *Salmonella typhi* (CIP 5535), *Escherichia coli* (CIP 54127), and *Vibrio cholerae* non-O1-non-O139 isolated from Tamoda Bay [18], while the tested yeasts were represented by *Candida albicans* (IPL CIM 861484) and *Candida tropicalis* (Pfizer CIM 4069).

2.3. Determination of Acidity and Total Polyphenolic Content (TPC). The acidity of different studied samples was determined by titration with 0.1 N NaOH. Results were expressed as a percentage of acetic acid equivalent, while the quantification of the polyphenolic content was carried out using the Folin–Ciocalteu method. Results were expressed as mg of gallic acid equivalent per mL of vinegar (mg GAE/mL) [19].

2.4. Antimicrobial Assay

2.4.1. Spread Plate Method. The test was performed by mixing 100 μL of inocula diluted in physiological saline from broth grown overnight and 4 mL of each sample. The mixture was left for 5 min, and then 100 μL of each mixture was inoculated onto an agar medium. The tests were replicated three times [20].

2.4.2. Sensitivity Assay

*(1) Agar Well Diffusion Method.* The antimicrobial activity of different samples of vinegar was evaluated using the well diffusion method [10, 21]. The isolates were subjected to antimicrobial activity by the method mentioned above. *Salmonella typhi*, *Escherichia coli* O157:H7, and *Vibrio cholerae* were grown in the Tryptone Casein Soja (TCS) medium, and yeasts *Candida albicans* and *Candida tropicalis* were grown in Sabouraud Broth. 100 μL of the active culture of different isolates consisting of 0.5 McFarland 1 × 10⁸ CFU/mL was prepared in physiological saline. 40 μL of the vinegar sample was placed in 5 mm diameter wells that have been cut in the agar of each Petri dish. Negative control wells were filled with sterile physiological water. Petri dishes were incubated at 37°C for 24 h for bacterial strains and at 30°C during 24–48 h for yeasts. Thereafter, the diameter of inhibition zones (DIZ) was measured.

2.4.3. Minimum Inhibitory Concentration Assay (MIC). The MIC was determined using a microdilution method according to the NCCLS method [21]. Firstly, dilution series of vinegar samples were prepared (S (initial solution), 1/2, 1/4, 1/6, 1/8, 1/10, 1/12, 1/100, and 1/150). Ten μL of each prepared dilution was added to 180 μL of TCS broth and 10 μL of suspension of the active culture of different microorganisms tested (1 × 10⁸ CFU/mL) in microplate wells. The plates were incubated at 37°C for 20 h for bacterial strains and were incubated at 30°C during the same time for yeasts. To reveal the growth of different studied microorganisms, we added to each well 20 μL of the aqueous solution of 0.5% TTC (2,3,5-triphenyltetrazolium chloride), and then plates were incubated at 37°C for 30 min. The lowest dilution with no growth observed was defined as MIC (disappearance of red color after TTC addition) [22].

2.5. Statistical Analysis. ANOVA followed by the Tukey test was used for statistical analysis (*P* < 0.05) to show if there is any significant difference between samples.

3. Results

3.1. Sample Characterization. Table 2 represents the results of acidity and total phenolic content of different vinegar samples. It is shown that sample S1 had the highest acidity (4.02 ± 0.04%), while the lowest acidity value was registered in sample S4 (0.78 ± 0.07%). Concerning TPC, the highest value (1.98 ± 0.05 mg GAE/mL) was recorded in sample S1, while S4 recorded the lowest value (0.47 ± 0.06 mg GAE/mL).

3.2. The Antimicrobial Ability of Apple Vinegar

4. Results of the Spread Plate Method. The results of the spread plate revealed that the growth of different organisms tested was not observed in plates in the presence of samples S1 and S2 as presented in Table 3. The outcomes indicate that samples S1 and S2 were the most effective against all microbes because they inhibited the growth of all tested microorganisms.

4.2. Results of the Sensitivity Assay. Concerning antimicrobial activity, the vinegar samples were found to have a strong ability to inhibit the growth of microorganisms tested in the present work. Sample S1 produced the best antimicrobial effect against all strains with an inhibition diameter ranging between 12 and 19 mm, whilst sample S4 established the lowest antimicrobial activity. *Escherichia coli* O157:H7 was the most sensible bacterial strain. On the contrary, samples

| Table 1: The sampling of apple vinegar. |
|-----------------------------|-----------------------------|
| Samples | Source | Varieties of apple | Stations |
| S1 | Herbalist Iklil Al-Jabal | Red delicious | Midelt |
| S2 | Herbalist Iklil Al-Jabal | Golden delicious | Midelt |
| S3 | Cooperative Al Jazeera | Different varieties | Midelt |
| S4 | Cooperative Domaine Chêne Vert | Gala royal | Sefrou |
The obtained results are in line with previous than those determined by Moroccan legislation [25]. Furthermore, the obtained results are in line with previous reports [26, 27]. Acidity is a key factor to determine the quality of apple vinegar, and the minimum of vinegar acidity is set at 5 grams of acetic acid equivalent per 100 mL [25]. Organic acids’ content determines the flavoring profile of vinegar and depends on the raw material [26]. In addition, vinegar’s organic acids play a crucial role in different properties of vinegar such as antimicrobial activities, anti-diabetic effects, and anticancer activities [6, 10, 11, 28].

Concerning the phenolic content, the highest value was found in S1. It is considered as an important criterion for quality evaluation [3]. The phenolic content values varied in the range of 1.98 ± 0.05 (S1) to 0.47 ± 0.06 mg GAE/100 mL (S4). These results are in agreement with those reported by Du et al. and Kim et al. [29, 30]. The polyphenolic content of apple vinegar is highly related to the variety of apples, maturity, and geographic location [31]. Secondary metabolites constitute a defense weapon of plants against different pathogenic agents such as fungi, viruses, and bacteria [32]. The efficacy of natural products could be related to their content of bioactive compounds which provide their broad spectrum of antimicrobial activities [33, 34].

Many pathogenic microbes can survive despite the use of antimicrobial chemicals. In the present study, we examined the ability of four vinegar samples to eradicate different pathogenic microbes (bacteria and yeasts). S1 exerted a good activity against all tested microbes with diameter zones ranging from 11 to 12 mm for yeasts and 16 to 19 mm for bacteria, while other samples showed weak antimicrobial activity. The antimicrobial activity could be due to the presence of organic acids, especially acetic acid. Sample S1 registered the highest value of acidity and showed remarkable antimicrobial potency. Our findings are partially coherent with the published studies reporting the efficacy of apple vinegar against Salmonella and Escherichia coli [35], Strep-tococcus pyogenes [36], Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes, Enterococcus faecalis, Streptococcus pneumoniae, Pseudomonas aeruginosa, Pseudomonas fluorescens, Enterobacter aerogenes, Klebsiella pneumoniae, Escherichia coli, Salmonella typhi, Proteus mirabilis, Proteus vulgaris, and Acinetobacter [10], Aspergillus niger, Aspergillus flavus, and Candida albicans [37].
Table 4: Diameters of inhibition zones (DI) of vinegar samples.

| Organism          | S1     | S2     | S3     | S4     |
|-------------------|--------|--------|--------|--------|
| **Bacteria**      |        |        |        |        |
| Salmonella typhi  | 15 ± 0.3 | 14 ± 0.9 | No effect | No effect |
| Escherichia coli O157:H7 | 19 ± 0.5 | 13 ± 0.6 | 8 ± 0.3 | 8 ± 0.7 |
| Vibrio cholerae   | 16 ± 0.8 | 14 ± 0.4 | 11 ± 0.5 | 9 ± 0.2 |
| Candida albicans  | 12 ± 0.5 | No effect | No effect | No effect |
| Candida tropicalis| 11 ± 0.7 | No effect | No effect | No effect |
| **Yeast**         |        |        |        |        |
| Candida tropicalis|        |        |        |        |

Table 5: Minimum inhibitory concentration (MIC) of vinegar samples.

| Sample | S. typhi | E. coli | V. cholerae | C. albicans | C. tropicalis |
|--------|---------|---------|-------------|-------------|---------------|
| S1     | 1/6     | 1/100   | 1/2         | 1/2         | 1/2           |
| S2     | S       | S       | S           | No effect   | No effect     |
| S3     | No effect | S      | No effect   | No effect   | No effect     |
| S4     | No effect | S      | No effect   | No effect   | No effect     |

6. Conclusion

In the present work, the evaluated apple vinegar samples, especially S1, demonstrated an adequate antimicrobial potency against different studied strains. Functional properties of apple vinegar could be related to the presence of organic acids and phenolic compounds. Vinegar, as an organic product, could be used as a natural sanitizer and also as a bioactive ingredient in the food industry.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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References

[1] M. Ahmad and A. U. Khan, "Global economic impact of antibiotic resistance: a review," *Journal of Global Antimicrobial Resistance*, vol. 19, pp. 313–316, 2019.
[2] S. Bakir, G. Toydemir, D. Boyacioglu, J. Beekwilder, and E. Capanoglu, "Fruit antioxidants during vinegar processing: changes in content and in vitro bio-accessibility," *International Journal of Molecular Sciences*, vol. 17, no. 10, p. 1658, 2016.
[3] D. Ousaaid, H. Imtara, H. Laaroussi, B. Lyoussi, and I. Elarabi, "An investigation of Moroccan vinegars: their physico-chemical properties and antioxidant and antibacterial activities," *Journal of Food Quality*, vol. 2021, Article ID e6618444, 2021.
[4] J. Lheman, A. Sutiono, Y. Yanti, R. R. Tjandrawinata, and B. W. Lay, "Functional Bignay Ciders inhibit key enzymes linked to obesity and diabetes for metabolic syndrome protection," *Journal Teknologi*, vol. 83, no. 2, pp. 67–75, 2021.
[5] H. Ahmadniaye Motlagh, A. Javadmanesh, and O. Safari, "Improvement of non-specific immunity, growth, and activity of digestive enzymes in *Carassius auratus* as a result of apple cider vinegar administration to diet," *Fish Physiology and Biochemistry*, vol. 46, no. 4, pp. 1387–1395, 2020.
[6] D. Ousaaid, H. Laaroussi, M. Bakour et al., "Beneficial effects of apple vinegar on hyperglycemia and hyperlipidemia in..."
hypercaloric-fed rats," Journal of Diabetes Research, vol. 2020, Article ID 9284987, 7 pages, 2020.

[7] S. Tripathi and P. M. Mazumder, "Apple cider vinegar (ACV) and their pharmacological approach towards alzheimer’s disease (AD): a review," Indian Journal of Pharmaceutical Education and Research, vol. 54, no. 25, pp. 667–74, 2020.

[8] R. Urtasun, J. Díaz-Gómez, M. Araña et al., "A combination of apple vinegar drink with Bacillus coagulans ameliorates high fat diet-induced body weight gain, insulin resistance and hepatic steatosis," Nutrients, vol. 12, no. 9, pp. 2504, 2020.

[9] W. A. Rutala, S. L. Barbee, N. C. Aguiar, M. D. Sobsey, and D. J. Weber, "Antimicrobial activity of home disinfectants and natural products against potential human pathogens," Infection Control & Hospital Epidemiology, vol. 21, no. 1, pp. 33–38, 2000.

[10] N. K. Hindi, "In vitro antibacterial activity of aquatic garlic extract, apple vinegar and apple vinegar-garlic extract combination," American Journal of Phytomedicine and Clinical Therapeutics, vol. 1, no. 1, pp. 42–51, 2013.

[11] N. K. Hindi, Z. K. A. Al-Mahdi, and Z. A. G. Chaback, "Antibacterial activity of the aquatic extract of fresh, dry powder ginger, apple vinegar extract of fresh ginger and crude oil of ginger (zingiber officinale) against different types of bacteria in Hilla City, Iraq," Prostate, vol. 3, p. 6, 2014.

[12] J. Zhang, Z. G. Tian, J. H. Wang, and A. R. Wang, "Advances in antimicrobial molecular mechanism of organic acids," Acta Veterinaria et Zootecnica Sinica-Chinese Journal of Animal Science and Veterinary, vol. 42, no. 3, pp. 323–328, 2011.

[13] C. A. Cherrington, M. Hinton, G. C. Mead, and I. Chopra, "Organic acids: chemistry, antibacterial activity and practical applications," Advances in Microbial Physiology, vol. 32, pp. 87–108, 1991.

[14] T. X. Xia, B. Zhang, W. Duan, J. Zhang, and M. Wang, "Nutrients and bioactive components from vinegar: a fermented and functional food," Journal of Functional Foods, vol. 64, Article ID 103681, 2020.

[15] B. Zhang, T. Xia, W. Duan et al., "Effects of organic acids, amino acids and phenolic compounds on antioxidiant characteristic of Zhenjiang aromatic vinegar," Molecules, vol. 24, no. 20, p. 3799, 2019.

[16] A. Adamczak, M. Ozarowski, and T. M. Karpiński, "Antibacterial activity of some flavonoids and organic acids widely distributed in plants," Journal of Clinical Medicine, vol. 9, no. 1, p. 109, 2020.

[17] M. M. Cowan, "Plant products as antimicrobial agents," Clinical Microbiology Reviews, vol. 12, no. 4, pp. 564–582, 1999.

[18] B. Abdelkhaled, M.-L. Quilici, F. Abdelaziz, and C. Nozha, "Occurrence of pathogenic Vibrio species in Tamouda Bay (Morocco)," Journal of Microbiology Research, vol. 3, no. 6, pp. 240–246, 2013.

[19] D. Ousaid, I. Mansouri, H. Laaroussi, B. Lyoussi, and I. El Arabi, "Physicochemical properties and antioxidiant activity of two varieties of apple cultivated in different areas in Morocco," Mediterranean Journal of Chemistry, vol. 10, no. 4, pp. 371–377, 2020.

[20] J. M. Jay, M. J. Loessner, and D. A. Golden, Modern Food Microbiology, Springer Science & Business Media, Berlin, Germany, 2008.

[21] Clinical and Laboratory Standards Institute, "Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard," CLSI Document M07-A9, Vol. 32, Clinical and Laboratory Standards Institute, Wayne, PA, USA, 2012.

[22] D. Dimitrijević, M. Stanković, Z. Stojanović-Radić, V. Ranđelović, and D. Laksarcon, "Antioxidant and antimicrobial activity of different extracts from leaves and roots of Jovibara heuffelii (Schott.) A. Lve and D. Lve," Journal of Medicinal Plants Research, vol. 6, no. 33, pp. 4804–4810, 2012.

[23] J. S. Molton, P. A. Tambah, B. S. P. Ang, M. L. Ling, and D. A. Fisher, "The global spread of healthcare-associated multidrug-resistant bacteria: a perspective from Asia," Clinical Infectious Diseases, vol. 56, no. 9, pp. 1310–1318, 2013.

[24] C. Veeresham, "Natural products derived from plants as a source of drugs," Journal of Advanced Pharmaceutical Technology & Research, vol. 3, no. 4, p. 200, 2012.

[25] "DEC.2-10-385.FR.pdf," 2020, http://www.onssa.gov.ma/images/reglementation/reglementation-sectorielle/vegetaux-et-produits-dorigine-vegetaux/Produits_dalimentaires/DEC.2-10-385.FR.pdf.

[26] V. K. Joshi and S. Sharma, "Cider vinegar: microbiology, technology and quality," in Vinegars of the World, pp. 197–207, Springer, Berlin, Germany, 2009.

[27] N.-H. Sung, S.-M. Woo, J.-H. Kwon, S.-H. Yeo, and Y.-J. Jeong, "Quality characteristics of high acidity apple vinegar manufactured using two stage fermentation," Journal of the Korean Society of Food Science and Nutrition, vol. 43, no. 6, pp. 877–883, 2014.

[28] N. Baba, Y. Higashi, and T. Kanekura, "Japanese black vinegar "Izumi" inhibits the proliferation of human squamous cell carcinoma cells via necroptosis," Nutrition and Cancer, vol. 65, no. 7, pp. 1093–1097, 2013.

[29] G. Du, Y. Zhu, X. Wang et al., "Phenolic composition of apple products and by-products based on cold pressing technology," Journal of Food Science & Technology, vol. 56, no. 3, pp. 1389–1397, 2019.

[30] S.-H. Kim, H.-K. Cho, and H.-S. Shin, "Physicochemical properties and antioxidant activities of commercial vinegar drinks in Korea," Food Science and Biotechnology, vol. 21, no. 6, pp. 1729–1734, 2012.

[31] L. Solieri and P. Giudici, Vinegars of the World, Springer, Berlin, Germany, pp. 1–16, 2009.

[32] B. C. Freeman and G. A. Beattie, "An overview of plant defenses against pathogens and herbivores," The Plant Health Instructor, 2008.

[33] W. Bilahutra, T. Techowisan, F. J. Peberdy, and S. Lumyong, "Antimicrobial activity of bioactive compounds from Periconia siamensis CMUGE015," Research Journal of Microbiology, vol. 2, no. 10, pp. 749–755, 2007.

[34] M. B. G. Viswanathan, J. D. Jeya Ananthi, and P. Sathish Kumar, "Antimicrobial activity of bioactive compounds and leaf extracts in Jatropha tanjorensis," Fitoterapia, vol. 83, no. 7, pp. 1153–1159, 2012.

[35] Q. A. Shah, F. Bibi, and A. H. Shah, "Anti-microbial effects of olive oil and vinegar against salmonella and Escherichia coli," PIST, vol. 14, no. 2, pp. 479–486, 2013.

[36] N. F. Ismael, ""Vinegar" as anti-bacterial biofilm formed by Streptococcus pyogenes isolated from recurrent tonsillitis patients, in vitro," Jordan Journal of Biological Sciences, vol. 6, no. 3, 2013.

[37] H. B. Jabir, F. N. Abbas, and R. M. Khalaf, "In vitro assessment of antifungal potential of apple cider vinegar and acetic acid versus fluconazole in clinical isolates of otomycosis," Thi-Qar Medical Journal, vol. 5, no. 1, pp. 126–133, 2011.

[38] M. K. Wali and M. M. Abed, "Antibacterial activity of acetic acid against different types of bacteria causes food spoilage," Plant Archives, vol. 19, no. 1, pp. 1827–1831, 2019.
[39] A. Adamczak, M. Ozarowski, and T. M. Karpiński, "Antibacterial activity of some flavonoids and organic acids widely distributed in plants," Journal of Clinical Medicine, vol. 9, no. 1, 2019.
[40] D. H. Carroll, F. Chassagne, M. Dettweiler, and C. L. Quave, "Antibacterial activity of plant species used for oral health against Porphyromonas gingivalis," PLoS One, vol. 15, no. 10, Article ID e0239316, 2020.
[41] A. Borges, C. Ferreira, M. J. Saavedra, and M. Simões, "Antibacterial activity and mode of action of ferulic and gallic acids against pathogenic bacteria," Microbial Drug Resistance, vol. 19, no. 4, pp. 256–265, 2013.
[42] L. Bouarab-Chibane, V. Forquet, P. Lantéri et al., "Antibacterial properties of polyphenols: characterization and QSAR (quantitative structure-activity relationship) models," Frontiers in Microbiology, vol. 10, p. 829, 2019.
[43] A. Pernin, L. Guillier, and F. Dubois-Brissonnet, "Inhibitory activity of phenolic acids against Listeria monocytogenes: deciphering the mechanisms of action using three different models," Food Microbiology, vol. 80, pp. 18–24, 2019.
[44] B. Botton, A. Breton, M. Febre et al., Useful and Harmful Moulds. Industrial Importance, Masson, Paris, France, 2nd edition, 1990.
[45] A. Djilani and A. Dicko, "The therapeutic benefits of essential oils," Nutrition: Tips for Improving Your Health, vol. 7, pp. 155–179, 2012.
[46] S. Tan, J. Gao, Q. Li et al., "Synergistic effect of chlorogenic acid and levofloxacin against Klebsiella pneumonia infection in vitro and in vivo," Scientific Reports, vol. 10, no. 1, p. 20013, 2020.
[47] R. S. Carvalho, C. A. Carollo, J. C. de Magalhães et al., "Antibacterial and antifungal activities of phenolic compound-enriched ethyl acetate fraction from Cochlospermum regium (mart. Et. Schr.) Pilger roots: mechanisms of action and synergism with tannin and gallic acid," South African Journal of Botany, vol. 114, pp. 181–187, 2018.