JMBFS

Journal of Microbiology, Biotechnology and Food Sciences

International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

IN-VITRO ANTIOXIDANT AND CYTOTOXIC POTENTIAL OF BANANA WINE

Durva Panchal [1], Aakifa Ansari [1], Apoorva Pawar [1], Shivali Patkar [1], Clarissa Rodrigue[2], Samidha Patil[1], Miriam Stewart[1], Avik Chakraborty [2], Pampi Chakraborty[1]*

Address(es):

[1] St. Xavier's college, Department of Microbiology, Mahapalika road, Mumbai - 400001, Maharashtra, India, Contact: +91-22-2262-0661.

[2] Radiation medicine centre (RMC), Bhabha Atomic Research Centre, Tata Memorial Centre Annex, Mumbai - 400012, Maharashtra, India.

*Corresponding author: pampi.chakraborty@xaviers.edu

https://doi.org/10.55251/jmbfs.10391

ARTICLE INFO

Received 23. 7. 2023
Revised 28. 2. 2025
Accepted 13. 3. 2025
Published 1. 6. 2025

Regular article

OPEN ACCESS

ABSTRACT

Fruit wines are being studied extensively for their biological properties. Banana wine is one of the less studied wines that possess antioxidant properties. The aim of the current study was to characterize and compare white (grape) wine with less investigated banana wine in terms of its polyphenol concentration, antioxidant capacity, and anti-cancer potential. Using the Folin Ciocalteu test, the total polyphenolic content of grape wine (white) and banana wine was found to be 817 mg/l and 1136 mg/l gallic acid equivalents (GAE), respectively. In both types of wine samples, the amounts of important polyphenolic substances such as quercetin, catechin, and gallic acid were estimated by HPLC. The antioxidant capacity of banana wine was assessed *in vitro* using FRAP assay. Further, the antioxidant potential was validated in a macrophage cell line (RAW264.7) after the induction of the oxidative stress with 0.5mM H_2O_2. The *in-vitro* cytotoxic effects of banana and white wines on human lung (A-549) and breast (MDA-MB-231) cancer cell lines were examined using the MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay, and propidium iodide (PI) - based flow cytometric analysis. An increase in the proportion of sub-G1 phase cells after PI labeling demonstrated that the proliferation of A-549 cells was significantly inhibited in the presence of banana wine compared to white wine. This study reveals that banana wine may have higher anti-oxidant and anti-cancer properties.

Keywords: Banana wine, polyphenols, anti-cancer activity, antioxidant activity

INTRODUCTION

Banana is one of the world's most popular tropical climacteric fruits and the fourth most important agricultural food crop produced globally **(Aurore *et al.*, 2009)**. It is high in sugars, vitamins, minerals, and dietary fibers, as well as phytochemical constituents (polyphenols) that have significant health benefits. According to several reports, bananas contain a wide range of phytochemicals, including carotenoids, flavonoids, phenolics, phytosterols, and biogenic amines **(Singh *et al.*, 2016)**. Because of the high concentrations of these phytochemicals, banana has greater antioxidant potential than some berries, herbs, and vegetables, and this quality improves as the fruit ripens **(Amini Khoozani *et al.*, 2019).** Bananas contain numerous bioactive compounds, including apigenin, β--sitosterol, flavonoids, and lectin, which have been shown to have anti-cancer properties. Furthermore, kaempferol and (S)-(+)-6-methoxy--methyl-2-naphthalene acetic acid in bananas contribute to anti-inflammatory properties **(Mathew & Negi, 2017)**. The most promising polyphenols, however, are quercetin, catechin, and gallic acid in fruit wines, which have received special attention due to their pharmacological properties **(de Souza Dias *et al.*, 2013)**. Notably, the quantity of phenolic components in the wine can be increased through the proper selection of yeast species, extending the maceration period, and utilizing enzyme preparations ***(Petrovic et al., 2019).***

Cancer is a complicated disease that has emerged as one of the leading causes of death in the modern era. Many researchers have shifted their focus in recent years in developing new strategies for cancer prevention. One promising strategy is the identification of natural biologically active compounds that can preferentially suppress or prevent the early stages of tumorigenesis or cancer progression while having minimal adverse effects on healthy cells **(Ranjan *et al.*, 2019)**. Several epidemiological studies have suggested that a diet high in fruits and vegetables reduces the risk and incidence of oxidative stress-related degenerative diseases such as cancer, cardiovascular disease, and neurodegenerative disease. The polyphenolic content of such a diet contributes significantly to its beneficial effects **(Castaneda & Kinne, 2004, Bigelow & Cardelli, 2006, Carocho & Ferreira, 2013)**. The need for finding a link between preventing cancer in humans and harnessing the potential of anti-oxidants as treatment or therapy is increasing by the hour, and there has been a paradigm shift in the scientific research world towards a less explored group of plant secondary metabolites known as polyphenols **(El Gharras, 2009)**.

Wines made from fruits are a potentially significant source of polyphenols. Wine may contain both fruit-derived polyphenols and new phenolic products accumulated due to fermentation process. White wine, has received a lot of attention in recent years for its potential anti-cancer properties. Numerous experimental studies using cell lines (*in-vitro*) and whole animals (*in-vivo*) have demonstrated the anti-proliferative, apoptosis induction, cell-cycle arrest, and modulation of signal transduction pathways properties of white wine **(De Souza Dias *et al.*, 2013, D'Angelo *et al.*, 2017, Choudhari *et al.*, 2020)** . Although, no reports on the characterization of polyphenolic components and the anti-cancer potential of banana wine have been published. The current study aimed to focus on the same.

MATERIAL AND METHODS

Chemicals and Reagents

All chemicals and reagents were of analytical grade and sourced from Sigma Aldrich, unless stated otherwise. Overripe bananas (Musa acuminata 'Robusta') were sourced from a local supermarket in Girgaon, Mumbai, India. For the fermentation process, *Saccharomyces cerevisiae* (standardized baker's yeast for wine production) was used at a concentration of 1 gram per litre of filtrate, along with 5% dextrose. Lactic acid bacteria (500 mg dried powder of *Lactobacillus acidophilus*) were utilized for malolactic fermentation. The Folin-Ciocalteau reagent, used for determining total polyphenolic content (TPC), and saturated sodium bicarbonate solution were procured as per the assay requirements. Gallic acid served as the reference standard for TPC determination. High-performance liquid chromatography (HPLC) analyses were conducted using a Eurosphere C-18 reversed-phase cartridge, with solvents comprising 0.1% formic acid in HPLC-grade water (Solvent A) and 0.1% formic acid in methanol (Solvent B). For the antioxidant activity assay, FRAP reagent was prepared and used. Cell culture media and supplements included Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), penicillin, and sodium bicarbonate, all necessary for maintaining cell viability. The MTT assay involved MTT reagent (0.5 g/mL) and sodium dodecyl sulfate (SDS) for solubilizing formazan crystals. For determining reactive oxygen species (ROS), 2', 7'-Dichlorofluorescein diacetate (DCFDA)

staining was performed. Propidium iodide (PI) was used for cell cycle analysis via flow cytometry.

Preparation of Banana wine

Overripe bananas (Musa acuminata 'Robusta') were purchased from a local supermarket in Girgaon, Mumbai, India, for the preparation of banana wine. The fruits were washed, peeled and cut. A benchtop food processor was used to prepare a mash after grinding the overripe bananas. The banana mash was blended with water in 1:3 ratio (1 part banana must to 3 parts water), then boiled for 45 min and filtered through a muslin cloth. *S. cerevisiae* (1 gram per litre of filtrate) and 5% dextrose were added to the pH-5 filtrate. The fermentation was carried out for 5 days at 28ºC. Malolactic fermentation was accomplished by adding lactic acid bacteria (500 mg dried powder Lactobacillus acidophilus) to the fermented product. The prepared banana wine was refrigerated at 4°C until use. Sauvignon Blanc was used as the standard for comparative studies because the wine made from bananas resembled the taste and characteristics of white wine. Experts from Sula Vineyards Private Limited, Nashik, India suggested the same for our study and it was a kind gift from them.

Determination of total polyphenolic compounds by Folin-Ciocalteau reagent

The total polyphenolic content (TPC) of the wine samples were estimated by the Folin-Ciocalteau reagent as described earlier **(Araújo *et al.*, 2013)** with a minor modification. The assay was performed in 96-well plate. 20 µL of undiluted wine samples were added to each of the wells, followed by addition of 100 µL of the Folin-Ciocalteau reagent. After 10 min, 80 µL of saturated sodium bicarbonate solution was added. After 30 min of incubation at room temperature (25-28 °C), the absorbance was measured at 690 nm. The reference standard used for plotting the standard curve was Gallic acid. A range of different standard concentrations (10-100 µg/mL) were prepared using water as the solvent. The TPC was determined using the linear equation obtained from the standard calibration curve prepared with Gallic acid and it was expressed as mg/L of gallic acid equivalents (GAE). All tests were performed in triplicate.

Determination of Polyphenolic compounds by High-performance liquid chromatography

Quantification of individual polyphenolic compounds (Quercetin, Catechin and Gallic acid) in the banana wine was performed by high performance liquid chromatography (HPLC). Separation was performed using a Eurosphere C-18 reversed-phase cartridge {Dimension: 300mn (length) × 4mm (diameter), particle size: 5µm} (KNAUER, Germany system). The mobile phase was as follows, Solvent A: 0.1% formic acid in HPLC water and Solvent B: 0.1% formic acid in methanol. At a flow rate of 0.5ml/min, the elution gradient was as follows: for zero time and 2 min, 95% solvent A and 5% solvent B; for 2 min and 20 min gradient changes to 5% solvent A and 95% solvent B. The UV detector, connected in series, was set to absorbance at 255nm. The ChromGate software was used for the analysis of the data. Wine samples were filtered through 0.45µm membrane filter and 10µl of each sample was directly injected into HPLC.

In vitro assays for evaluating the biological activities of wine prepared from bananas

Determination of antioxidant activity

The antioxidant potential of wine prepared from bananas was determined by FRAP assay using a UV-VIS spectrophotometer (BioTek, Epoch2). The FRAP assay was carried out in accordance with a previously published protocol **(Mildner-Szkudlarz *et al.*, 2009)** . In brief, the samples were prepared by combining 150µl of FRAP reagent and 50µl of banana wine, and the absorbance was measured at 592nm after 40 min of incubation in the dark at 37ºC. The antioxidant activity of banana wine was compared to that of a standard white wine processed in the same manner. The tests were carried out in triplicate, and the results were expressed in milligrams of Quercetin equivalent per liter of banana wine or standard white wine (mg/liter).

Cell culturing

The cyto-toxic effect of the wine prepared from bananas was tested on two human cancer cell lines; A-549 (human alveolar basal epithelial Adenocarcinoma) and MDA-MB231 (human metastatic mammary adenocarcinoma). Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% v/v fetal bovine serum (FBS), 0.2% (2 mg/mL) of penicillin and adjusted to pH-7.2 by sodium bicarbonate. The cells were grown at 37°C under 5% CO_2.

Determination of the cell cytotoxicity

Cell viability was used to determine cell cytotoxicity using the MTT assay. Cells were seeded in a 96-well microtiter plate at a density of 1 x 105 cells/mL and incubated for 24 h at 37°C in CO_2 incubator. Except for the untreated controls, banana wine and standard white wine were added to the respective wells (100µl) after cell adhesion along with 100µl of double-strength complete media. The adhered cell confluency was at least 90% before starting the treatment. As a positive control, 0.5mM H_2O_2 was added to the wells with or without wine samples and incubated for 24 h at 37°C. After the incubation, 100 µl of medium was removed from each well and replaced with 100µl of MTT (0.5g/mL) and incubated at 37°C with 5% CO_2 for 4 h. Purple formazan crystals accumulated in viable cells which were solubilized by adding 100µl of 10% SDS (100 mg/mL) to each well and incubated for 60 min at 37°C. The amount of formazan produced was measured using an ELISA plate reader at 570 nm **(Kleiveland, 2015**). The same conditions were used to grow control cells. We kept the 4% and 12% alcohol controls because banana wine and standard white wine had alcohol content of 4% and 12%, respectively, and to rule out the possibility of cell death due to the inherent presence of this alcohol content. The experiments were carried out in triplicate three times. The equation was used to calculate the percentage of cell viability.

$$\%\textbf{viability} = [\textbf{Absorbance of Test/ Absorbance of control}]\ \textbf{x 100}$$

Determination of ROS by 2', 7'- Dichlorofluorescein diacetate (DCFDA) staining

Cells were seeded in a 96-well microtiter plate (black) at a density of 1 x 10^5 cells/mL and incubated for 24 h at 37°C in CO_2 incubator. The cells were given wine treatment as mentioned above along with 0.5mM H_2O_2 for 12 h. After that the media was replaced by 100µl of DCFH-DA (10 µmol/mL) in serum free media was added to each well and incubated for 30 min at 37 °C in 5% CO_2. Then the cell layer was washed with PBS and 100µl of serum-free medium was added to each well before taking the fluorescence intensity with an excitation wavelength of 485nm and emission wavelength of 535nm in fluorescence plate reader (iD3 spectradrop, Molecular devices).

Cell cycle analysis

The ability of banana wine to induce cell death in A-549 cells was determined by PI staining coupled with flow cytometry as described before **(Riccardi & Nicoletti, 2006)**. Briefly, exponentially growing cells were adjusted to a cell density of 1.5 x 10^6 cells per cell culture dish (60 mm) in the complete Iscove's modified Dulbecco's medium (IMDM) and were incubated at 37°C in CO_2 incubator. After cell monolayer formation, cells were treated with banana wine, standard white wine, 4% alcohol, and 12% alcohol for 24 h. Cells grown in complete IMDM (pH 3.5-4) at the same seeding density and incubation conditions were considered as untreated vehicle control (pH of banana wine- 4). After 24 h, the cells were collected, washed with 1X PBS, fixed with chilled 70% ethanol, and held at -20°C for 24 h. The fixed cells were harvested, followed by the removal of excess ethanol by washing with 1X PBS. These cells were stained with 50 µg/ml PI (T4170, Sigma) for 30 min and analyzed by a flow cytometer (Cyflow space-SysmexPartec). A minimum of 25,000 cells were analyzed per sample.

Statistical analysis

The statistical analysis was performed with Sigma stat 3.5 and Microsoft Excel software. All the results were expressed as mean ± SD values. The means values for all the tests were analyzed by one-way ANOVA with a post hoc (Tukey's honest significant difference) test for pairwise multiple comparisons. Differences were considered significant at p was < 0.05.

RESULTS AND DISCUSSION

The physicochemical parameters of the banana wine employed in this study were meticulously characterized: alcohol content was determined to be 5.75%, pH measured at 3.67, total sugars quantified at 2.34 g/L, total acids at 1.57 g/L, total SO_2 recorded at 10 ppm, with free SO_2 at 5 ppm, and total polyphenol content measured at 163 ± 5 mg/L. Additionally, specific organic acids such as lactic acid were identified. These parameters were previously comprehensively characterized in our published work (**Panchal *et al.*, 2020**) highlighting the robust characterization of banana wine. This study focuses on exploring the in-vitro antioxidant and cytotoxic potential of banana wine.

Phenolic content of wine prepared from Bananas

Polyphenolic components are considered as one of the most important contributors to antioxidant and anticancer activities. Thus, in the present study, total polyphenolic content of the wines was estimated by Folin Ciocalteau and Fig. 1A depicts the total polyphenolic composition of the wine samples in terms of GAE. The total polyphenolic content of banana wine was significantly higher *(P value < 0.001)* than that of white wine. Further, three polyphenols quercetin, gallic acid, and catechin were chosen for estimation in banana wine due to their abundance in banana fruit **(Sidhu and Zafar, 2018).** These three polyphenols are also found in

commercial fruit wines such as apple, blackberry, raspberry, and strawberry **(Ljevar *et al.*, 2016)**, as well as grape wines **(Li & Sun, 2019).** Furthermore, the selected polyphenols have been linked to anti-cancer activity in a number of studies **(Huh *et al.*, 2004, Kang *et al.*, 2016, Pang *et al.*, 2017, Li & Sun, 2019)**.

Among the polyphenols, quercetin is abundant in bananas and is known to act not only as an anti-oxidant but also as a cytotoxic pro-oxidant **(D'Angelo *et al.*, 2017b)** . This pro-oxidant property contributes to its chemotherapeutic potential, and it has been reported to induce apoptosis in various cancer cell lines **(Hashemzaei *et al.*, 2017)**. Quercetin activates p53 in the MDA-MB-453 breast cancer cell line, resulting in cell cycle arrest. It also increases the expression of the pro-apoptotic protein Bax while decreasing the expression of the anti-apoptotic protein Bcl-2 and induces cytochrome-c-mediated apoptosis **(Hardwick & Soane, 2013)**. Catechin, a major polyphenol found in green tea, fruits, and vegetables, has been shown to inhibit the cancerous growth of the prostate, breast, and skin **(Athar *et al.*, 2007)**. It induces the cyclin kinase inhibitor p21 in lung epithelial cancer cells (A-549), leading to G1 cell cycle arrest and accumulation of cells in the G_0/G_1 phase, and suppresses cyclin E1 and P-AKT, which are responsible for cancer development, tumorigenesis, and metastatic spread **(Sun *et al.*, 2020)**. Gallic acid, another important polyphenol found in fruits and vegetables, has been linked to anti-cancer activity. It increases the expression of p21 and p53, alters the expression of Mcl-1, and induces apoptosis in breast cancer cells (MCF-7) **(Rezaei *et al.*, 2016)**. Although these polyphenols have anti-cancer potential on their own, studies have shown that their anti-cancer efficacy is maximized when used in combination **(Niedzwiecki *et al.*, 2016)**. As a result, fruit wines containing a combination of these important polyphenols may be considered safer and more effective anticancer agents.

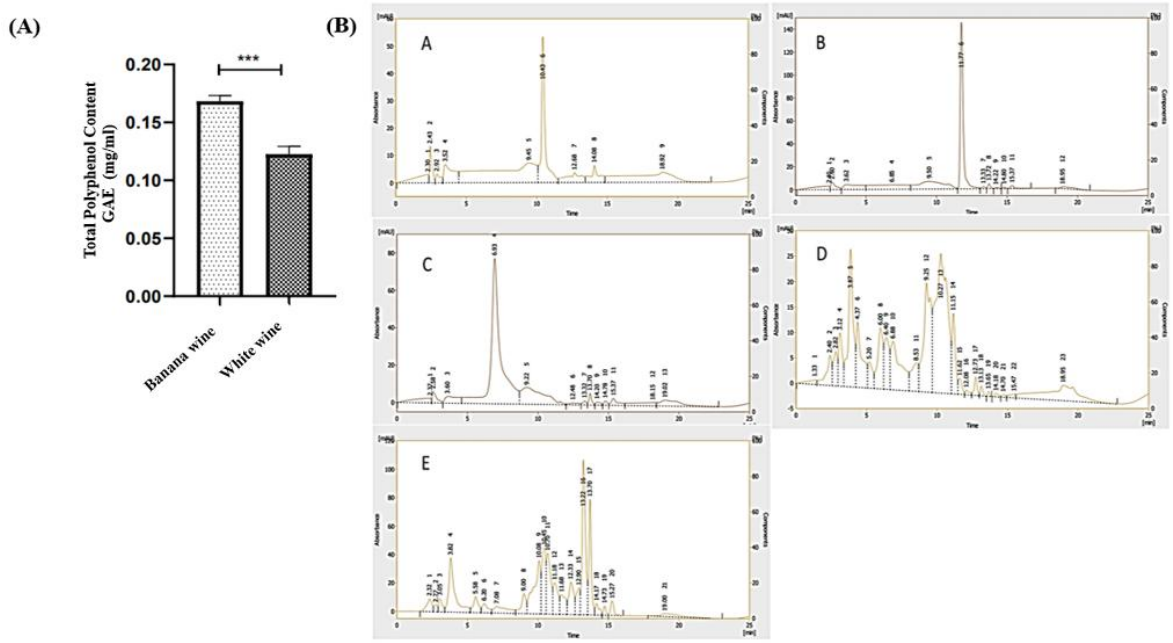


Figure 1 Phenolic content of wine prepared from Bananas **(**A) Estimation of total polyphenolic content of banana wine and white wine by Folic Ciocalteau method in terms of gallic acid equivalent. Each bar represents the mean ± SD for three replicates. (B) HPLC chromatogram of standard catechin (a), quercetin (b) gallic acid(c) and banana wine (d) and white wine (e) at –255 nm. The x-axis represents retention time and the y-axis represents the absorbance.

HPLC was used to quantify the polyphenols in the banana and white wine (standard). In less than 22 min, standards of quercetin, gallic acid, and catechin were well separated in a single run (Fig. 1B. A , Fig 1B. B and Fig 1B. C). The chromatogram of banana wine revealed that it contained more catechin (1.1 ± 0.8 mg/L) and quercetin (0.289 ± 0.8 mg/L) than white wine (0.19 ± 0.1 mg/L and 0.04 ± 0.02 mg/L respectively (Fig. 1B. D and Fig. 1B. E). White wine, on the other hand, had a higher concentration of gallic acid (2.19 ± 0.4 mg/L) than banana wine (0.44 ± 0.12mg/L). The differences in the profiles of the individual polyphenolic compounds could be attributed to maturity, wine-making technology, phenolic changes that occur during the ageing process, and other factors such as the type of fruit used in the preparation of wine. Therefore, banana wine was chosen for further research due to its high polyphenolic content and unique polyphenolic composition.

Antioxidant activity of banana wine

Phenolic compounds boost antioxidant activity and improve human health by lowering the risk of chronic diseases such as diabetes, cardiovascular and inflammatory disease, and cancer. In-vitro antioxidant activity of banana wine was assessed using the FRAP assay (Fig. 2A) and cellular ROS detection using the DCFDA (Fig. 2B). The antioxidant activity of the two wines tested, banana wine and white wine, was not significantly different in vitro using the FRAP assay. Our correlation analysis between FC and FRAP assay revealed that antioxidant activity is related to polyphenolic content. Furthermore, ROS detection using DCFDA revealed that treating the macrophage cell line with H_2O_2 and then with Banana wine or white wine significantly reduced ROS production (P value < 0.0001). After H_2O_2 treatment, the ROS levels in banana wine and white wine were comparable, with no statistical difference between them. As a result, it is correct to state that the polyphenolic compounds determine the antioxidant activity of these wines, which varies greatly depending on the individual compound in the wine. Because antioxidants have been shown to affect several signalling cascades and targets associated with anti-proliferative effects, our next goal was to investigate their effectiveness on cancer cell lines.

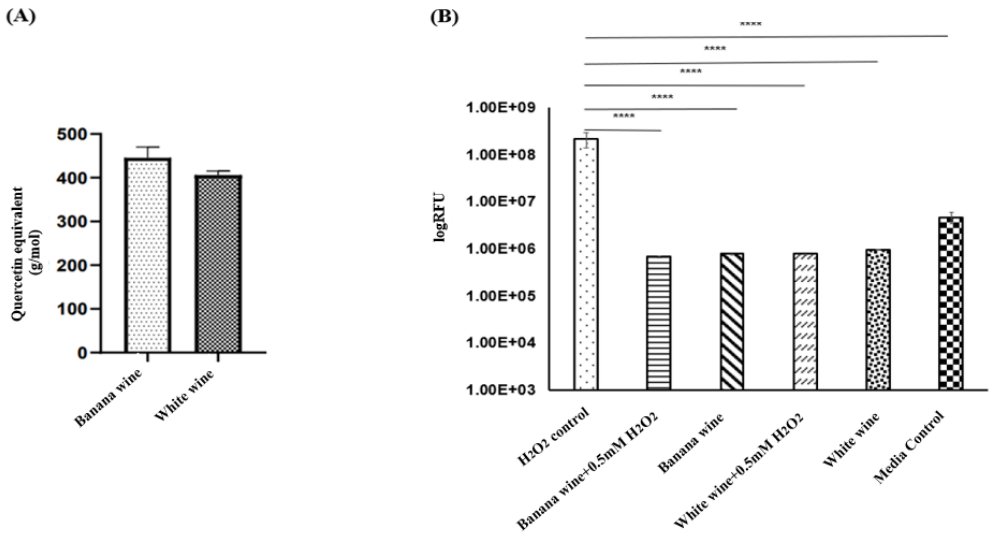


Figure 2 Antioxidant activity of banana wine (A) Estimation of antioxidant potential of banana wine and white wine by FRAP assay keeping quercetin as a standard. Each bar represents the mean ± SD for three replicates. (B) Mouse macrophage cells (RAW246.7) were treated with/without 0.5mM H_2O_2 and wine samples for 24 h and ROS production of the treated and untreated cells were determined by DCFDA staining and relative fluorescence unit estimation in fluorescence plate reader (iD3 spectradrop, Molecular devices). Each bar represents the mean ± SD for three replicates.

Cytotoxic effect of Banana wine on human cancer cell lines

Depending on the source of free radicals and their concentration, the polyphenolic compounds in wine can exert both pro-oxidant and anti-oxidant activity. Wine's cytotoxic activity could be linked to polyphenol's pro-oxidant properties. By producing reactive oxidative species, pro-oxidant activity can induce apoptosis and accelerate oxidative damage. Numerous studies have shown that polyphenols can inhibit cancer initiation via a variety of mechanisms, including blocking specific mutagen-transforming enzymes, regulating heme-containing phase I, and preventing the formation of DNA adducts **(Abdal Dayem *et al.*, 2016)**. In this study, the cytotoxic activity of banana wine on two human cancer cell lines was investigated. A-549 and MDA-MB231 cells were treated for 24 h with banana wine samples, and cytotoxicity was determined by assessing cell viability using the MTT assay. Here, hydrogen peroxide (H_2O_2) was used as a positive control to cause oxidative damage-induced cell death **(Heo *et al.*, 2020)**. Both banana wine and white wine treatments significantly reduced cell viability (P-value < 0.0001) in cancer cell lines when compared to the no-treatment control. The percentage cell viability of A-549 cells treated with banana wine and white wine did not differ significantly (8.86 ± 0.74 and 6.96 ± 0.55, respectively) (Fig 3A, 3B). The observed increase in cytotoxicity towards the two cancer cell lines following treatment with banana wine could be attributed to polyphenols targeting different aspects of the oncogenic signaling pathway. Some studies have also shown that polyphenols can help prevent malignant transformation from benign tumors **(Cháirez-Ramírez *et al.*, 2021; Yagi *et al.*, 2013).** Another study confirmed the ability of fruit wines to mitigate experimentally induced oxidative stress by inducing specific enzymes **(Cakar *et al.*, 2021)**. More research is needed into the molecular interactions of protein pathways and understand how the active components of banana wine affect target genes.

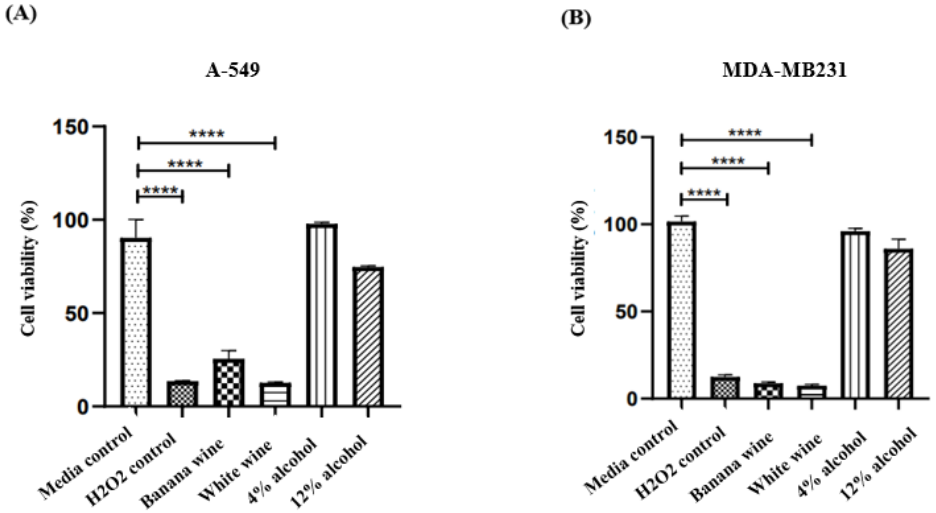


Figure 3 Cytotoxic effect of Banana wine on human cancer cell lines **(**A) A-549, and (B) MDA-MB-231 cell lines. For both the bar graphs A and B, the cells were either untreated (media control), or were treated for 24 h with- standard white wine (undiluted), 12% alcohol, banana wine (undiluted), 4% alcohol, and H_2O_2. The data is represented as the mean viability ± SD of the three replicates. (*P < 0.05 and **P < 0.01)

The viability of A-549 cells after treatment with banana wine was also evaluated using PI staining-based flow-cytometric assay as shown in Figure 4. Viability of A-549 cells after treatment with banana wine showed a broad sub-G1 peak as compared to the other treatments, indicating a larger proportion of cell death with this treatment (Fig. 4A-D). There was a significant (P-value < 0.01) increase in the percentage of sub-G1 cells (Fig. 4A) for banana wine treatment (58.54 ± 3.2%), over the untreated control (6.3 ± 0.24%) and standard white wine (35.38 ± 0.7%). Furthermore, to determine if the alcohol concentration in banana wine is one contributing factor for cell death, A-549 cells treated with 12% alcohol were also analyzed. The result from this experiment indicated that the sub-G1 phase cells in banana wine treatment were significantly (P-value < 0.01) higher as compared to 12% alcohol (32.9 ± 1.3%). These findings confirm the cytotoxic activity of banana wine on A-549 cells and also suggest that the cytotoxic effect is independent of its alcohol concentration. Although, this result is not exactly following the MTT assay result for the A-549 cell line in Fig 3. The viability of A549 cells was higher after banana wine treatment compared to white wine. There can be many reasons behind the observation. A-549 cell line is a human non-small cell lung cancer (NSCLC) cells which have been reported to express high levels of multiple isoforms of aldehyde dehydrogenase enzyme that induces the concentration of NADH in these cells **(Kang *et al.*, 2016)**. An enhanced NADH concentration in

the cells can lead to the nonspecific reduction of MTT and underestimates the cytotoxicity that is caused solely by ethanol.

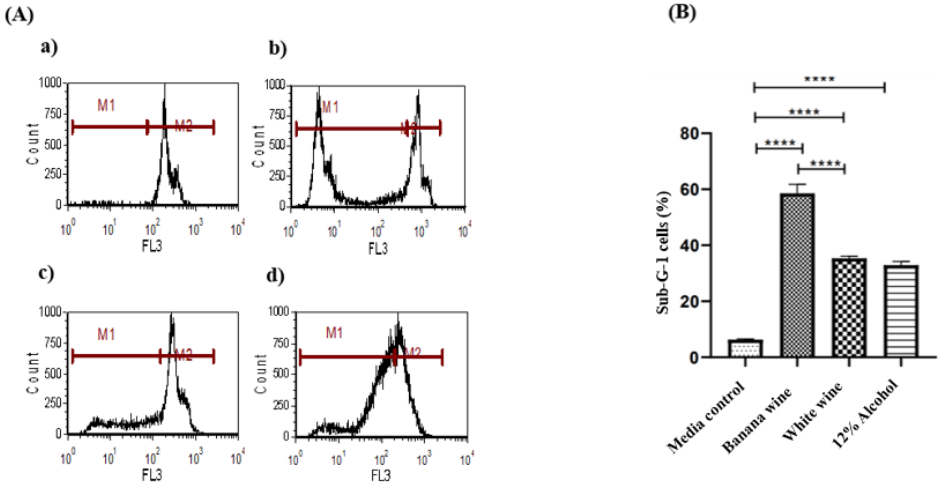


Figure 4 Cytotoxic effect of Banana wine on human cancer cell lines **(A)** Flow cytometry plots indicating the measure of A-549 cells (stained with PI) on treatment with wine samples. Untreated cells - vehicle control (a), and cells treated for 24 h with banana wine (b), standard white wine (c), and 12% alcohol (d). (B) Bar graph depicting a marked increase in sub-G1 cell population when cells are treated with banana wine and 12% alcohol over other treatments (F). For the bar graph (B), the data is represented as the mean ± SD of the three replicates. (****$P < 0.001$)

Several studies have found that isolated polyphenolic fractions of wines and fruit extracts have cytotoxic activity in cancer cell lines **(Choudhari *et al.*, 2020)**. One study also found that blackberry, cherry, raspberry, blackcurrant, strawberry, and apple wine had anti-proliferative and apoptotic effects on cervical cancer, breast cancer, and colon cancer cell lines **(Ljevar *et al.*, 2016)**. Plum and Andean berry wines have also been shown to be cytotoxic against various cancer cell lines **(Hakimuddin *et al.*, 2004)**. However, to the best of our knowledge, this is the first study to show that banana wine has anti-cancer potential.

CONCLUSION

This study compared banana wine's antioxidant and cytotoxic potential to standard white wine. Our findings reveal that banana wine contains significantly higher levels of quercetin and catechin—essential polyphenolic compounds known for their antioxidant and anti-cancer properties—compared to white wine. Thus, banana wine exhibits superior antioxidant and anti-cancer properties. These results highlight the importance of investigating non-traditional fruit wines derived from sources other than grapes.

Acknowledgments: The authors sincerely thank the Department of Microbiology, St. Xavier's College (Autonomous), Mumbai, for providing essential facilities that supported this study. We are also deeply grateful to Dr. Neil Fernandes and Ms. Pooja Pote from Sula Vineyards, Nashik, for their generous support in granting access to laboratory facilities and for sharing their invaluable expertise. This research was funded by Mumbai University (Project No. 362) and the Department of Biotechnology, Government of India (DBT-Builder-BT/INF/22/SP41293/2020).

REFERENCES

Abdal Dayem, A., Choi, H., Yang, G.-M., Kim, K., Saha, S., & Cho, S.-G. (2016). The Anti-Cancer Effect of Polyphenols against Breast Cancer and Cancer Stem Cells: Molecular Mechanisms. *Nutrients*, *8*(9), 581. https://doi.org/10.3390/nu8090581

Amini Khoozani, A., Birch, J., & Bekhit, A. E.-D. A. (2019). Production, application and health effects of banana pulp and peel flour in the food industry. *Journal of Food Science and Technology*, *56*(2), 548–559. https://doi.org/10.1007/s13197-018-03562-z

Araújo, C. R. R., Silva, T. de M., Lopes, M., Villela, P., Alcântara, A. F. de C., & Dessimoni-Pinto, N. A. V. (2013). Total antioxidant capacity, total phenolic content and mineral elements in the fruit peel of Myrciaria cauliflora. *Brazilian Journal of Food Technology*, *16*(4), 301–309. https://doi.org/10.1590/S1981-67232013005000036

Athar, M., Back, J., Tang, X., Kim, K., Kopelovich, L., Bickers, D., & Kim, A. (2007). Resveratrol: A review of preclinical studies for human cancer prevention. *Toxicology and Applied Pharmacology*, *224*(3), 274–283. https://doi.org/10.1016/j.taap.2006.12.025

Aurore, G., Parfait, B., & Fahrasmane, L. (2009). Bananas, raw materials for making processed food products. *Trends in Food Science & Technology*, *20*(2), 78–91. https://doi.org/10.1016/j.tifs.2008.10.003

Bigelow, R. L. H., & Cardelli, J. A. (2006). The green tea catechins, (−)-Epigallocatechin-3-gallate (EGCG) and (−)-Epicatechin-3-gallate (ECG), inhibit HGF/Met signaling in immortalized and tumorigenic breast epithelial cells. *Oncogene*, *25*(13), 1922–1930. https://doi.org/10.1038/sj.onc.1209227

Čakar, U., Čolović, M., Milenković, D., Medić, B., Krstić, D., Petrović, A., & Đorđević, B. (2021). Protective effects of fruit wines against hydrogen peroxide—Induced oxidative stress in rat synaptosomes. Agronomy, 11(7), 1414. https://doi.org/10.3390/agronomy11071414

Carocho, M., & Ferreira, I. C. F. R. (2013). A review on antioxidants, prooxidants and related controversy: Natural and synthetic compounds, screening and analysis methodologies and future perspectives. *Food and Chemical Toxicology*, *51*, 15–25. https://doi.org/10.1016/j.fct.2012.09.021

Castaneda, F., & Kinne, R. K.-H. (2004a). Ethanol Treatment of Hepatocellular Carcinoma: High Potentials of Low Concentrations. *Cancer Biology & Therapy*, *3*(5), 430–433. https://doi.org/10.4161/cbt.3.5.806

Castaneda, F., & Kinne, R. K.-H. (2004b). Ethanol Treatment of Hepatocellular Carcinoma: High Potentials of Low Concentrations. *Cancer Biology & Therapy*, *3*(5), 430–433. https://doi.org/10.4161/cbt.3.5.806

Cháirez-Ramírez, M. H., De La Cruz-López, K. G., & García-Carrancá, A. (2021). Polyphenols as Antitumor Agents Targeting Key Players in Cancer-Driving Signaling Pathways. *Frontiers in Pharmacology*, *12*, 710304. https://doi.org/10.3389/fphar.2021.710304

Choudhari, A. S., Mandave, P. C., Deshpande, M., Ranjekar, P., & Prakash, O. (2020a). Phytochemicals in Cancer Treatment: From Preclinical Studies to Clinical Practice. *Frontiers in Pharmacology*, *10*, 1614. https://doi.org/10.3389/fphar.2019.01614

Choudhari, A. S., Mandave, P. C., Deshpande, M., Ranjekar, P., & Prakash, O. (2020b). Phytochemicals in Cancer Treatment: From Preclinical Studies to Clinical Practice. *Frontiers in Pharmacology*, *10*, 1614. https://doi.org/10.3389/fphar.2019.01614

D'Angelo, S., Martino, E., Ilisso, C. P., Bagarolo, M. L., Porcelli, M., & Cacciapuoti, G. (2017a). Pro-oxidant and pro-apoptotic activity of polyphenol extract from Annurca apple and its underlying mechanisms in human breast cancer cells. *International Journal of Oncology*, *51*(3), 939–948. https://doi.org/10.3892/ijo.2017.4088

D'Angelo, S., Martino, E., Ilisso, C. P., Bagarolo, M. L., Porcelli, M., & Cacciapuoti, G. (2017b). Pro-oxidant and pro-apoptotic activity of polyphenol extract from Annurca apple and its underlying mechanisms in human breast cancer cells. *International Journal of Oncology*, *51*(3), 939–948. https://doi.org/10.3892/ijo.2017.4088

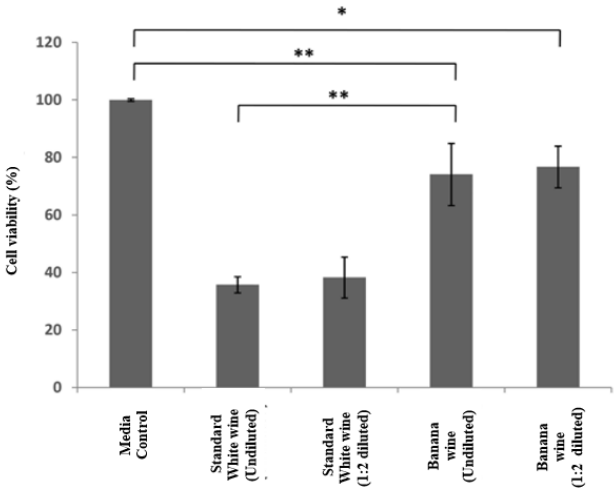
De Souza Dias, F., Silva, M. F., & David, J. M. (2013). Determination of Quercetin, Gallic Acid, Resveratrol, Catechin and Malvidin in Brazilian Wines Elaborated in the Vale do São Francisco Using Liquid–Liquid Extraction Assisted by Ultrasound and GC-MS. *Food Analytical Methods*, *6*(3), 963–968. https://doi.org/10.1007/s12161-012-9507-2

De Souza Dias, F., Silva, M. F., & David, J. M. (2013). Determination of Quercetin, Gallic Acid, Resveratrol, Catechin and Malvidin in Brazilian Wines Elaborated in the Vale do São Francisco Using Liquid–Liquid Extraction Assisted by Ultrasound and GC-MS. *Food Analytical Methods*, *6*(3), 963–968. https://doi.org/10.1007/s12161-012-9507-2

Durva Panchal, Aakifa Ansari, Apoorva Pawar, Anila Gorge, Pampi Chakroborty. (2020). *Antioxidant and Antibacterial properties of wine prepared from bananas*. *15*, 88–97.

El Gharras, H. (2009). Polyphenols: Food sources, properties and applications - a review: Nutraceutical polyphenols. *International Journal of Food Science & Technology*, *44*(12), 2512–2518. https://doi.org/10.1111/j.1365-2621.2009.02077.x
Hakimuddin, F., Paliyath, G., & Meckling, K. (2004). Selective Cytotoxicity of a Red Grape Wine Flavonoid Fraction Against MCF-7 Cells. *Breast Cancer Research and Treatment*, *85*(1), 65–79. https://doi.org/10.1023/B:BREA.0000021048.52430.c0
Hardwick, J. M., & Soane, L. (2013). Multiple Functions of BCL-2 Family Proteins. *Cold Spring Harbor Perspectives in Biology*, *5*(2), a008722–a008722. https://doi.org/10.1101/cshperspect.a008722
Hashemzaei, M., Far, A. D., Yari, A., Heravi, R. E., Tabrizian, K., Taghdisi, S. M., Sadegh, S. E., Tsarouhas, K., Kouretas, D., Tzanakakis, G., Nikitovic, D., Anisimov, N. Y., Spandidos, D. A., Tsatsakis, A. M., & Rezaee, R. (2017). Anticancer and apoptosis-inducing effects of quercetin in vitro and in vivo. *Oncology Reports*, *38*(2), 819–828. https://doi.org/10.3892/or.2017.5766
Heo, S., Kim, S., & Kang, D. (2020). The Role of Hydrogen Peroxide and Peroxiredoxins throughout the Cell Cycle. *Antioxidants*, *9*(4), 280. https://doi.org/10.3390/antiox9040280
Huh, S. W., Bae, S. M., Kim, Y.-W., Lee, J. M., Namkoong, S. E., Lee, I. P., Kim, S. H., Kim, C. K., & Ahn, W. S. (2004). Anticancer effects of (−)-epigallocatechin-3-gallate on ovarian carcinoma cell lines. *Gynecologic Oncology*, *94*(3), 760–768. https://doi.org/10.1016/j.ygyno.2004.05.031
Kang, J. H., Lee, S.-H., Hong, D., Lee, J.-S., Ahn, H.-S., Ahn, J.-H., Seong, T. W., Lee, C.-H., Jang, H., Hong, K. M., Lee, C., Lee, J.-H., & Kim, S.-Y. (2016a). Aldehyde dehydrogenase is used by cancer cells for energy metabolism. *Experimental & Molecular Medicine*, *48*(11), e272–e272. https://doi.org/10.1038/emm.2016.103
Kang, J. H., Lee, S.-H., Hong, D., Lee, J.-S., Ahn, H.-S., Ahn, J.-H., Seong, T. W., Lee, C.-H., Jang, H., Hong, K. M., Lee, C., Lee, J.-H., & Kim, S.-Y. (2016b). Aldehyde dehydrogenase is used by cancer cells for energy metabolism. *Experimental & Molecular Medicine*, *48*(11), e272–e272. https://doi.org/10.1038/emm.2016.103
Kleiveland, C. R. (2015). Peripheral Blood Mononuclear Cells. In K. Verhoeckx, P. Cotter, I. López-Expósito, C. Kleiveland, T. Lea, A. Mackie, T. Requena, D. Swiatecka, & H. Wichers (Eds.), *The Impact of Food Bioactives on Health* (pp. 161–167). Springer International Publishing. https://doi.org/10.1007/978-3-319-16104-4_15
Li, L., & Sun, B. (2019a). Grape and wine polymeric polyphenols: Their importance in enology. *Critical Reviews in Food Science and Nutrition*, *59*(4), 563–579. https://doi.org/10.1080/10408398.2017.1381071
Li, L., & Sun, B. (2019b). Grape and wine polymeric polyphenols: Their importance in enology. *Critical Reviews in Food Science and Nutrition*, *59*(4), 563–579. https://doi.org/10.1080/10408398.2017.1381071
Ljevar, A., Ćurko, N., University of Zagreb, Faculty of Food Technology and Biotechnology, Pierottijeva 6, HR-10000 Zagreb, Croatia, Phenolic Composition, Antioxidant Capacity and in vitro Cytotoxicity Assessment of Fruit Wines. *Food Technology and Biotechnology*, *54*(2). https://doi.org/10.17113/ftb.54.02.16.4208
Mathew, N. S., & Negi, P. S. (2017). Traditional uses, phytochemistry and pharmacology of wild banana (Musa acuminata Colla): A review. *Journal of Ethnopharmacology*, *196*, 124–140. https://doi.org/10.1016/j.jep.2016.12.009
Mildner-Szkudlarz, S., Zawirska-Wojtasiak, R., Obuchowski, W., & Gośliński, M. (2009). Evaluation of Antioxidant Activity of Green Tea Extract and Its Effect on the Biscuits Lipid Fraction Oxidative Stability. *Journal of Food Science*, *74*(8), S362–S370. https://doi.org/10.1111/j.1750-3841.2009.01313.x
Niedzwiecki, A., Roomi, M., Kalinovsky, T., & Rath, M. (2016). Anticancer Efficacy of Polyphenols and Their Combinations. *Nutrients*, *8*(9), 552. https://doi.org/10.3390/nu8090552
Petrović, A., Lisov, N., Čakar, U. D., Marković, N., Matijašević, S., Cvejić, J. M., ... & Gojković-Bukarica, L. (2019). The effects of Prokupac variety clones and vinification method on the quantity of resveratrol in wine. Food and Feed research, 46(2), 189-198. https://doi.org/10.5937/FFR1902189P
Pang, J.-H. S., Yen, J.-H., Wu, H.-T., & Huang, S.-T. (2017). Gallic Acid Inhibited Matrix Invasion and AP-1/ETS-1-Mediated MMP-1 Transcription in Human Nasopharyngeal Carcinoma Cells. *International Journal of Molecular Sciences*, *18*(7). https://doi.org/10.3390/ijms18071354
Ranjan, A., Ramachandran, S., Gupta, N., Kaushik, I., Wright, S., Srivastava, S., Das, H., Srivastava, S., Prasad, S., & Srivastava, S. K. (2019). Role of Phytochemicals in Cancer Prevention. *International Journal of Molecular Sciences*, *20*(20), 4981. https://doi.org/10.3390/ijms20204981
Rezaei, M., Elyasi, F., Janbabai, G., Moosazadeh, M., & Hamzehgardeshi, Z. (2016). Factors Influencing Body Image in Women with Breast Cancer: A Comprehensive Literature Review. *Iranian Red Crescent Medical Journal*, *18*(10). https://doi.org/10.5812/ircmj.39465
Riccardi, C., & Nicoletti, I. (2006). Analysis of apoptosis by propidium iodide staining and flow cytometry. *Nature Protocols*, *1*(3), 1458–1461. https://doi.org/10.1038/nprot.2006.238
Singh, B., Singh, J. P., Kaur, A., & Singh, N. (2016). Bioactive compounds in banana and their associated health benefits—A review. *Food Chemistry*, *206*, 1–11. https://doi.org/10.1016/j.foodchem.2016.03.033
Sun, H., Yin, M., Hao, D., & Shen, Y. (2020). Anti-Cancer Activity of Catechin against A549 Lung Carcinoma Cells by Induction of Cyclin Kinase Inhibitor p21 and Suppression of Cyclin E1 and P–AKT. *Applied Sciences*, *10*(6), 2065. https://doi.org/10.3390/app10062065
Yagi, H., Tan, J., & Tuan, R. S. (2013). Polyphenols suppress hydrogen peroxide-induced oxidative stress in human bone-marrow derived mesenchymal stem cells. Journal of cellular biochemistry, 114(5), 1163-1173. https://doi.org/10.1002/jcb.24459

SUPPLEMENTARY FIGURE



Supplementary Figure 1 Cytotoxic activity of banana wine on human PBMC. The cells were either untreated (media control), or were treated for 24 h with banana wine (un diluted and 1:2 diluted) and standard white wine (undiluted and 1:2 diluted). The data is represented as the mean viability ± SD of the three replicates. (*P < 0.05 and **P < 0.01)