

Antioxidant and antimicrobial activities of muscadine grape extracts

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머스커다인 포도 추출물의 항산화 및 항균 활성

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Abstract

The objective of this research was to investigate the antioxidant and antimicrobial activities of muscadine grape extracts. Three different cultivars of muscadine grapes including Higgings, Jumbo, and Noble were selected. The skin/pulp and seed parts of three selected muscadine grape cultivars were used for extraction. The total phenolic contents of muscadine grape extracts were expressed as gallic acid equivalents (GAE). The antioxidant activity of muscadine grape extracts were determined by scavenging activity of diphenylpicrylhydrazyl (DPPH) radical and expressed as effective concentration (EC₅₀), which represented the concentration of the extract exhibiting 50% DPPH radical scavenging. The antimicrobial activity against *E. coli* K12 was determined and expressed as the minimum inhibition concentration (MIC). The seed extracts exhibited greater total phenolic contents than the skin/pulp extracts, ranging from 231.24 to 294.81 mg/mL GAE. The seed extracts exhibited greater antioxidant activities than the skin/pulp extracts (EC₅₀ of Higgins seed extract=0.026 mg/mL). However, the skin/pulp extracts exhibited greater antimicrobial activities than the seed extracts, exhibiting the minimum inhibition concentration (MIC) in Higgins skin/pulp extract (MIC=4.0 mg/mL). This research indicated that the seed part and skin/pulp parts of the muscadine grapes possessed antioxidant activity and antimicrobial activity, respectively. Therefore, it was concluded that muscadine grapes possess the potential to be utilized as functional foods or nutraceuticals.

Key words : antioxidant activity, antimicrobial activity, muscadine grapes, polyphenols, extract

Introduction

Naturally occurring antimicrobial compounds play an important role in the natural defense or competition systems of living organisms including microorganisms, insects, animals and plants (1). Many plant-derived foods including vegetables, fruits, and spices and herbs contain indigenous compounds that possess antioxidant and antimicrobial

activities. Natural compounds found in plants with potentially antimicrobial activity can be divided into phenolics, polyphenols, quinones, flavons, flavonoids, flavonols, tannins, coumarines, terpenoids, alkaloids, lectins, and polypeptides (2). Naturally occurring antimicrobial agents in foods may be desirable alternatives to replace synthetic preservatives food products.

Muscadine grapes (*Vitis rotundifolia*) are one type of grape varieties grown in the south and southeastern areas of the United States (3). Due to the unique tastes and flavors, muscadine grapes are more often consumed as fresh table fruits, rather than as grape juice or muscadine wines (4). The production and utilization of muscadine grapes are limited to the cotton-belt areas of the southeastern United States,

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Received 21 October 2014; Revised 22 December 2014;
Accepted 25 January 2015.
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primarily due to the necessity of a moderate climate condition. In addition, since muscadine grapes have a relatively shorter storage periods compared to grapes, muscadine grapes were sold locally. Thus, the nutritional information and health benefits of muscadine grapes have not been publicized (4).

Recently, however, the interests in muscadine grapes are growing due to the unique phytochemical compositions in muscadine grapes (4-7). Muscadine grapes are believed to possess strong antioxidant compounds (8). The major antioxidant compounds in muscadine grapes include monomeric procyanidin compounds such as (+)-catechins, (-)-epicatechin, (-)-epicatechin-3-O-gallate, and dimeric, trimeric, and tetrameric procyanidins (9). The other antioxidant compounds in muscadine grapes are phenolics including ellagic acid, gallic acid, myricetin, kaempferol, and quercetin (4).

Grape seeds are known to possess strong antioxidant and antimicrobial activities (10). The antimicrobial activities of grape seeds are mainly attributed to monomeric phenolic compounds such as (+)-catechins, (-)-epicatechins and (-)-epicatechin-3-O-gallate and dimeric, trimeric and tetrameric procyanidins (11,12). The antibacterial and antioxidant activities of grape seed extract are reported by several investigators (12-14). Gram-positive bacteria such as *Bacillus* and *Staphylococcus* were completely inhibited by grape seed extract at the concentration of 850~1,000 ppm, while gram-negative bacteria such as *E. coli* and *Pseudomonas* required greater concentrations of 1,250~1,500 ppm for similar inhibition (12).

Muscadine grapes contain considerable amounts of polyphenolic compounds distinguished by ellagic, gallic, and flavonoid concentrations, similar to other grapes (4,15,16). However, compared to the extensive research on antimicrobial activity of other grapes, the antimicrobial activities of muscadine grapes are not well investigated. The information on the antimicrobial activities of muscadine grapes as well as the antioxidant activities will benefit the growers and consumers, and increase the production and consumption of the muscadine grapes. Therefore, the main objective of this research was to determine the antioxidant and antimicrobial activities of selected muscadine grape cultivars.

Materials and Methods

Materials

Three cultivars of muscadine grapes were selected, including

bronze-skinned Higgins cultivar, and purple-skinned Jumbo and Noble cultivars. The muscadine fruits were harvested from the George Washington Carver Agricultural Experiment Station farm at Tuskegee University, Tuskegee, AL, USA in late August. The collected muscadine grapes were kept frozen at -20°C prior to use. Gallic acid and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Tryptic soy broth (TSB) and tryptic soy agar (TSA) were purchased from Difco-Laboratories (Detroit, MI, USA). All other reagents and solvents (Sigma Chemical Co., St. Louis, MO, USA) were analytical/HPLC grades.

Extraction of muscadine grape

A portion of 1,500 g of each fresh muscadine grape cultivar was weighed and freeze-dried. The seeds were separated from the dried pulp and skin after freeze-drying. The dried skin/pulp and seed parts were ground and powdered using a blender. The muscadine extraction was performed following the protocols for the grape seed extraction developed by Jayaprakasha et al. (12) with minor modifications. A portion of 150 g of each skin/pulp and seed powder was placed into a 500 mL-Erlenmeyer flask, and the fat extracted were removed from each sample using 250 mL petroleum ether by shaking three times. The defatted samples were filtered and dried over night, and stored in the dark in a freezer at -20°C until further analyses.

A portion of 60.0 g of defatted skin/pulp powder was taken for extraction. The extraction was performed with a mixture solvent (140 mL) of methanol:water:acetic acid (90:9.5:0.5) in a Soxhlet apparatus for 8 hr at 75°C. The extract was separated by centrifugation at 3,000×g for 15 min, and the supernatant was concentrated under the vacuum, flushed with N₂ gas, and the extract was kept in dark place prior to further analyses. A similar procedure was applied to obtain seed extracts; muscadine seed powders from Higgins (28.8 g), Jumbo (29.3 g) and Noble (58.0 g) were taken and extracted with 93.2, 94.0 and 137.0 mL mixed solvents, respectively.

Total phenolic content

The total phenolic contents of muscadine grape extracts were determined following the Folin-Ciocalteu method with minor modifications (10,17). A portion of 0.50 mL of the diluted extracts was mixed with 0.25 mL Folin-Ciocalteu reagent, followed by the addition of 1.25 mL sodium carbonate (20% aqueous solution). The mixture was kept in the dark for 40 min, and the absorbance was measured at

725 nm using a spectrophotometer (Shimadzu UV-Vis 2401 PC, Suzhou Instruments Manufacturing Co., Suzhou, China). A standard curve was obtained using gallic acid with selected concentrations of 0.0, 0.02, 0.04, 0.06, 0.08, and 0.1 mg/mL. The total phenolic content of the extracts was expressed as gallic acid equivalents (GAE), and the GAE represented the phenolic content as the amount of gallic acid (mg) in 1.0 g sample.

Scavenging activity of diphenylpicrylhydrazyl (DPPH) radical

An aliquot of 1.0 mL of 0.1 mM DPPH radical solution was dissolved in methanol and mixed with 0.5 mL each muscadine grape extract with a concentration of 0.05 mg/mL or blank methanol for negative control. The reaction solution was mixed, and the absorbance was recorded at 520 nm using a spectrophotometer. Gallic acid (1.5 mg/mL) was used as a standard. The DPPH radical-scavenging activity (%) was calculated by the following equation:

$$\text{Scavenging activity (\%)} = \{1 - (\text{Abs at 520 nm sample} / \text{Abs at 520 nm of the control})\} \times 100.$$

The scavenging activity of sample was also expressed as 50% effective concentration (EC₅₀), which represented the concentration of sample exhibiting 50% DPPH radical scavenging activity (17).

Antimicrobial activity

Escherichia coli K12 was obtained from the Food Microbiology Culture Collection in the Department of Food and Nutritional Sciences at Tuskegee University, Tuskegee, AL, USA. From the stock culture of *E. coli* K12, one colony of bacteria was transferred to 9.9 mL TSB, and incubated for overnight at 37°C. The bacterial culture was diluted in TSB to contain 3.0 log CFU/mL. The muscadine grape extracts were also diluted with TSB to obtain selective concentrations of 0.0, 1.0, 2.0, 3.0, 4.0, 5.0 and 10.0 mg/mL. The diluted extracts were filtered with sterilized filter (pore size 0.45 μm) before adding to the bacterial culture. An aliquot of 120 μL *E. coli* culture and 120 μL diluted extracts were incorporated into Microplate wells (UV Star, Greiner bio-one, Frickenhausen, Germany). The absorbance was read at 620 nm immediately using a plate reader (Bio-Tek Instrumenting Co., Winooski, VT, USA). The microplate was then incubated at 37°C for 24 hr, and the absorbance was read at 620 nm again after incubation. A minimum inhibition concentration (MIC) at 24 hr was defined as the lowest concentration of antimicrobial that exhibited a complete growth inhibition. The growth inhibition was defined as the absorbance of the test

wells at 24 hr minus the absorbance of the test wells at 0 hr was less than 0.05 (18).

Statistical analysis

Each experiment was a completely randomized design. The treatment factors were three selected cultivars (Higgins, Jumbo, and Noble) and two selected fruit parts (skin/pulp and seed). The overall F test was conducted following the general linear model of SAS Software package (SAS Inc., Cary, NC, USA). The difference between the means was analyzed using the Least Significant Procedure of SAS Software Package. The entire analyses were repeated three times. The level of significance used was p<0.05.

Results and Discussion

The total phenolic contents in muscadine seed extracts exhibited approximately four-fold greater contents than the total phenolic contents in skin/pulp extracts (Table 1). It is well reported that the seed extracts from grapes contain significantly greater amounts of phenolic compounds than the skin and/or pulp extracts (4,8,10). Even though direct comparison of total phenolic contents among selected studies is difficult, because the levels of phenolic compounds including flavonoids vary by cultivars, locations, prevailing climatic conditions, postharvest handling, and analysis methods (8), the total phenolic contents in seed extracts from muscadine grapes in this study were somewhat smaller than the total phenolic contents in seeds extracts from other grapes. Baydar et al. (10) reported the total phenolic contents in selected cultivars of other grape seed extracts ranged from 506.60 to 589.09 mg/g of GAE.

There was no significant difference in total phenolic contents in skin/pulp extracts among the selected muscadine grape cultivars. However, the purple-skinned Jumbo and Noble seed extracts exhibited significantly greater contents of total phenolics than the bronze-skinned Higgins seed extract (p<0.05). According to Pastrana-Bonilla et al. (4), the purple-skinned muscadine cultivars contain greater amounts of anthocyanin than the bronze-skinned cultivars, which would explain the greater total phenolic contents in the purple-skinned cultivars. The lack of significant differences of total phenolic contents in the skin/pulp extracts in our study indicated that the presence of other polyphenolic compounds rather than anthocyanins might also contribute to the total phenolic contents in the purple-skinned cultivars. Gallic acid, catechin, and epicatechin are believed to be the major phenolic compounds in muscadine grape seeds (16).

Yilmaz and Toledo (8) detected greater amounts of gallic acid in muscadine grape seeds than the amounts in other grape seeds.

Table 1. Total phenolic contents of muscadine grape extracts

Cultivar	Part	GAE ^z (mg/mL of GAE)
Higgins	Skin/pulp	50.36±2.06 ^c
	Seed	231.24±22.76 ^b
Jumbo	Skin/pulp	70.37±2.53 ^c
	Seed	294.81±4.02 ^a
Noble	Skin/pulp	84.60±2.53 ^c
	Seed	281.08±22.14 ^a

All values are mean±SD (n=3).

^zGallic acid equivalents.

Different letters (a, b, and c) reflect significant differences at p<0.05.

The muscadine seed extracts from the tested cultivars exhibited significant greater free radical scavenging activities than the muscadine skin/pulp extracts (p<0.05), resulting in approximately two-fold greater percentages of scavenging activities than the skin/pulp extracts (Table 2). A similar trend in antioxidant activity among selected parts was observed in effective concentration (EC₅₀). Jayaprakasha et al. (12) reported that the scavenging activities of grape seed extracts ranged from 41.3 to 45.6% at the concentration of 0.025 mg/mL extracts. The ranges of scavenging activities of muscadine grape seed extracts in this study were from 89.9 to 95.0% at the concentration of 0.05 mg/mL extracts, indicating similar scavenging activities to other grapes, when the concentrations of added extracts are compensated.

Greater scavenging activities in seed extracts than in skin/pulp extracts were expected based on greater total phenolic contents in seed extracts than in skin/pulp extract. Extensive studies also reported greater antioxidant activities in grape seed parts than in other selected parts such as skin, pulp, or leaves (4,8,10,14). Determined as Trolox equivalent antioxidant capacity (TEAC), muscadine seed extract exhibited 22, 116, and 18-fold greater antioxidant activity than skin, pulp, and the whole grape, respectively (4). Yilmaz and Toledo (8) reported that grape seed extracts exhibited greater oxygen radical absorbance capacity (ORAC) values compared with grape skin extracts, and the greater antioxidant activities of grape seeds might be due to the presence of polymeric procyanidins in addition to monomers. The authors also stated that antioxidant activity involving peroxy radical scavenging activity was comparative rather than quantitative, concluding that the peroxy radical scavenging activities of phenolic compounds present in grape seeds or skins in decreasing order were resveratrol > catechin > epicatechin

= gallic acid > gallic acid = ellagic acids.

In this study, the individual polyphenolic compounds were not identified. However, the fact that 2-fold greater antioxidant activity of muscadine grape seed extracts as compared to 4-fold greater total phenolic contents expressed as gallic acid equivalents implied that the antioxidant activities of muscadine grapes was not only from the phenolic compounds represented by gallic acid, but also from the presence of other antioxidant compounds.

Table 2. The scavenging activity and EC₅₀ of muscadine grape extracts at the concentration of 0.05 mg/mL

Cultivar	Part	Scavenging activity (%)	EC ₅₀ ^z (mg/mL)
Higgins	Skin/pulp	57.09±1.22 ^b	0.044±0.0005 ^b
	Seed	94.99±0.13 ^a	0.026±0.0025 ^d
Jumbo	Skin/pulp	57.34±0.55 ^b	0.040±0.0007 ^b
	Seed	89.88±4.03 ^a	0.027±0.0030 ^d
Noble	Skin/pulp	48.47±0.56 ^c	0.049±0.0010 ^a
	Seed	94.29±0.23 ^a	0.029±0.0005 ^c

All values are mean±SD (n=3).

^z50% effective concentration, representing the concentration of the sample exhibiting 50% DPPH radical scavenging activity.

Different letters (a, b, c, and d) reflect significant differences at p<0.05.

The antimicrobial effects of muscadine seed extracts against *E. coli* K12 are presented in Fig. 1. As the concentrations of muscadine seed extracts increased, the growth of *E. coli* K12 was gradually reduced, however the growth did not reach the levels of complete growth inhibition except for Higgins seed extract. As presented in Table 3, the bronze-skinned Higgins exhibited a minimum inhibitory concentration (MIC) of 10.0 mg/mL, however the purple-skinned Jumbo and Noble exhibited MICs greater than 10.0 mg/mL. Jayaprakasha et al. (12) reported that the MIC

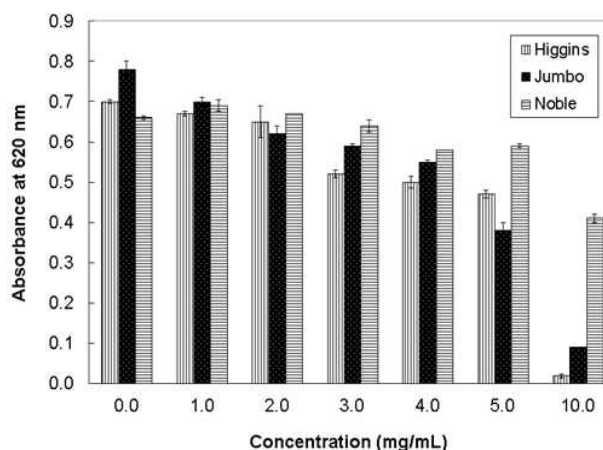


Fig. 1. Effect of muscadine grape seed extracts on the growth of *E. coli* K12 at selected concentrations.

of grape seed fraction extracted using a mixture of methanol, water and acetic acid was determined as 1.25 mg/mL against *E. coli*. According to Ahn et al. (19), the MIC of grape seed extract was determined to be 4.0 mg/mL against 4.43 log CFU/mL per plate of *E. coli* O157:H7. Baydar et al. (10) also reported that the grape seed extracts exhibited bacteriostatic activities at the concentrations of 0.5% and 1.0%, and bactericidal activities at the concentration greater than 2.5% against *E. coli* O157:H7. The MICs of muscadine seed extracts were greater than the MICs from other grape seed extracts in the literature, exhibiting less antimicrobial activities of muscadine grape seed than other grape seed extracts.

Table 3. Minimum inhibition concentration (MIC) of muscadine grape extracts against *E. coli* K12

Cultivar	Part	MIC ^a (mg/mL)
Higgins	Skin/pulp	4.0
	Seed	10.0
Jumbo	Skin/pulp	5.0
	Seed	> 10.0
Noble	Skin/pulp	10.0
	Seed	> 10.0

^aMinimum inhibition concentration.

While the antimicrobial activities of muscadine seed extracts in this study were not comparable to the antimicrobial activities of other grape seed extracts, the muscadine skin/pulp extracts exhibited greater antimicrobial activities than seed extracts against *E. coli* K12 (Fig. 2). The bronze-skinned Higgins exhibited a complete growth inhibition at the concentration of 4.0 mg/mL, and the purple-skinned Jumbo and Noble exhibited complete growth inhibition at the

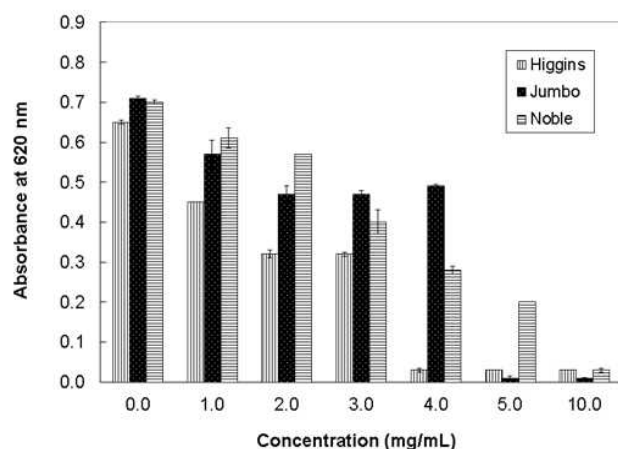


Fig. 2. Effect of muscadine grape skin/pulp extracts on the growth of *E. coli* K12 at selected concentrations.

concentrations of 5.0 and 10.0 mg/mL, respectively. In general, seed parts in the grapes are believed to contain greater amounts of total phenolic compounds than other parts of grapes, presumably resulting in greater antimicrobial activities. Our results also demonstrated greater phenolic compounds in muscadine seed extracts than in muscadine skin/pulp extracts (Table 1).

According to Jayaprakasha et al. (12), the antimicrobial activities of grape seed extracts might be the result of phenolic compounds in the seed extracts, inducing phenoldienones, epicatechin, epigallocatechin, epigallocatechin gallate, ferulic acid, caffeic acid and so on. Therefore, it was anticipated that muscadine grape seed extracts containing greater concentrations of phenolic compounds could exhibit greater antimicrobial activities against *E. coli* K12. However, our results did not support antimicrobial activities from polyphenolic compounds. The total phenolic compounds were expressed as gallic acid equivalents, meaning that most phenolic compounds determined were represented by gallic acid contents. There are controversial reports on the antimicrobial activities from phenolic compounds. The active compound of grape seed extracts to inhibit *E. coli* and *Salmonella enteritidis* was identified as gallic acid (12). However, when tannic acid, propyl gallate, gallic acid and ellagic acid were tested, gallic and ellagic acid did not exhibit antimicrobial activities against pathogenic bacteria (20). The fact that muscadine skin/pulp extracts containing smaller amounts of total phenolic compounds exhibited greater antimicrobial activities may imply that the major compounds responsible for antimicrobial activities in muscadine grapes could be other than polyphenolic compounds expressed as gallic acid. The variations in antimicrobial activities of muscadine grape extracts could be also due to the unique properties from the variations of cultivar, part, agroecology in muscadine grapes, as compared to other grapes.

Muscadine seed extracts contained greater amounts of total phenolic compounds than muscadine skin/pulp extracts, resulting in greater antioxidant activities determined by free radical scavenging activities and effective concentration. However, the antioxidant activities of muscadine grapes were not proportional to the total phenolic contents, implying that other than gallic acid might contribute to the antioxidant activities of muscadine grape extracts. The skin/pulp extracts of the muscadine grapes exhibited greater antimicrobial activities than the muscadine seed extracts against *E. coli* K12 in entire three cultivars. This research indicated that

muscadine grapes contained considerable amounts of antimicrobial compounds other than phenolic compounds. Since the whole fruit parts of muscadine grape including skin/pulp and seed parts exhibit antioxidant and antimicrobial activities, therefore muscadine grapes possess the potential to be utilized as functional foods or nutraceuticals.

요 약

본 논문은 미국 남부지역 특화작물인 머스커다인 포도의 부분별 추출물의 항산화능 및 항균활성을 구명하기 위한 목적으로, Higgins, Jumbo, Noble 3종의 머스커다인 품종을 대상으로 하여 포도의 과피/과육부분과 종자부분을 추출하여 실험에 사용하였다. 각 추출물에 대하여, 항산화능은 총페놀함량 및 라디칼 소거능(Scavenging activity 및 EC₅₀)을, 항균활성은 *E.coli* K12에 대한 최소생육저해농도(minimum inhibitory concentration, MIC)를 조사하였다. 종자 추출물의 총페놀함량은 231.24~294.81 mg/mL GAE로 과피/과육 추출물에 비하여 높은 함량을 보였다. 또한 Higgins 품종의 종자 추출물이 가장 우수한 라디칼소거능(EC₅₀=0.026 mg/mL)을 나타내, 종자추출물이 과피/과육추출물보다 우수한 항산화능을 보유하고 있다. 반면, *E.coli* K12에 대한 항균활성은 Higgins 품종의 과피/과육추출물이 40 mg/mL의 MIC를 보임으로써 가장 우수하였다. 따라서, 본 연구는 머스커다인 포도 추출물이 천연 유래의 항산화 및 항균 기능을 보유한 소재로 활용될 수 있는 잠재적 가치를 제시하였다.

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