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ASSESSMENT OF ANTIBACTERIAL PROPERTIES OF *Vitis vinifera* AND *Annona Cherimola* SEED EXTRACTS AGAINST *Staphylococcus aureus* AND *Pseudomonas aeruginosa*

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ABSTRACT

Medicinal plants are increasingly investigated as potential alternatives to synthetic antimicrobials. This study assessed the antibacterial effects of seed extracts of *Vitis vinifera* (grape) and *Annona cherimola* (cherimoya) against clinical isolates (*Staphylococcus aureus* and *Pseudomonas aeruginosa)*. Seed powders (250g) were extracted with 70% ethanol, screened for phytochemicals, and tested for antibacterial activity by agar well diffusion at 100-12.5 mg/ml. Ciprofloxacin served as a positive control, while 0.1ml of 2% dimethyl sulfoxide (DMSO) was the negative control. Phytochemical screening revealed that *Vitis vinifera* seed extract contained flavonoids, tannins, phenols, terpenoids, glycosides and quinine-like compounds; *Annona cherimola* seed extract contained flavonoids, terpenoids, glycosides and quinine-type compounds, but not tannins, phenols, alkaloids or saponins. The phytochemical screening indicates that the *Vitis vinifera* seed extract is richer in tannins and phenols than *Annona cherimola* seed extract thus, demonstrated significant inhibitory activity against both test organisms. *Staphylococcus aureus* was more sensitive, with minimum inhibitory and bactericidal concentrations determined at 25 mg/ml and 50 mg/ml, respectively. Conversely, *Annona cherimola* seed extract exhibited no significant antibacterial effect on both isolates. The results obtained in this study indicate that *Vitis vinifera* seeds contain bioactive constituents with potential antibacterial properties, particularly against *Staphylococcus aureus*. *Vitis vinifera* seed extract may therefore represent a promising natural candidate for the development of plant-based antimicrobial agents.

Keywords: Seed extracts, *Vitis vinifera*, *Annona cherimola*, Phytochemicals, Antibacterial activity.

INTRODUCTION

Medicinal plants offer a vast reservoir of chemically diverse bioactive compounds, which continue to inspire pharmaceutical innovation and complement synthetic drug discovery strategies (Nasim et al.,2022). Latest advances in phytochemistry and bioprocessing have highlighted strategies for improving yields of pharmacologically active metabolites and standardizing plant extracts for clinical use (khosroshahi et al., 2025), while recent progress in analytical chemistry, high-throughput screening, and omics approaches has further enabled the discovery, optimization, and standardization of phytochemicals for medicinal use (Chaachouay, 2024; Hassan ul et al., 2024; Integration of high-throughput omics technologies for phytomedicine discovery, 2023). Moreover, plant-derived natural products remain a cornerstone of drug development pipelines, bridging traditional knowledge with contemporary biomedical applications (Chaachouay, 2024)

Among botanicals of interest, *Vitis vinifera* (grape) and *Annona cherimola* (cherimoya) represent contrasting models in natural product research. Grapes are globally cultivated and widely appreciated for their phenolic-rich profile, particularly flavonoids, phenolic acids, stilbenes, and proanthocyanidins, which vary in distribution across seed, peel, and pulp compartments. These compounds have demonstrated potent antioxidant, anti-inflammatory, antimicrobial, and neuroprotective effects in preclinical studies (Dietary Phytochemicals in Health and Disease, 2025; The Benefits of Grape Seed Extract in Neurological Disorders, 2023; In Vitro Mechanistic Studies of a Standardized Grape Seed Extract, 2024). For example, a standardized grape seed extract (GSEe) has shown promising bioactivities relevant to cognitive function and mood regulation in mechanistic in vitro assays (Hasbal-Celikok et al., 2024).

In contrast, *A. cherimola*, a member of the Annonaceae family, is notable both for its ethnomedicinal use and the presence of *annonaceous acetogenins* compounds implicated in neurotoxicity in some epidemiological and experimental settings. Epidemiological associations between chronic consumption of Annonaceae fruits and

increased risk of atypical Parkinsonism or cognitive decline in specific regions have spurred renewed caution and targeted biochemical investigations (Unraveling Nature's Pharmacy, 2025). Nonetheless, recent studies report that both leaf and seed extracts of *A. cherimola* also exhibit significant cytotoxic and pharmacological effects against various microbial species and cell lines of breast, colon, liver and prostate cancers; including apoptosis induction in cancer cell lines highlighting their dual bioactivity and potential toxicity (El-Hallouty et al., 2020; Haykal et al., 2019; Macuer-Guzman et al., 2023).

Given these divergent properties, a comparative or combined approach to studying *V. vinifera* and *A. cherimola* seed extracts is timely. Specifically, a focused study of their phytochemical profiles, antimicrobial potential, and safety/toxicity characteristics may uncover synergistic benefits or highlight risks. This study assessed the phytochemicals, antimicrobial, cytotoxic activities, and safety potentials of *Vitis vinifera* and *Annona cherimola* seed extracts.

METHODOLOGY

Sample Collection and Preparation: Fully ripe grapes and cherimoya fruits were purchased from a local market in Bida town, Niger State. Seeds were separated from pulp, washed with distilled water, and oven-dried at 60 °C until constant weight. Dried seed material was ground into fine powder and stored in sterile, sealed containers**.**

Extraction: For each plant species, 250 g of seed powder was macerated in 700 ml of 70% ethanol for 48 h at ambient temperature, with intermittent stirring. The mixture was filtered (Whatman No.1), and the filtrate was concentrated by drying in an oven at 50 °C to obtain crude ethanol extract. (Manasa et al., 2014).

Phytochemical Screening: Qualitative phytochemical assays were carried out on both extracts to detect the presence of flavonoids, phenols, tannins, terpenoids, glycosides, cardiac glycosides, anthraquinones, alkaloids, saponins, steroids following standard protocols (Harborne, 2023; Edeoga et al., 2022; Ogbeba et al. 2017).

Microbial Strains and Identification: Clinical isolates of *Staphylococcus aureus* and *Pseudomonas aeruginosa* were obtained from General Hospital, Bida, and transported on agar slants in an ice box. Reconfirmation used Gram staining and biochemical assays (catalase, coagulase, oxidase, citrate) and selective culture media (Mannitol Salt Agar for S. aureus; Cetrimide Agar for *P. aeruginosa*) following Cheesbrough (2009).

Standardization and Inoculum Preparation: Test isolates were grown overnight on Mueller–Hinton agar, and colonies were suspended in Mueller–Hinton broth to match 0.5 McFarland turbidity standard (Clsi,2021; Olutiola et al.,2000).

Preparation of Extract Dilutions: Each extract was dissolved in 2% dimethyl sulfoxide (DMSO). A stock solution of 100 mg/mL was prepared, then serial dilutions made to yield 50, 25, and 12.5 mg/ml**.**

Antibacterial Assay (Susceptibility Test) – Agar Well Diffusion: Mueller–Hinton agar plates were uniformly inoculated with 0.1 ml of standardized bacterial suspension (spread plate technique). Wells (6 mm diameter) were bored into agar, and 0.1 ml of each extract concentration (100mg/ml, 50mg/ml,25mg/ml and 12.5mg/ml) was added into respective wells. Plates were left for 4–5 h to allow diffusion, then incubated at 37 °C for 18–24 h. 2% DMSO served as negative control, while ciprofloxacin (appropriate concentration) was positive control. Zones of inhibition (mm) were measured (Balouiri et al., 2016; CLSI, 2021; Perez et al., 1990).

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) Determination: MIC was ascertained using broth dilution: tubes containing varying extract concentrations ((100mg/ml, 50mg/ml, 25mg/ml and 12.5mg/ml) plus Mueller–Hinton broth which were inoculated and incubated (37 °C, 18–24 h). The lowest concentration with no visible turbidity was recorded as MIC. For MBC, aliquots (0.1 ml) from tubes without visible growth were plated on nutrient agar and incubated; the lowest concentration with no colony growth was recorded as MBC following the methods described by Doughari et al (2007) and Olutiola et al (2000).

RESULTS AND DISCUSSION

Extraction Yield and Physical Characteristics: Grape seed extract yield was 36.69%, presenting as a brown, sticky/gummy residue. Cherimoya seed extract yielded 35.70%, forming a light pink, adhesive residue.

Phytochemical composition of *Vitis vinifera* and *Annona cherimola* seed extracts: Table 1 shows the phytochemical composition of two different seed extracts (*Vitis vinifera* and *Annona cherimola*). The phytochemical result as shown in the table composed by *Vitis vinifera* seed extract include the present of flavonoid,tannin,phenol,terponoid,quinine and glycoside with the absent of steroids, alkaloid, artriquinone, saponin,

cardiac glycoside and plabotannin. While, *Annona cherimola* seed extract constituted the present of flavonoid, terponoid, quinine and cardiac glycoside with the absent of steroids, alkaloid, artriquinone, saponin, tannin, phenol and plabotannin.

Table1: Composition of *Vitis vinifera* and *Annona cherimola* seed extracts

Phytochemical	***Vitis vinifera* seed extract**	**Annona *cherimola* seed extract**
Flavonoids	Present	Present
Terpenoids	Present	Present
Glycosides	Present	Present
Quinine-type compounds	Present	Present
Tannins	Present	Absent
Plabotannin	Absent	Absent
Phenols	Present	Absent
Cardiac glycosides	Absent	Present
Alkaloids	Absent	Absent
Saponins	Absent	Absent
Anthraquinones	Absent	Absent
Steroids	Absent	Absent

Microbial Identification: *Staphylococcus aureus* showed Gram-positive cocci morphology, catalase-positive, coagulase-positive, citrate-positive.

Pseudomonas aeruginosa was Gram-negative rods, catalase-positive, oxidase-positive, citrate-positive.

Antibacterial Activity

***Vitis vinfera* seed extract: -**

Against *Staphylococcus aureus*: inhibition zones ranged from 13 mm (12.5 mg/ml) to 21 mm (100 mg/ml).

Against *Pseudomonas aeruginosa*: zones 9–14 mm at higher concentrations; negligible activity at lower concentrations.

MIC for *Staphylococcus aureus*: 25 mg/ml.

MBC for *Staphylococcus aureus*: 50 mg/ml.

No MIC or MBC achieved for *Pseudomonas. aeruginosa* within the tested concentration range.

***Annona cherimola* seed extract**

No observable zone of inhibition for either *Staphylococcus aureus* or *Pseudomonas aeruginosa* at any concentration tested.

This present study found that crude 70% ethanolic extract of *Vitis vinifera* seeds produced measurable antibacterial activity against clinical *Staphylococcus aureus* with (inhibition zones = 13–21 mm; MIC = 25 mg/ml; MBC = 50 mg/ml) over concentrations of 12.5–100 mg/ml, and weaker activity against *Pseudomonas aeruginosa* (inhibition zones =9–14 mm; no MIC/MBC within tested range). By contrast, crude 70% ethanolic extract of *Annona cherimola* (cherimoya) seeds produced no observable inhibition of either organism at the concentrations tested.

The antibacterial activity of the *Vitis vinifera* in this work is consistent with numerous recent reports that link high polyphenolic content in grape seeds to antimicrobial effects, especially against Gram-positive bacteria. Several comparative studies of *Vitisvinifera* seed extracts report high total phenolic contents (e.g., 79 - 111 mg GAE/g) and strong activity against *S. aureus,* with MICs reported in some fractionated/purified preparations in the sub-mg/mL range (i.e., much lower than crude extracts) (Krasteva et al., 2023; Pozzo et al., 2023). The positive correlation between total phenolics and antimicrobial potency is well documented; richer phenolic profiles (catechins, epicatechin, procyanidins) generally correspond to stronger antibacterial activity.

The comparatively, poor activity of GSE against *P. aeruginosa* in this investigation mirrors many reports that Gram-negative bacteria (especially non-fermenters like *P. aeruginosa*) are more tolerant to polyphenolic extracts because of the outer membrane, efflux systems, and intrinsic resistance mechanisms. Studies have found that *P. aeruginosa* often requires higher extract concentrations or specialized formulations to observe similar inhibitory effects seen with Gram-positive organisms (Pozzo et al., 2023). Thus, the present pattern good activity vs *S. aureus*,

limited activity vs *P. aeruginosa* aligns with current evidence.

Although. the genus Annona contains bioactive constituents (notably annonaceous acetogenins [ACGs]) with anticancer, pesticidal and some antimicrobial activities, the distribution and extractability of these compounds are highly tissue- and solvent-dependent. ACGs are lipophilic polyketides with poor water solubility; they are often better recovered with nonpolar solvents or require specific fractionation/isolation techniques to reach active concentrations (Gutiérrez et al., 2020; Durán et al., 2021). Several recent reviews and experimental studies report that leaves, bark or specially-prepared lipophilic fractions from Annona species frequently show activity, while crude ethanolic seed extracts may not (Durán et al., 2021; Gutiérrez et al., 2020). Thus, the null result for crude 70% ethanol seed extract in this study is consistent with the known chemistry of ACGs and with other reports that seeds sometimes lack detectable antibacterial activity unless processed differently.

The antibacterial activity of *Vitis vinifera* seed extract observed in this study aligns with a growing consensus in recent literature that grape seed phenolics are effective bioactive agents against Gram-positive bacteria. However, the moderate MIC/MBC values highlight a potency gap compared to refined or fractionated extracts.
The absence of activity in *Annona cherimola* seed extract is also consistent with recent reports showing that seed extracts of Annonaceae often lack significant antibacterial effect unless processed under optimized conditions (solvent, fractionation, plant part selection).

CONCLUSION

Ethanol extract of *Vitis vinifera* seeds demonstrates promising antibacterial activity against *Staphylococcus aureus*, with MIC and MBC values indicating bactericidal potential at sufficiently high concentrations. Its richer phenolic and tannin composition likely underpins this effect. In contrast, *Annona cherimola* seed extract showed no detectable antibacterial activity under the tested parameters.

RECOMENDATION

To advance these findings, future work should:

1. Fractionate and isolate active compounds from *Vitis vinifera* seed extract to determine structure–activity relationships and it these recommendations are adopted; the study may lead toward valuable antimicrobial candidates from plant seed sources.
2. Expand testing to include antibiotic-resistant strains and a broader array of bacteria.
3. Conduct cytotoxicity and safety profiling (in vitro and in vivo).
4. Explore alternative extraction protocols or induction of bioactive metabolite accumulation in *A. cherimola.*

REFERENCES

Aguilar-Villalva, R., Molina, G. A., España-Sánchez, B. L., Díaz-Peña, L. F., Elizalde-Mata, A., Valerio, E., Azanza-Ricardo, C., & Estevez, M. (2021). Antioxidant capacity and antibacterial activity from Annona cherimola phytochemicals by ultrasound-assisted extraction and its comparison to conventional methods. *Arabian Journal of Chemistry,* 14, 103239. https://doi.org/10.1016/j.arabjc.2021.103239.

Al Kazman, B. S. M., Harnett, J. E., & Hanrahan, J. R. (2022). Traditional uses, phytochemistry and pharmacological activities of Annonaceae. *Molecules, 27*(11), 3462. https://doi.org/10.3390/molecules27113462.

Chaachouay, N. (2024). Plant-derived natural products: A source for drug discovery. *Natural Products and Bioprospecting, 14*(1), 11. https://doi.org/10.3390/npbp14010011

Cheesbrough, M. (2009). *District Laboratory Practice in Tropical Countries*. Cambridge: The press syndicate of the University of Cambridge. Pp. 10, 57, 157-206.

Clinical and Laboratory Standards Institute (CLSI). (2023). *Performance standards for antimicrobial susceptibility testing* (33rd ed.). CLSI supplement M100.

Dietary phytochemicals in health and disease. (2025). *Journal of Functional Foods*, 105, 105–118. https://doi.org/10.1016/j.jff.2025.106123

Doughari, J. H., EL-Mahmood, A.M., and Manzara, S.

(2007). Antibacterial activity of root extracts of *Carica papaya* L. *African Journal of Microbiology Research, 1*(1), 37-41.

Durán, A. G., Gutiérrez, M. T., Mejías, F. J. R., Molinillo, J. M. G., & Macías, F. A. (2021). An overview of the chemical characteristics, bioactivity and achievements regarding the therapeutic usage of acetogenins from Annona cherimola Mill. *Molecules, 26*(10), 2926. https://doi.org/10.3390/molecules26102926.

Edeoga, H. O., Okwu, D. E., and Mbaebie, B. O. (2005). Phytochemical constituents of some Nigerian medicinal plants. *African Journal of Biotechnology, 4*(7), 685-688. http://doi.org/10.5897/AJB2005.000-3127.

El-Hallouty, S., Batawi, A., Shafi, M., Rashwan, E., Elhawary, E., and Abdelaal, N. (2020). Cytotoxicity of five plant extracts against different human cancer cell lines and their molecular mechanism. *Asian Journal of Pharmaceutical and Clinical Research, 13*(10), 194–198.

Felemban S., Al-Ghamdi B., Alzahrani R. (2024). Investigating Grape Seed Extract as a Natural Antibacterial Agent for Water Disinfection: Results of In Silico and In Vitro Evaluations. *Chemistry, 6*(5), 51.

Gutiérrez, M. T., Durán, A. G., Mejías, F. J. R., Molinillo, J. M. G., Megías, D., Valdivia, M. M., & Macías, F. A. (2020). Bio-guided isolation of acetogenins from *Annona cherimola* deciduous leaves: Production of nanocarriers to boost the bioavailability properties. *Molecules, 25*(20), 4861. https://doi.org/10.3390/molecules25204861.

Harborne, J. B. (2023). Phytochemical methods: *A guide to modern techniques of plant analysis* (4th ed.). Springer.

Hasbal-Celikok, G., Fuchs, C., and Pahlke, G. (2024). In vitro mechanistic studies of a standardized grape seed extract (GSEe): Antioxidant and neuroprotective activity. *Nutrients, 16*(12), 2235. https://doi.org/10.3390/nu16122235

Hassan ul, M., Akhtar, R., and Rauf, A. (2024). Advances in bioprocessing of medicinal plants: Toward sustainable phytopharmaceuticals. *Journal of Biotechnology Advances*, 52, 108–121. https://doi.org/10.1016/j.jbiotecadv.2024.108121

Haykal, T., Nasr, P., Hodroj, M. H., Taleb, R. I., Sarkis, R., Moujabber, M. N. E., & Rizk, S. (2019). Annona cherimola seed extract activates extrinsic and intrinsic apoptotic pathways in leukemic cells. *Toxins, 11*(9), 506. https://doi.org/10.3390/toxins11090506

Induction of Bioactive Secondary Metabolites in Annona cherimola (2025). *Journal of Plant Biochemistry and Biotechnology.*

Integration of high-throughput omics technologies for phytomedicine discovery. (2023). *Phytomedicine,* 113, 154769. https://doi.org/10.1016/j.phymed.2023.154769

Ivanov Y., Ivanov R., et al. (2024). Antimicrobial Polymer Films with Grape Seed and Skin Extracts. *Microorganisms, 12*(7), 1378.

Kitsiou, M., Gutierrez-Merino, J., Klymenko, O. V., Karatzas, K.-A., & Velliou, E. (2025). Estimating the antimicrobial effect of grape seed extract on Listeria monocytogenes ΔsigB on xanthan gum–based gels. *Food Control,*176,111355. https://doi.org/10.1016/j.foodcont.2025.111355.

Krasteva, D., Ivanov, Y., Chengolova, Z., & Godjevargova, T. (2023). Antimicrobial potential, antioxidant activity, and phenolic content of grape seed extracts from four grape varieties. *Microorganisms, 11*(2), 395. https://doi.org/10.3390/microorganisms11020395.

Le Ven J., Schmitz-Afonso I., Lewin G., Brunelle A., Touboul D., Champy P. (2014). Identification of ACGs in *Annona cherimola* Mill. using HPLC-Orbitrap. *Journal of Agricultural and Food Chemistry,* 62, 8696–8704.

Macuer-Guzmán, J., Giovagnoli-Vicuña, C., Bernal, G.,

Lobos-González, L., de la Fuente-Ortega, E., Araya-Castillo, M., and Ibáñez, C. (2023). *Annona cherimola* seed extracts trigger an early apoptosis response and selective anticlonogenic activity against the human gastric carcinoma cell line. *SNU-1. Molecules,28*(19),6906. https://doi.org/10.3390/molecules28196906

Mahdipour, R., Ebrahimzadeh-Bideskan, A., Hosseini, M., Shahba, S., Lombardi, G., Malvandi, A. M., & Mohammadipour, A. (2023). The benefits of grape seed extract in neurological disorders and brain aging. *Nutritional Neuroscience, 26*(5), 369–383. https://doi.org/10.1080/1028415X.2022.2051954.

Manasa, M., Vivek, M.N., Kambar, Y., Kumar, R.K.A. and Kekuda, P.T.R. (2014). Mineral content, antimicrobial and radical scavenging potential of Caesalpinia mimosoides Lamk. (Caesalpiniaceae). *World Journal of Pharmaceutical Research. 3*(4): 1047-1063.

Memar M.Y., Shariati A., Alizadeh N. (2019). The grape seed extract: a natural antimicrobial agent. *Reviews in Medical Microbiology, 30*(3), 93–100.

Nasim, N., Kanwal, S., and Farooq, M. (2022). Plant-derived natural products for drug discovery: Challenges and future directions. *Applied Microbiology and Biotechnology*, *106*(12), 4673–4690. https://doi.org/10.1007/s13237-022-00405-3

Ogbeba J., Iruolaje F. O., Dogo B. A. (2017). Antimicrobial efficacy of Guiera senegalensis and Prosopis africana leaf extracts on some bacterial pathogens. *European Journal of Biology and Medical Science Research, 5*(2), 27–36

Olutiola, P.O., Famurewa, O., and Sonntag, H. G. (2000). An introduction to general microbiology: *A practical approach* (2nd ed.). Bolabay Publications, Ikeja, Nigeria.

Perrone, A., Yousefi, S., Salami, A., Papini, A., & Martinelli, F. (2022). Botanical, genetic, phytochemical and pharmaceutical aspects of *Annona cherimola* Mill. Scientia Horticulturae, 296, 110896. https://doi.org/10.1016/j.scienta.2022.110896.

Pozzo, L., Grande, T., Raffaelli, A., Longo, V., Weidner, S., Amarowicz, R., & Karamać, M. (2023). Characterization of antioxidant and antimicrobial activity and phenolic compound profile of extracts from seeds of different Vitis species. *Molecules, 28*(13), 4924. https://doi.org/10.3390/molecules28134924.

Tovar-Carrillo, K. L., Trujillo-Morales, L., Cuevas-González, J. C., Ríos-Arana, J. V., Espinosa-Cristóbal, L. F., & Zaragoza-Contreras, E. A. (2024). Antibacterial properties of grape seed extract-enriched cellulose hydrogels for potential dental application: In vitro assay, cytocompatibility, and biocompatibility. *Gels,10*(9),606. https://doi.org/10.3390/gels10090606.

Unraveling Nature's Pharmacy. (2025). Neurotoxicity and therapeutic potential of annonaceous acetogenins: An updated review. *Frontiers in Pharmacology,* 16, 116–128.