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The Anticancer and Antioxidant Effects of Muscadine Grape Extracts on Racially Different Triple-negative Breast Cancer Cells

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Abstract. *Background/Aim: Triple-negative breast cancer (TNBC) is the most aggressive subtype, predominant in African American women. In this study, the antioxidant/anticancer activity of muscadine grape extracts and the role of their phenolic and flavonoid contents in exerting these properties were investigated in TNBC cells. Materials and Methods: Berry extracts from muscadine genotypes were investigated for total phenolic content (TPC), total flavonoid content (TFC), antioxidant capacity, and anticancer effects using breast cancer cell lines, representing Caucasians and African Americans. Results: The antioxidant activity was associated with high TPC content. Extracts showed cytotoxicity up to 78.6% in Caucasians and 90.7% in African American cells, with an association with high antioxidant capacity. There was a strong correlation between TPC and anticancer/antioxidant activities. Conclusion: The anticancer and antioxidant effects of muscadine grapes are attributed to the TPC of extracts, which showed a stronger positive correlation with growth inhibition of African American breast cancer cells compared to Caucasians.*

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Breast cancer is the second most frequent cause of death among American women, with an expected rise of new cases estimated at 268,600 in 2019 (1). Racial disparities demonstrate that African American and Caucasian women present a different disease outcome. In African Americans, the disease has an earlier onset, more advanced stage, more aggressive histologic features, poor survival (2), and a higher incidence of the triple-negative breast cancer (TNBC) subtype, compared to other ethnic groups (3, 4). TNBC corresponds to 15-20% of breast cancers (5) with the worst prognosis, no approved targeted therapies, and usual chemotherapy as the main systemic therapy (6). TNBC lacks three receptors: estrogen, progesterone, and Her2/neu, which are targets for pharmacological treatment (7). TNBC tumors are linked to an increased size of the tumor and higher incidence of axillary node positivity (8), presenting a more aggressive disease, and elevated chances of having axillary lymph node metastases (9). In African American women, frequently, there is a high-grade tumor, which is notably associated with higher disease stage (10), and the prevalence of TNBC. In addition, TNBC also prevails among women with a mutation in the *BRCA1* gene (11), which is a tumor suppressor able to repair DNA breaks (12-14). TNBC expresses cytokeratin 5 and 6, elevated levels of epidermal growth factor receptor, and exhibits worse clinical outcome. TNBC is also associated with a high occurrence of metastases and a poor rate of survival (11, 15).

Despite vast efforts to establish effective treatments for TNBC using chemotherapy and radiotherapy, complications deriving from the high toxicity and low selectivity of such treatments can cause serious side effects reducing efficacy once metastasis has occurred (16, 17). Phytochemicals are chemicals that derive from plants and have therapeutic properties (18, 19). Among them, the family of polyphenolics is the most abundant and widely studied group

that has received greater attention due to their ability to suppress the growth and initiate apoptosis of cancer cells (20-22). It has been described that breast cancer cell growth (specifically MDA-MB-231), can be effectively inhibited by natural phenolic compounds combined with chemotherapy drugs (23, 24). Therefore, natural polyphenolics may have therapeutic potential as anticancer agents.

Grape is one of the most abundantly consumed fruits worldwide, known also for its health benefits. *Muscadinia rotundifolia* Michx (muscadine grape), is native to the southeastern region of the United States (24, 25). Muscadine grape polyphenols have received increased attention because they contain biologically active compounds at much higher levels relative to other grapes (26-30). Their unique mix of bioactive elements is effective against inflammation, cancer, microbial infections, and oxidative stress (24, 31-33).

Meanwhile, there is a lack of sufficient knowledge of muscadine capacities as a source for biologically active compounds. Also, there is meager information on the diversity of beneficial compounds of muscadine grapes. The variety in structure and phytochemical composition indicate that these compounds may have a potential anticancer activity by acting on various cellular processes linked to tumor development (34). Therefore, in the present study, the antioxidant/anticancer activity of extracts from a wide array of genotypes from muscadine grapes was investigated in TNBC cells. Furthermore, the role of their content in phenolic and flavonoid compounds in exerting these properties was also examined.

Materials and Methods

Grape materials. All muscadine genotypes used in this study were developed under the grape breeding program of the Center for Viticulture and Small Fruit Research, Florida A&M University (FAMU), Tallahassee, FL, USA. Muscadine grape berries from 196 genotypes were collected at the time of optimum harvest maturity, as determined by total soluble sugars, total acidity, and color. Each plant was identified by one of the authors (IES). For each genotype, three clusters/replicate and three replicates/genotype were randomly collected. In order to prepare the extracts, whole berries (seeds, skin, and pulp) were frozen in liquid nitrogen and ground to a fine powder using Geno/Grinder 2010 (Metuchen, NJ, USA). Twelve grams of frozen powder was extracted using 100 ml methanol and shaking for 24 h at room temperature. By using Whatman filter papers no 41 ashless (Thomas Scientific, Swedesboro, NJ, USA), the supernatant was separated, concentrated under reduced pressure at 45˚C with a rotary evaporator, followed by dehydration in a speed vacuum. The extracts were stored at room temperature. Dried extracts (10 mg) dissolved in DMSO (Dimethyl sulfoxide) (1 ml) were used for the quantification of total phenolic/flavonoid contents and DPPH (1, 1 diphenyl 2-picrylhydrazyl) assay. For cytotoxicity assay, 10 mg of dried extracts were dissolved in 25 μl DMSO (stock solution). This was further diluted 10X with DMEM (Dulbecco's Modified Eagle Medium) (working solution) to determine the anticancer activity.

Estimation of total phenolic content (TPC). The content of total phenolic compounds was determined according to the Folin-Ciocalteu colorimetric method (35) with slight modification for the application in 96 microplates. Briefly, aliquots of 20 μl of appropriately diluted samples were placed into wells of microtitration plates (Genesee Scientific, San Diego, California, USA). Subsequently, 100 μl of Folin-Ciocalteu Phenol reagent was diluted with water (1:15, v/v), added to the mixture, and kept for 30 min in the dark at room temperature. Then, 80 μl of sodium carbonate (7.5%) were placed into each well, and the absorbance was measured at λ=630 nm with the microplate reader ACCURIS SmartReader (Edison, NJ, USA) using DMSO as a blank. TPC was determined from extrapolation of a standard curve prepared with 100, 200, 300, and 400 mg/l of gallic acid solution. Phenolic compound estimation was performed for each one of the biological replicates in triplicate and expressed as mg of gallic acid equivalents (GAE) per gram of fresh materials (FM) [mg GAE/g(FM)].

Estimation of total flavonoid content (TFC). The total flavonoid content was determined using 96 microplates as previously described with slight modifications (36). Twenty μl of respective samples were mixed with 60 μl of 96% methanol (v/v). Then, 4 μl of 10% $AlCl_3*6H_2O$ and 4 μl of potassium acetate (1 M) were added to the mixture and brought to 200 μl with deionized water. After a 30 min-incubation time in the dark at room temperature, the absorbance of the supernatants was measured in triplicates using a microplate reader (λ=405 nm), and deionized water was used as a blank. Determination of TFC was based on a standard curve prepared using different concentrations of quercetin ranging from 3.12 to 100 mg/l. Flavonoid estimation was performed for each one of the biological replicates in triplicate. The TFC was presented as milligrams of quercetin equivalents (QE) per gram of fresh materials [(mg QE/g(FM)].

Measurement of the antioxidative activity. The antioxidative activity was measured by DPPH radical scavenging activity (37). Ten mg of the dried grape extracts dissolved in DMSO (1 ml) was used as a stock solution. A serial dilution was prepared in a 96-well plate, obtaining final concentrations of 100, 50, and 25 μg/ml for each extract. Briefly, 198 μl of a methanol-DMSO solution (1% DMSO) was added to the first well and 100 μl to the second and third well. Then, 2 μl of the stock solution was added to the first well (total volume of 200 μl) to reach the concentration of 100 μg/ml. By mixing and transferring 100 μl to the second and third wells, the concentrations of 50 μg/ml and 25 μg/ml were obtained. Next, 100 μl of freshly prepared DPPH methanolic solution (200 μM) were added to each well. After a 30 min-incubation time in the dark at room temperature, the absorbance of the triplicates was measured using a microplate reader (λ=405 nm). Trolox was used as a control. The DPPH results were expressed as % DPPH inhibition using the following formula: % inhibition=$[(A_{blank}-A_{sample})/(A_{blank})]$ *100. The calibration curve was established using the inhibition rate of Trolox solution. IC_{50} was determined for extracts of muscadine genotypes exhibiting the highest anticancer activity, as the concentration of the sample required to inhibit the formation of the DPPH radical by 50%.

Cell culture. Triple-negative breast cancer cells (MDA-MB-231 (MM-231) (Caucasian) and MDA-MB-468 (MM-468) (African American)) were purchased from American Type Culture Collection (ATCC)

(Manassas, VA, USA). The cells were grown in 75 mm flasks using DMEM, 10% heat inactivated fetal bovine serum (FBS-HI) and 1% penicillin/streptomycin (100 U/ml penicillin and 0.1 mg/ml streptomycin). Cell cultures were incubated in an atmosphere of 5% CO_2 at 37˚C. Experimental media consisted of DMEM supplemented with 2.5% of FBS-HI.

Anticancer activity. Cells (density of 3×104 cells/well) were incubated overnight in experimental media in 96-well plates. All muscadine extracts were prepared in 3 biological replicates, and each replicate was tested in triplicate on the 96-well plate. Muscadine extracts (dissolved in DMSO) were added to the 96-well plates at final concentrations of 400 µg/reaction (4 µg/µl). Cells were incubated at 37˚C for 24 h. Next day, 10 µl of Alamar Blue solution (0.5 mg/ml) was pipetted into the plate (final concentration 10%, v/v), and incubated for an additional 4 h. The fluorescence signal was measured (550/580 nm) using Infinite M200 microplate reader (Tecan, Männedorf, Switzerland). Controls were treated with DMSO at the same concentration used in the extracts (<1%). Blank wells contained only media, without cells. The cytotoxicity rate was calculated based on cell viability, using the following formula:

% viability=(treated cells – blank cells) / (control cells – blank cells) × 100
% cytotoxicity=100 – (% cell viability)
The IC_{50} for cell growth inhibition was calculated for extracts of muscadine genotypes exhibiting the highest activity.

Data analysis. Graph Pad Prism software (version 6.07) was used for statistical analysis, and all data presented as the mean±SEM of 3 biological replicates. IC_{50} values for cell growth inhibition were calculated by nonlinear regression using the dose-response curve plot, log of compound concentration, and percentage of inhibition of cell growth. Principal component analysis (PCA) and hierarchical clustering were carried out using XLSTAT software to examine the grouping of genotypes, outliers and to visualize the relative distribution of the biological activities and total phenolic/flavonoid content. PCA was run on the log2-transformed area using the individual variables. Hierarchical clustering was run using complete linkage method with correlation.

Results

In this study, different muscadine genotypes were selected to ensure diversity regarding flower structure (female/perfect), reproductive parameters (berry/cluster size), phenological characters (early/late) and target of use (fresh/wine). The methanolic extracts of the whole muscadine grape berries (seeds, skin, and pulp) were evaluated for nutraceutical properties. All the studied traits varied considerably among muscadine genotypes. Based on the visual characterization of berry color, 41.8% of the population was either bronze or black, 3.1% green, and 13.3% were red (Suppl. Material).

TPC levels in muscadine berries ranged from 14.7±0.02 to 169±0.05 mg GAE/g(FM) with a mean of 32.06±1.17 mg GAE/g(FM). Based on the minimum content of 70 mg GAE/g(FM), 26% (51/196) of the population exhibited high TPC accumulation (Suppl. Material). Among them, 27 and 24 genotypes displayed black/red and bronze/green color, respectively. Similarly, TFC levels ranged from 14.9±0.02 to 97.8±0.04 mg QE/g(FM) with a mean of 30.32±0.52 mg QE/g(FM). Based on the minimum content of 30 mg QE/g(FM), 27% (53/196) of the population displayed high TFC accumulation (Suppl. Material). Among them, 38 and 15 genotypes were black/red and bronze/green, respectively. Analysis of TPC/TFC relationship revealed that both traits showed a positive correlation (r=0.391, $p<0.001$) among the 196 genotypes (Table I).

Table I. *Simple correlation coefficient (r) between total polyphenolic and flavonoid content of muscadine berry extracts and anticancer and antioxidant activities.*

	TPC	TFC	DPPH	MM-231	MM-468
TPC	1	0.391***	0.820***	0.658***	0.793***
TFC		1	0.410***	0.173*	0.181*
DPPH			1	0.559***	0.583***
MM-231				1	0.627***
MM-468					1

Total phenolic content (TPC), total flavonoid content (TFC); DPPH antioxidant activity; and anticancer activity in Caucasian (MM-231) and African American (MM-468) breast-cancer cell lines were analyzed. Statistically significant differences represented by probability levels are indicated as *$p<0.05$, **$p<0.01$, and ***$p<0.001$ [n=196, $r_{0.05}$=0.140, $r_{0.01}$=0.184, $r_{0.001}$=0.233].

Assessing the DPPH scavenging capacity of the extracts revealed that the antioxidant activity ranged from 7.1 to 56.9% with a mean of 25.96%±0.69. Based on minimum oxidant inhibition activity of 30%, 27.6% (54/196) of the population demonstrated high antioxidant activity (Suppl. Material). Among them, 28 and 26 genotypes displayed black/red and bronze/green color, respectively. Evaluation of the association between muscadine genotypes exhibiting high antioxidant activity and their TPC/TFC levels revealed that 40.1% were associated with high TPC/TFC levels. Specifically, 29.6% and 3.7% were associated with high TPC and TFC levels, respectively. Correlation analysis demonstrated a stronger positive correlation between DPPH inhibition and TPC (r=0.820, $p<0.001$) compared to TFC (r=0.410, $p<0.001$) (Table I).

The screening for anticancer activity demonstrated that the cytotoxicity levels of muscadine extracts ranged from 0-78.6% for MM-231 cells and 0.3-90.7% for MM-468 cells. Based on 50% minimum cell growth inhibition, 20.4% (40/196) of the population demonstrated high cytotoxicity for MM-231 and/or MM-468 cell lines (Suppl. Material). Among them, 17 and 23 genotypes displayed black/red and bronze/green color, respectively. The evaluation of the association between muscadine genotypes exhibiting high anticancer activity and their antioxidant capacity revealed that 64.1% were associated with high antioxidant activity.

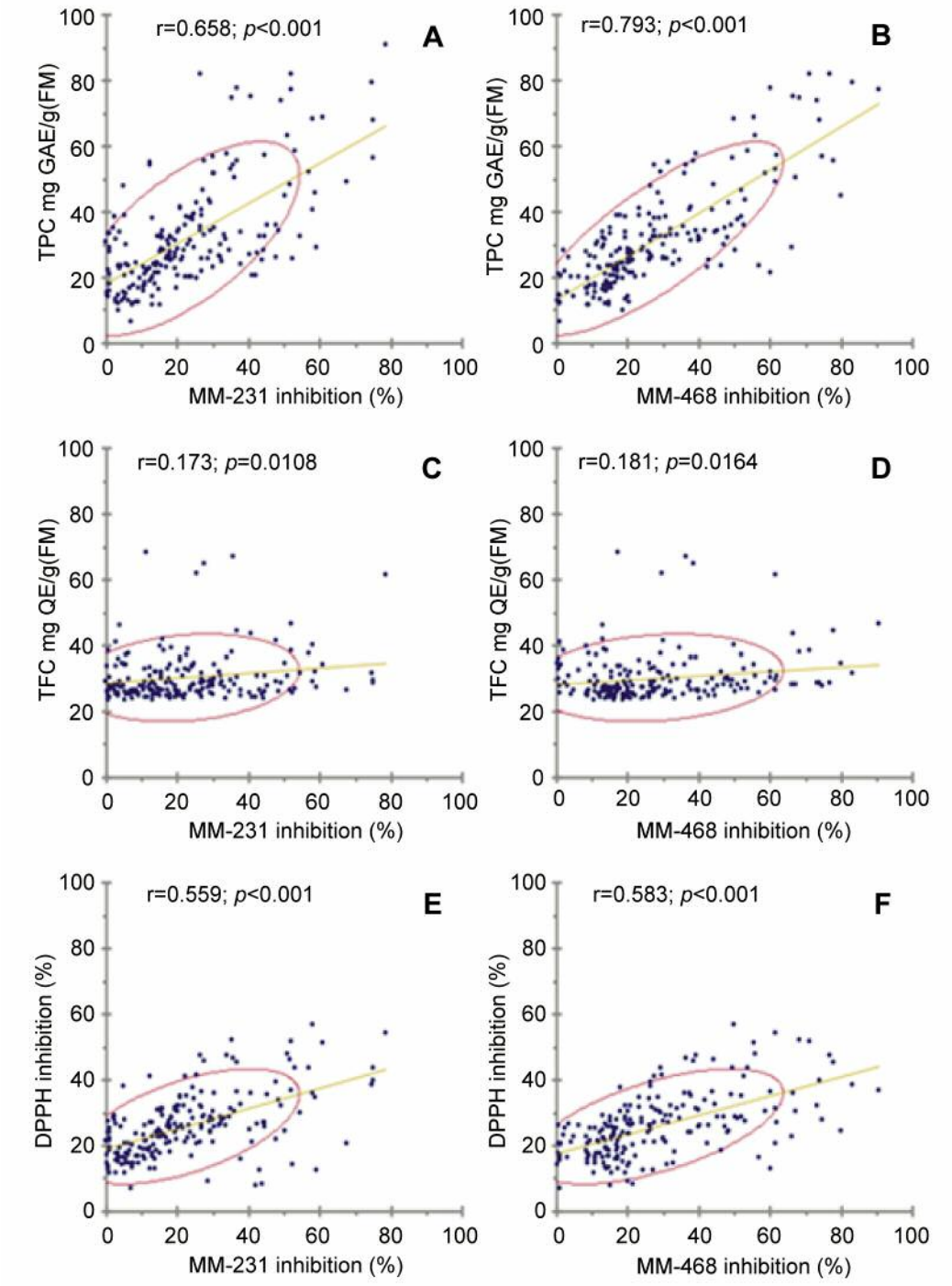

Figure 1. *Illustration showing the correlation of MM-231/MM-468 cell growth inhibition versus TPC (A, B), TFC (C, D) and DPPH inhibition (E, F). Each point represents the genotype value for each of the two traits. Statistically significant differences represented by probability levels at *p<0.05, **p<0.01, and ***p<0.001 are calculated [n=196, $r_{0.05}$=0.140, $r_{0.01}$=0.184, $r_{0.001}$=0.233].*

Correlation analysis demonstrated a significant positive linear correlation between TPC and the inhibition of cell growth in the two breast cancer cell lines (Figure 1A and B; Table I); however, the correlation was stronger with MM-468 cells (r=0.793, p<0.001) than that with MM-231 cells (r=0.658, p<0.001). Surprisingly, TFC levels showed moderate correlation with the cell growth inhibition of MM-231 (r=0.173, p<0.05) and MM-468 cells (r=0.181, p<0.05)

(Figure 1C and D; Table I). Interestingly, the antioxidant capacity showed similar correlation trend as that of TPC with the growth inhibition of both breast-cancer cell lines (Figure 1E and F; Table I). The antioxidant activity exhibited strong positive linear correlation with cell growth inhibition of MM-231 (r=0.559, $p<0.001$) and MM-468 cells (r=0.583, $p<0.001$).

Principle component analysis (PCA) was performed using the results from the assessment of 196 muscadine genotypes in order to establish the relationship between the six variables, including berry color, TPC/TFC levels and the consequent biological activities represented by antioxidant capacity and anticancer activity, using MM-231 and MM-468 breast-cancer cells (Figure 2). As over 71.48% of the PCA, the variance was covered by the first (PC1: TPC; 53.16%) and second (PC2: TFC; 18.32%) principal components. Therefore, we considered that the variables used for this study were represented by these two components. The PCA model indicated that the variables were separated into four clusters based on their correlation. Cluster-I represented by two very close patterns of MM-231 and MM-468 cell growth inhibition. Cluster-II, represented by the close patterns of TPC levels and DPPH inhibition, was located nearby the first cluster. Cluster-III, represented by TFC levels, was located slightly far from the other traits. Cluster-IV was located in a different region separated from the rest of the variables.

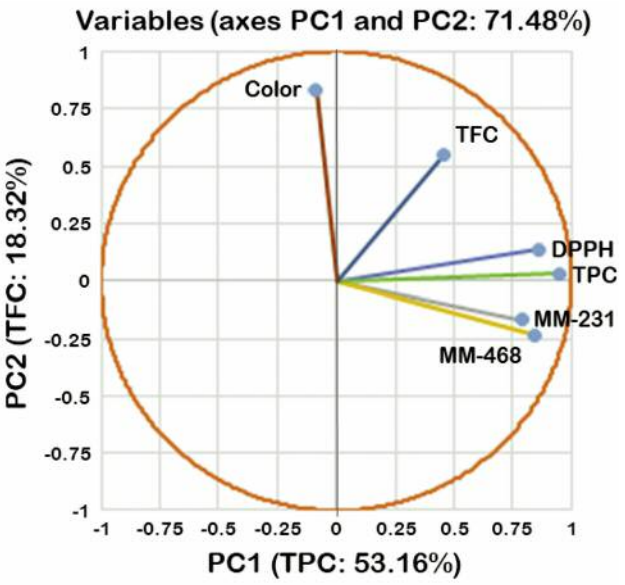

Figure 2. *PCA scatter plots of different traits. TPC: Total phenolic content; TFC: total flavonoid content; DPPH: antioxidant effect; MM-231 (Caucasian breast-cancer cells) and MM-468 (African American breast-cancer cells) anticancer activity. According to the PCA model, 53.16% and 18.55% of the variance were explained by the PC1 (TPC) and the PC2 (TFC) principal components, respectively.*

A hierarchical cluster map was constructed to elucidate the pattern of MM-231 and MM-468 cell growth inhibition along with TPC/TFC measurements and antioxidant capacity (Figure 3). Interestingly, the hierarchical clustering allowed us to identify four different groups of muscadine genotypes based on their anticancer activities. The dendrogram shows relationships based on abundance levels of the four cytotoxicity pattern groups. Group-I, represented by 6.6% (8 black/red and 5 bronze/green) of the population, showed high anticancer activities for both types of breast cancer cells. Group-II, represented by 3.6% (4 black/red and 3 bronze/green) of the population, demonstrated selective high cytotoxicity for the MM-231 cell line. Group-III, represented by 10.2% (5 black/red and 15 bronze/green) of the population, displayed selective high cytotoxicity for the MM-468 cell line. Lastly, group-IV represented by 79.6% (91 black/red and 65 bronze/green) of the population, exhibited low activities for both types of breast cancer. The results indicated that breast anticancer activity of muscadine extracts occurred in a cell line-dependent manner.

Finally, we analyzed the distribution of fruit traits and consequent biological activities in the muscadine population (Figure 4). Excluding MM-231 cell growth inhibition, the distribution frequencies of all traits resembled a normal distribution with transgressive segregation. However, MM-231 cell growth inhibition trait displayed a typical geometric negative binomial distribution pattern (Figure 4D). The distribution pattern for TPC, TFC, DPPH radical scavenging activity and MM-468 cell growth inhibition traits were skewed to the right departing slightly from normality (Figure 4A, B, C and E), where most of the muscadine genotypes shifted towards lower content (TPC and TFC) or bioactivity (DPPH and MM-468 cell growth inhibition). This pattern of distribution suggests that the inheritance mode of these traits is most likely coordinated quantitatively by polygenes in our study population. Only the TFC trait exhibited a truncated distribution pattern due to the absence of TFC values in the range of 0 and 20 mg QE/g(FM) (Figure 4B).

The obtained data (Table II) showed the seven most potent muscadine genotypes with high inhibitory effect over the cell growth of Caucasian (MM-231) and/or African American (MM-468) breast cancer cell lines. In general, the identified seven extracts illustrate close IC_{50} values in relation to the anticancer activity (Table II). The IC_{50} values demonstrated that the genotypes A22-4-1 exhibited the highest cytotoxicity in MM-231 cells with an IC_{50} of 2.7 mg/ml. However, the D7-16-1 genotypes displayed the highest cytotoxicity against the MM-468 cell line with an IC_{50} value estimated at 2.8 mg/ml. The same extracts showed significant antioxidant capacity with IC_{50} values ranging from 33.1±1.3 to 77.7±3.1 μg/ml (Table III). These findings showed the potential of the seven genotypes in inhibiting cell growth and their antioxidant properties in the studied breast cancer cell lines, which represent Caucasians and African Americans.

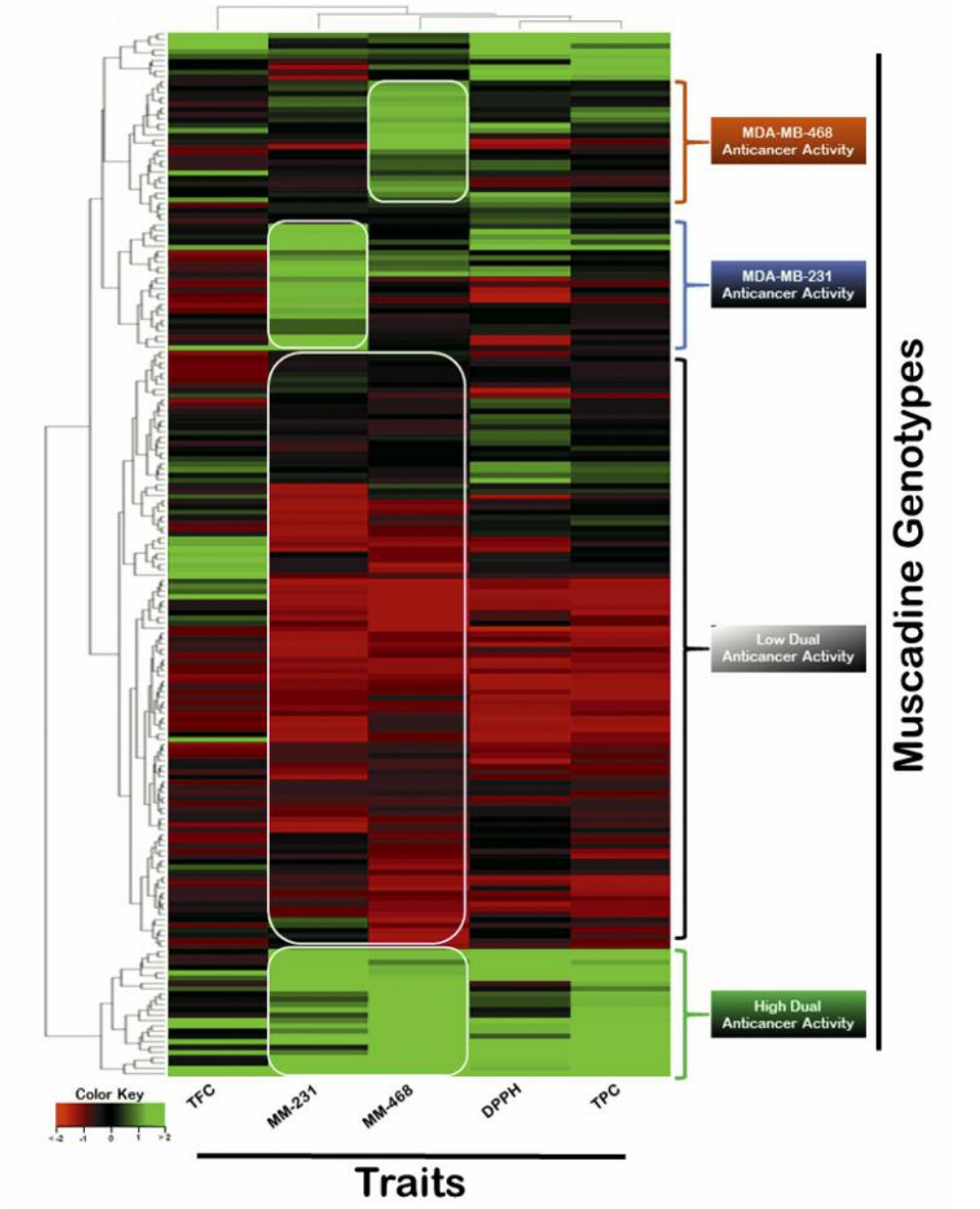

Figure 3. *Hierarchical clustering of phytochemical content and biological activities of muscadine population (196 genotypes). Data related to TPC, TFC, antioxidant activity, and anticancer activity for Caucasian (MM-231) and African American (MM-468) breast-cancer cells are presented as a percentage. The log2-transformed values of each character are represented by colors. Green boxes indicate higher levels and red boxes indicate lower levels compared to the control. The color change is proportional to the accumulation/activity levels (see the color scale at the bottom of the figure).*

Discussion

Various studies on the fruit of muscadine grape revealed that their berries contain a large variety of bioactive constituents. This typically includes nutritive compounds (sugars and vitamins), and also flavonoids, anthocyanins, tannins, catechins, stilbenes, phenolic acids and procyanidins (26-30, 38-40). It has been shown that the bioactive constituents of muscadine berries have activities against cancer and inflammation, and potent antioxidant properties (24, 32, 33). The current investigation aimed to determine the diversity, capability, and cross-correlation of beneficial compounds present in muscadine berries and consequent their biological activities. To achieve this goal, the total phenolic and

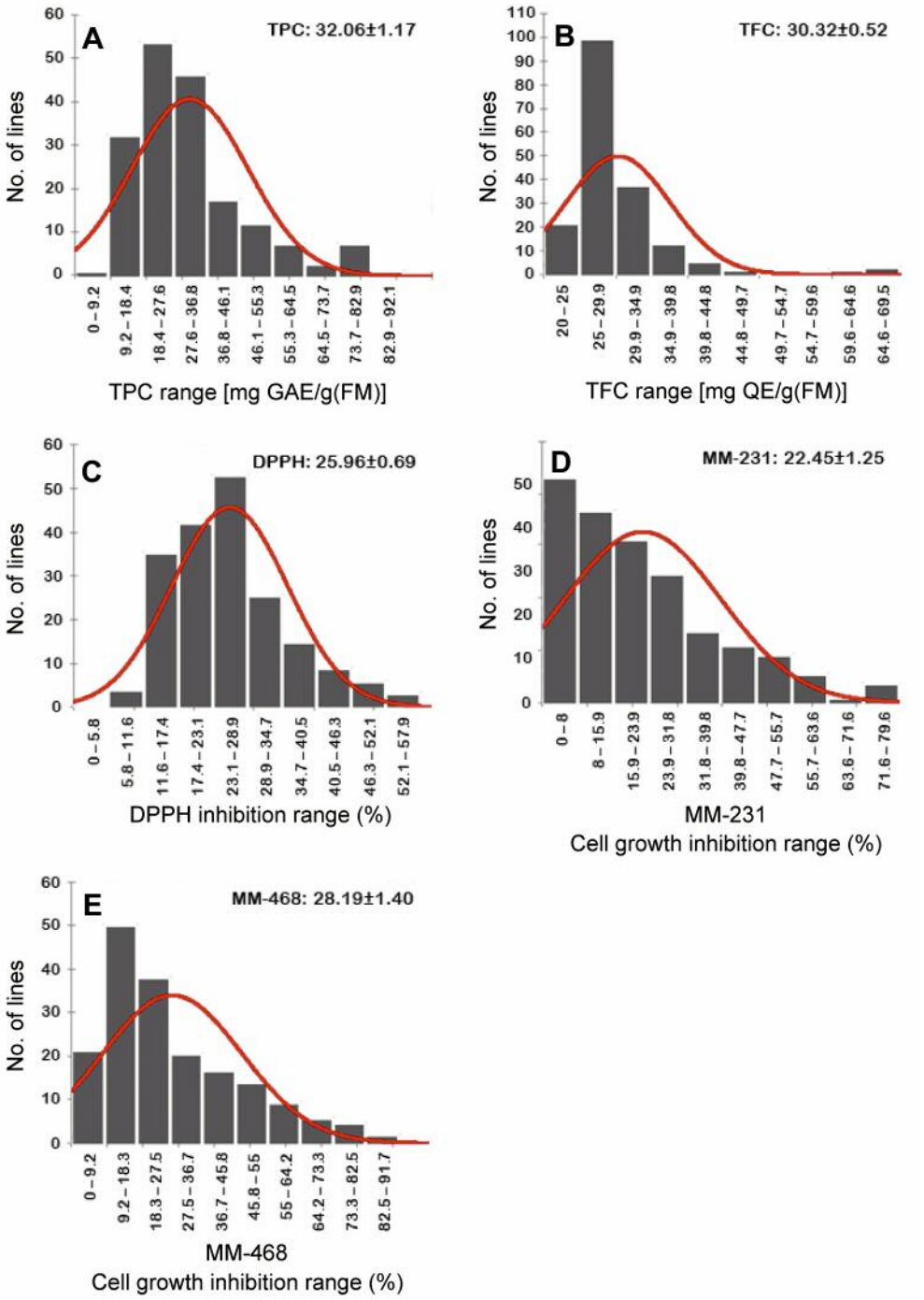

Figure 4. *Frequency distribution of berry metabolomic traits and consequent biological activities of the muscadine population (n=196). Population means of each trait are indicated.*

flavonoid content, DPPH radical scavenging activity, and cytotoxicity were simultaneously profiled in a population of 196 muscadine genotypes using two different breast cancer cell lines, representing Caucasian and African American women.

Phenolic and flavonoid contents are important fruit constituents because of their potent antioxidant activity (41-43). The bioactive properties of phenolic compounds (PCs) of individual plants are influenced by their structure, providing a range of antioxidant activities (44). PCs are attractive targets

Table II. *Percentage of cell growth inhibition of MM-231 and MM-468 breast-cancer cell lines, and IC_{50} values for selected muscadine genotypes.*

A) MM-231 breast-cancer cell line

Genotypes	Cell growth inhibition (%)				IC_{50} (μg/μl)
	4 μg/μl	3 μg/μl	2 μg/μl	1 μg/μl	
O34-21-3	76.5±3.0	22.0±1.0	7.5±0.5	0.5±0.2	3.6
A19-13-8	74.6±3.4	56.5±4.5	18.0±2.0	12.5±1.7	3.1
D7-16-1	74.6±2.3	14.3±2.2	8.0±0.7	2.5±0.8	3.5
Granny Val	66.9±2.0	32.0±2.0	26.0±1.0	17.5±1.5	3.7
O43-16-1	63.7±4.3	42.4±3.9	9.6±1.9	3.8±0.8	3.4
O41-3-1	82.6±1.2	58.0±1.1	43.7±1.1	37.0±1.2	3.1
A22-4-1	83.0±1.9	61.6±2.0	41.7±1.5	27.8±1.2	2.7

B) MM-468 breast-cancer cell line

O34-21-3	69.0±3.0	44.7±2.3	38.5±2.5	36.0±2.0	4.0
A19-13-8	46.8±2.0	38.5±3.0	18.0±2.0	12.5±1.6	4.7
D7-16-1	85.5±1.9	80.0±3.0	62.5±3.1	49.8±0.8	2.8
Granny Val	61.9±2.9	28.0±1.8	8.0±1.6	3.0±0.7	3.6
O43-16-1	82.6±2.0	61.0±3.4	48.5±1.5	39.0±2.0	3.2
O41-3-1	61.8±3.1	44.3±1.8	39.5±1.5	33.3±0.7	3.1
A22-4-1	74.5±3.1	35.3±2.8	31.0±2.3	25.7±2.4	3.4

The data are presented as the mean±SEM biological (n=3) and technical replicates (n=9). IC_{50} values were calculated from the dose-response curve plot of the extract concentration log (μg/μl) and % inhibition of cell growth. Data were calculated using the nonlinear regression with Graph Pad Prism statistical software.

Table III. *Percentage of DPPH inhibition and IC_{50} values for selected Muscatine genotypes.*

Genotypes	DPPH inhibition (%)			IC_{50} (μg/ml)
	50 μg/ml	25 μg/ml	12.5 μg/ml	
O34-21-3	62.7±0.02	43.8±0.02	28.4±0.02	33.1±1.3
A19-13-8	56.6±0.03	36.1±0.01	20.2±0.02	42.1±1.2
D7-16-1	56.8±0.07	39.2±0.01	20.2±0.03	40.9±3.1
Granny Val	35.9±0.04	22.7±0.03	13.4±0.01	77.7±3.1
O43-16-1	46.0±0.04	32.3±0.03	24.4±0.02	57.6±2.4
O41-3-1	53.7±0.01	42.1±0.03	22.8±0.06	46.3±2.6
A22-4-1	36.7±0.04	22.4±0.04	17.0±0.01	69.6±1.8
Trolox (μM)	83±0.08	60.2±0.06	25.0±0.02	13.9±1.5

The data are presented as the mean±SEM of biological (n=3) and technical replicates (n=9).

in the phytochemicals search since their isolation and characterization helps to comprehend the numerous antioxidant action mechanisms (45). Our results showed that 27% of the extracts presented high antioxidant activity, and among those, 40.1% were associated with high TPC/TFC contents. However, the contribution of TPC seems to be more significant.

Muscadine grape is characterized by elevated TPC levels in the skin (ellagic acid, quercetin, and others) and in the seeds (gallic acid, resveratrol, catechin, proanthoacyanidins, and others) (28, 40, 46-49). Accordingly, muscadine grapes exhibited different degrees of antioxidant and anticancer abilities depending on genotype and the type of challenging cancer (33, 50-54). Grape polyphenols are attractive cancer therapeutics with the potential to be included in combination therapy. It has been shown that high concentrations of individual grape polyphenols may play a role in cancer prevention, having antiproliferative, antioxidant, antiangiogenic, anti-invasive, and pro-apoptotic activities (55). Furthermore, grape polyphenols may specifically inhibit breast-cancer cell growth with low cytotoxicity towards normal mammary epithelial cells (16, 56).

Since no comparative study about the effects of muscadine extracts in Caucasian and African American TNBC cells have been previously described, we explored whether muscadine extracts contain compounds that can inhibit the growth of these cells. The MM-231 (Caucasian) and the more aggressive MM-468 (African American) cell lines were treated with crude extracts. Results showed that muscadine grape extracts were able to inhibit cell growth *in vitro* with high efficiency. Even more, the extracts showed more potency in African American breast-cancer lines, displaying 90.7% cytotoxicity in MM-468 (African American) cells *versus* 78.6% cytotoxicity in MM-231 cells (Caucasian). Correlation analysis showed a stronger positive correlation between the TPC/antioxidant capacity and the inhibition of cell growth in MM-468 compared to MM-231 cells. However, TFC levels displayed only a moderate correlation with the inhibition of cell growth in both breast cancer cell lines. Even though natural flavonoids play major roles in defining the ultimate antioxidant/anticancer activities, and muscadine grapes accumulate high TFC concentrations (28, 39, 46-50, 57, 58), it seems that muscadine flavonoids slightly contribute to breast anticancer activity, at least under our experimental conditions.

In previous studies, researchers have used muscadine grape pomace to study different phenolic fractions and assess their antioxidative effect and capacity to induce apoptosis and cause cell cycle arrest in MM-231 breast cancer cells. It has been shown that the fraction exhibiting the highest anti-oxidative activity and the strongest ability to induce cell cycle arrest, and apoptosis was a mixture of anthocyanidins and ellagic acid (24). Also, Yi and colleagues, using colon cancer cell lines, have determined the anticancer activity of polyphenols in various cultivars of muscadine grapes. The IC_{50}s of crude extracts ranged from 1 to 7 mg/ml. However, when the extracts were partitioned, the IC_{50}s were lowered as expected, ranging from 0.5 to 3 mg/ml and 0.3 to 0.6 mg/ml in the phenolic and flavonoid fractions, respectively

(53). In the present study, the seven genotypes with the highest anticancer activity presented IC_{50}s of the crude extracts ranging from 2.7 to 4.7 mg/ml, showing a potent inhibitory effect on the growth of Caucasian and African American cell lines. The antioxidant results showed that the genotypes O34-21-3, A19-13-8, and D7-16-1 presented the lowest IC_{50}, showing the higher potency. On the other hand, the genotypes A22-4-1/O41-3-1/A19-13-8 and D7-16-1/O41-3-1 showed the highest potency in inhibiting cell growth in MM-231 and MM-468, respectively. Taking in account that the beneficial properties of muscadine grapes are related to a variety of bioactive components that enhance antioxidant capacity and consequently anticancer activity (37, 59, 60), the genotypes A19-13-8 and D7-16-1 may be strong candidates for future breast cancer studies, having high antioxidant and anticancer activity, as well as a high phenolic content.

Conclusion

The identification of new compounds with antitumor and antioxidant activities, but minimum toxicity is vital to discover substances that may be significant in cancer prevention and possible treatment. In this study, the potential bioactivities of grape phytochemicals were examined in two racially different breast cancer cell lines. Our results showed a differential effect of muscadine extracts in Caucasian and African American breast cancer cell lines. Our results showed a high association between anticancer and antioxidant activities and total phenolic content in selected genotypes. Future studies are needed to examine the effect of different fractions of the most effective extracts to identify the phenolic components that are responsible for the antioxidant and anticancer activities described in this work.

Conflicts of Interest

The Authors declare that there is no conflict of interest regarding the publication of this paper.

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Authors' Contributions

Conceptualization: PM, AD, IES, KS; Methodology: PM, AD; Formal analysis: PM, AD, IES; Funding acquisition: IES, KS; Project administration: IES, KS; Resources: IES, KS; Software: PM, AD, IES; Supervision: IES, KS; Writing – original draft: PM, AD, IES, KS; Writing – review & editing: PM, AD, VT, IES, KS.

Supplementary Material

Muscadine grape population used for evaluation of nutraceutical properties. Available at: http://pharmacy.famu.edu/wp-content/uploads/2019/06/Supplementary-Materials.pdf

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