Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts

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Abstract

Guava fruit extracts were analyzed for antioxidant activity measured in methanol extract (AOAM), antioxidant activity measured in dichloromethane extract (AOAD), ascorbic acid, total phenolics, and total carotenoids contents. The ABTS, DPPH, and FRAP assays were used for determining both AOAM and AOAD, whereas the ORAC was used for determining only AOAM. Averaged AOAM (\textmu{M Trolox equivalent (TE)/g fresh mass (FM)}) were 31.1, 25.2, 26.1, and 21.3 as determined by the ABTS, DPPH, FRAP, and ORAC assays, respectively. Averaged AOAD (\textmu{M TE/g FM}) were 0.44, 0.27, and 0.16 as determined by the ABTS, DPPH, and FRAP assays, respectively. AOAM determined by all assays were well correlated with ascorbic acid ($0.61 \leq r \leq 0.92$) and total phenolics ($0.81 \leq r \leq 0.97$) and also among themselves ($0.68 \leq r \leq 0.97$) but had negative correlation with total carotenoids ($-0.67 \leq r \leq -0.81$).

Keywords: Ascorbic acid; Phenolic; Carotenoid; \textit{Psidium guajava} L.

1. Introduction

Natural antioxidants, particularly in fruits and vegetables have gained increasing interest among consumers and the scientific community because epidemiological studies have indicated that frequent consumption of natural antioxidants is associated with a lower risk of cardiovascular disease and cancer (Renaud et al., 1998; Temple, 2000). The defensive effects of natural antioxidants in fruits and vegetables are related to three major groups: vitamins, phenolics, and carotenoids. Ascorbic acid and phenolics are known as hydrophilic antioxidants, while carotenoids are known as lipophilic antioxidants (Halliwell, 1996).

Guava (\textit{Psidium guajava} L.) fruit is considered a highly nutritious fruit because it contains a high level of ascorbic acid (50–300 mg/100 g fresh weight), which is three to six times higher than oranges. Red-fleshed Brazilian guava has several carotenoids such as phytofluene, \textbeta-carotene, \textbeta-cryptoxanthin, \gamma-carotene, lycopene, rubixanthin, cryptoflavin, lutein, and neochrome (Mercadante et al., 1999). Setiawan et al. (2001) reported that Indonesian guava is an excellent source of provitamin A carotenoids. Phenolic compounds such as myricetin and apigenin (Mian and Mohamed, 2001), ellagic acid, and anthocyanins (Misra and Seshadri, 1968) are also at high levels in guava fruits. Therefore, producing guava specially bred for higher levels of antioxidant compounds, is a realistic approach to increase dietary antioxidant intake. Evaluation in any plant-breeding program, however, has to deal with numerous plants, particularly at the early selection stage. Therefore, the assay for screening germplasm and hybrids should be simple, inexpensive, rapidly performed, and provide a high degree of precision.

Several assays have been frequently used to estimate antioxidant capacities in fresh fruits and vegetables and their products and foods for clinical studies including 2,2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS)
(Leong and Shui, 2002; Miller and Rice-Evans, 1997), 2,2-
diphenyl-1-picrylhydrazyl (DPPH) (Brand-Williams et al., 1995; Gil et al., 2002), ferric reducing antioxidant power
(FRAP) (Bende and Strain, 1999; Guo et al., 2003; Jimenez-Escrig et al., 2001), and the oxygen radical
absorption capacity (ORAC) (Cao et al., 1993; Ou et al., 2001; Prior et al., 2003). The ORAC assay is said to be
more relevant because it utilizes a biologically relevant
radical source (Prior et al., 2003). These techniques have
shown different results among crop species and across
laboratories. Ou et al. (2002) reported no correlation of
antioxidant activity between the FRAP and ORAC
techniques among most of the 927 freeze-dried vegetable
samples, whereas these methods revealed high correlation
in blueberry fruit (Connor et al., 2002). Similarly, Awika
et al. (2003) observed high correlation between ABTS,
DPPH, and ORAC among sorghum and its products.

The aim of this research was to compare the efficiency of
ABTS, DPPH, FRAP, and ORAC assays to estimate
antioxidant activities and their correlations with ascorbic
acid, total phenolics, and total carotenoids contents in
guava fruit extracts.

2. Materials and methods

2.1. Plant materials

Guava fruits were harvested at maturity from one white-
fleshed (‘Allahabad Safeda’) and three pink-fleshed (‘Fan
Retief’, ‘Ruby Supreme’ and an advanced selection) clones
at Weslaco, TX, USA with the cooperation of Dr. Kevin
Crosby. Whole fruit was stored at −20 °C for 6 months
before extraction.

2.2. Extractions

Fruit extracts for ascorbic acid analysis were obtained by
homogenizing 3 g of guava tissue (pulp and peel) in 20 mL
cold solution of 3% (w/v) oxalic acid plus 8% glacial acetic
acid (v/v) until uniform consistency, using an Ultra-Turrax
homogenizer (T25, Ika Works Inc., USA). The homo-
genates were centrifuged at 15,000 rpm at 4 °C for 10 min.
The supernatants were recovered and ascorbic acid
immediately measured.

Fruit extracts for total phenolics and antioxidant activity
measured in methanol extract (AOAM) analysis were
prepared using the method of Swain and Hillis (1959),
with some modifications. Three grams of guava tissue were
mixed with 25 mL methanol and homogenized using the
Ultra-Turrax homogenizer. The homogenates were kept at
4 °C for 12 h and then centrifuged at 15,000 rpm for 20 min
using a vacuum micro centrifuge (Beckman, J2-21, Beck-
man Instruments Inc., USA). The supernatants were
recovered and stored at −20 °C until analysis. The pellet
was re-dissolved with 20 mL dichloromethane and homo-
genized for antioxidant activity measured in dichloro-
methane extract (AOAD) analysis. The homogenates were
centrifuged at 15,000 rpm for 20 min. The supernatants
were recovered and stored at −20 °C until analysis. In
general, methanol extraction and dichloromethane extrac-
tion are used for determining hydrophilic and lipophilic
antioxidant activities (Arnao et al., 2001).

Fruit extracts for total carotenoids analysis were
prepared by the method of Wilberg and Rodriguez-Amaya
(1995), with some modifications. Three grams of guava
tissue were mixed with 20 mL ethanol–hexane (1:1)
solution containing 200 mg/L 2,6-di-ter-butyl-p-cresol to
avoid carotenoid oxidation and then homogenized using
the Ultra-Turrax homogenizer until uniform consistency.
The homogenates were filtered using a Whatman No. 4
filter paper and re-extracted two or three times, depending
on the clone, with 20 mL solvent. The extracts were washed
three times with nanopure water. The supernatants were
recovered and added with hexane to a final volume of
10 mL, and then stored at −20 °C until analysis.

2.3. Antioxidant determinations

Ascorbic acid content was determined using the 2, 6-
dichlorophenol-indophenol titration method described in
Association of Office Analytical Chemists (1996). L-
ascorbic acid was used to prepare a standard solution
(1 mg/mL). The ascorbic acid concentration was calculated
by comparison with the standard and expressed as mg/
100 g fresh mass.

Total phenolics content was determined by the Folin–
Ciocalteu method, which was adapted from Swain and
Hillis (1959). The 150 µL of extract, 2400 µL of nanopure
water, and 150 µL of 0.25 N Folin–Ciocalteu reagent were
combined in a plastic vial and then mixed well using a
Vortex. The mixture was allowed to react for 3 min then
300 µL of 1 N Na2CO3 solution was added and mixed well.
The solution was incubated at room temperature (23 °C) in
the dark for 2 h. The absorbance was measured at 725 nm
using a spectrophotometer (Hewlett Packard 8452A, Diode
Array, USA) and the results were expressed in gallic acid
equivalents (GAE; mg/100 g fresh mass) using a gallic acid
(0–0.1 mg/mL) standard curve. Additional dilution was
done if the absorbance value measured was over the linear
range of the standard curve.

Total carotenoids content was determined by the
spectrophotometric method at 470 nm, which was adapted
from Talcott and Howard (1999) using a β-carotene
(0.001–0.005 mg/mL) standard curve. The total carotenoids
content was expressed based on β-carotene equivalents (β-
carotene; mg/100 g fresh mass). Additional dilution was
done if the absorbance value measured was over the linear
range of the standard curve.

2.4. Antioxidant activity determinations

For ABTS assay, the procedure followed the method of
Arnau et al. (2001) with some modifications. The stock
solutions included 7.4 mM ABTS+ solution and 2.6 mM
obtain an absorbance of 1.1 by mixing 1 mL ABTS solution with 60 mL methanol to obtain an absorbance of 1.1 ± 0.02 units at 734 nm using the spectrophotometer. Fresh ABTS⁺ solution was prepared for each assay. Fruit extracts (150 μL) were allowed to react with 2850 μL of the ABTS⁺ solution for 2 h in a dark condition. Then the absorbance was taken at 734 nm using the spectrophotometer. The standard curve was linear between 25 and 600 μM Trolox. Results are expressed in μM Trolox equivalents (TE)/g fresh mass. Additional dilution was needed if the ABTS value measured was over the linear range of the standard curve.

The DPPH assay was done according to the method of Brand-Williams et al. (1995) with some modifications. The stock solution was prepared by dissolving 24 mg DPPH with 100 mL methanol and then stored at −20 °C until needed. The working solution was obtained by mixing 10 mL stock solution with 45 mL methanol to obtain an absorbance of 1.1 ± 0.02 units at 515 nm using the spectrophotometer. Fruit extracts (150 μL) were allowed to react with 2850 μL of the DPPH solution for 24 h in the dark. Then the absorbance was taken at 515 nm. The standard curve was linear between 25 and 800 μM Trolox. Results are expressed in μM TE/g fresh mass. Additional dilution was needed if the DPPH value measured was over the linear range of the standard curve.

The FRAP assay was done according to Benzie and Strain (1996) with some modifications. The stock solutions included 300 mM acetate buffer (3.1 g C4H3NaO2·3H2O and 16 mL C2H2O2), pH 3.6, 10 mM TPTZ (2, 4, 6-tripyridyl-s-triazine) solution in 40 mM HCl, and 20 mM FeCl₃·6H2O solution. The fresh working solution was prepared by mixing 25 mL acetate buffer, 2.5 mL TPTZ solution, and 2.5 mL FeCl₃·6H2O solution and then warmed at 37 °C before using. Fruit extracts (150 μL) were allowed to react with 2850 μL of the FRAP solution for 30 min in the dark condition. Readings of the colored product [ferrous tripyridyltriazine complex] were then taken at 593 nm. The standard curve was linear between 25 and 800 μM Trolox. Results are expressed in μM TE/g fresh mass. Additional dilution was needed if the FRAP value measured was over the linear range of the standard curve.

The ORAC procedure used an automated plate reader (KC4, Bio Tek, USA) with 96-well plates (Prior et al., 2003). Analyses were conducted in phosphate buffer pH 7.4 at 37 °C. Peroxyl radical was generated using 2, 2'-azobis (2-amidino-propane) dihydrochloride which was prepared fresh for each run. Fluorescein was used as the substrate. Fluorescence conditions were as follows: excitation at 485 nm and emission at 520 nm. The standard curve was linear between 0 and 50 μM Trolox. Results are expressed as μM TE/g fresh mass.

2.5. Statistical analysis

Each antioxidant activity assay was done three times from the same extract in order to determine their reproducibility. Analysis of variance was used to test any difference in antioxidant activities resulting from these methods. Duncan’s new multiple range test was used to determine significant differences. Correlations among data obtained were calculated using Pearson’s correlation coefficient (r).

3. Results and discussion

3.1. Ascorbic acid, total phenolic, and total carotenoid contents

The amount of ascorbic acid (AA), total phenolics (TPH), and total carotenoids expressed as β-carotene (BET) were significantly different among guava clones (Table 1). The AA was 378.6 mg/100 g in ‘Allahabad Safeda’ and ranged from 174.2 to 396.7 mg/100 g in the pink pulp clones. The TPH was 344.9 mg GAE/100 g in ‘Allahabad Safeda’ and ranged from 170.0 to 308.0 mg GAE/100 g in the pink pulp clones. The BET ranged from 0.78 to 2.93 mg/100 g in the pink pulp clones, while it was not present in the white pulp clone. Luximon-Ramma et al. (2003) have also reported that white pulp guavas had higher AA and TPH than pink pulp guavas in which the AA was 142.6 and 72.2 mg/100 g in white and pink pulp, respectively, and the TPH was 247.3 and 126.4 mg GAE/100 g in white and pink pulp, respectively. The AA, TPH, and BET contents in guavas were very high compared to other fruit crops. The ranges of AA contents (mg/100 g) were 4.8–13.2 in nectarines, 3.6–12.6 in peaches and 2.5–10.2 in plums (Gil et al., 2002), 19.0 in starfruit, 27.5 in pineapple, 60.5 in mango, 92.9 in papaya, 13.8 in litchi (Luximon-Ramma et al., 2003). The ranges of TPH contents (mg/100 g) were 14–102 in nectarines, 21–111 in peaches and 42–109 in plums (Gil et al., 2002), 142.9 in starfruit, 47.9 in pineapple, 56.0 in mango, 57.6 in papaya, 28.8 in litchi (Luximon-Ramma et al., 2003). The ranges of BET contents (mg/100 g) were 0.01–0.19 in nectarines, 0.01–0.26 in plums (Gil et al., 2002).

3.2. Reproducibility of ABTS, DPPH, FRAP, and ORAC assays

Antioxidant activities measured in methanol extract obtained using ABTS, DPPH, FRAP, and ORAC assays from a single extract were measured three times to test the reproducibility of the assays. The DPPH and FRAP assays showed no differences among determinations, while the ABTS and ORAC assays differed among runs (Table 2). All assays, however, had no genotype x time interaction, indicating that all techniques gave a comparable ranking of antioxidant activity among clones within each time of determination. Therefore, the DPPH and FRAP assays...
could be used to determine antioxidant activity in guava as both showed high reproducibility. Working solutions of the DPPH, FRAP, and ORAC were used immediately after preparation while that of ABTS needed to be kept in the dark for 12 h to generate free radicals from the ABTS salt and then was used within 4 h (Awika et al., 2003; Arnao et al., 2001). Since the ABTS working solution was not always the same age, the activity of the solution to react with guava extracts might have been different among the determination times. For the ORAC, a 96-well plate machine (KC4, Bio Tek, USA) was used in this research. Reading value tended to be higher at the top than that at the bottom and also from the left than the right of the 96-well plates (data not shown). Prior et al. (2003) noted that a lower coefficient of variance (CV) is obtained using the 48-well format compared to the 96-well format. The 48-well plate data had a CV about 50% of the CV of the data generated in a 96-well plate. Therefore, the location of samples in the plate induced an increased error rate in the assays.

In terms of cost and time of running these methods, the main disadvantage of the ORAC technique is that it required the use of expensive equipment (Awika et al., 2003), whereas the other three methods required a simple machine, a spectrophotometer, which is commonly available in most laboratories. Another advantage of the ABTS and FRAP was that extracts reacted rapidly with ABTS (2 h) or ferric ion (30 min), respectively, whereas the DPPH reaction took much longer (24 h).

3.3. Antioxidant activity measured in methanol extract

The genotypes and the assays resulted in different antioxidant activity measured in methanol extract (AOAM) (Table 3). The white pulp clone, ‘Allahabad Safeda’, had the highest AOAM value (32.25 μM TE/g). The pink pulp clones had 28.45, 18.03, and 25.13 μM TE/g for ‘Fan Retief’, ‘Ruby Supreme’ and the advanced selection, respectively (Table 4). Higher level of AOAM in the white pulp clone was found in all assays as compared to the pink pulp clones due to its higher AA and TPH (Table 1). It, however, cannot be generalized that white pulp guava has a higher level of antioxidant activity than pink pulp guava because limited numbers of samples were studied in this research. There are three major pulp colored types: white, pink and maroon. Each consists of many genotypes, especially the white and pink pulp types. Therefore, more genotypes of all of the classes need to be studied in this research. There are three major pulp colored types: white, pink and maroon. Each consists of many genotypes, especially the white and pink pulp types. Therefore, more genotypes of all of the classes need to be measured for antioxidant activity to properly assess the variation of antioxidant activity among guava types.

The antioxidant activity as determined by ORAC assay of guavas (18.03–32.25 μM TE/g) was comparable to that of blueberries (13.9–45.9 μM TE/g) which contain an exceptionally high antioxidant activity (Prior et al., 1998).

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Table 1

<table>
<thead>
<tr>
<th>Genotypea</th>
<th>Ascorbic acidb</th>
<th>Total phenolicsc</th>
<th>Total carotenoidsd</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allahabad Safeda</td>
<td>378.6 ± 29.2 a</td>
<td>344.9 ± 33.6 a</td>
<td>na</td>
</tr>
<tr>
<td>Fan Retief</td>
<td>396.7 ± 25.0 a</td>
<td>300.8 ± 12.7 b</td>
<td>1.59 ± 0.12 b</td>
</tr>
<tr>
<td>Ruby Supreme</td>
<td>174.2 ± 5.8 c</td>
<td>170.0 ± 5.6 c</td>
<td>2.93 ± 0.35 a</td>
</tr>
<tr>
<td>Advanced selection</td>
<td>258.9 ± 49.9 b</td>
<td>270.6 ± 2.9 b</td>
<td>0.78 ± 0.16 c</td>
</tr>
<tr>
<td>P value</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

na = not available.

a‘Fan Retief’, ‘Ruby Supreme’, and advanced selection are pink pulp; ‘Allahabad Safeda’ is white pulp.
bAscorbic acid expressed in mg/100 g fresh mass.
cTotal phenolics content expressed in mg gallic acid equivalents/100 g fresh mass.
dTotal carotenoids content expressed in mg β-carotene equivalents/100 g fresh mass.
eMean separation within columns by Duncan’s new multiple range test.

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Table 2

ANOVA for antioxidant activity among three determinations of a single methanol extract by ABTS, DPPH, FRAP, and ORAC assays from four guava genotypes

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>MS</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABTS</td>
<td>Guava</td>
<td>3</td>
<td>410.95</td>
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<tr>
<td></td>
<td>Repeatability</td>
<td>2</td>
<td>82.69</td>
</tr>
<tr>
<td></td>
<td>Guava × Repeatability</td>
<td>6</td>
<td>11.73</td>
</tr>
<tr>
<td></td>
<td>Error</td>
<td>24</td>
<td>8.68</td>
</tr>
<tr>
<td>DPPH</td>
<td>Guava</td>
<td>3</td>
<td>393.37</td>
</tr>
<tr>
<td></td>
<td>Repeatability</td>
<td>2</td>
<td>13.76</td>
</tr>
<tr>
<td></td>
<td>Guava × Repeatability</td>
<td>6</td>
<td>8.95</td>
</tr>
<tr>
<td></td>
<td>Error</td>
<td>24</td>
<td>12.76</td>
</tr>
<tr>
<td>FRAP</td>
<td>Guava</td>
<td>3</td>
<td>554.81</td>
</tr>
<tr>
<td></td>
<td>Repeatability</td>
<td>2</td>
<td>2.35</td>
</tr>
<tr>
<td></td>
<td>Guava × Repeatability</td>
<td>6</td>
<td>0.81</td>
</tr>
<tr>
<td></td>
<td>Error</td>
<td>24</td>
<td>2.87</td>
</tr>
<tr>
<td>ORAC</td>
<td>Guava</td>
<td>3</td>
<td>85.54</td>
</tr>
<tr>
<td></td>
<td>Repeatability</td>
<td>2</td>
<td>75.67</td>
</tr>
<tr>
<td></td>
<td>Guava × Assay</td>
<td>9</td>
<td>16.8</td>
</tr>
<tr>
<td></td>
<td>Error</td>
<td>32</td>
<td>4.7</td>
</tr>
</tbody>
</table>

---

Table 3

ANOVA for antioxidant activity by the ABTS, DPPH, FRAP, and ORAC assays based on methanol extraction from four guava genotypes

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>MS</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guava</td>
<td>3</td>
<td>433.0</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Assay</td>
<td>3</td>
<td>192.9</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Guava × Assay</td>
<td>9</td>
<td>16.8</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Error</td>
<td>32</td>
<td>4.7</td>
<td></td>
</tr>
</tbody>
</table>
Therefore, guava is another fruit that has an exceptionally high antioxidant activity. The antioxidant activities obtained in the present study were very high compared to other fruit crops. Wang et al. (1996) reported the high antioxidant activity. The antioxidant activities as determined by the ABTS, DPPH, and ORAC assays, respectively (Table 4). The different AOAM levels obtained from the assays may reflect a relative difference in the ability of antioxidant compounds in the extracts to quench aqueous peroxyl radicals and to reduce ABTS$^+$, the DPPH free radical and ferric iron in vitro systems. Although the interaction of guava and assay was significant for the AOAM, it only explained a small amount of the total variation as compared to either guava or assay (Table 3). The interaction of guava and assay, however, was not significant for the AOAM when the ORAC technique was excluded from the analysis (data not shown). These indicated that the other three techniques yielded comparable results between clones.

3.4. Antioxidant activity measured in dichloromethane extract

Only three assays, ABTS, DPPH, and FRAP, were used to measure AOAD. The ANOVA showed that AOAD level was significantly different among assays ($P<0.01$) but not between guava clones, with no interaction between guavas and assays (Table 5). Means of the AOAD levels from the four guavas were 0.44, 0.27, and 0.16 μM TE/g as determined by the ABTS, DPPH, and FRAP assays, respectively (Table 6). The AOAD levels were very low: less than 2% of the total. Means of the AOAD levels from the four guavas as determined by the ABTS were 2 and 3 times of DPPH and FRAP assays, respectively (Table 6).

3.5. Correlations

Correlations between AOAM obtained from all assays, TPH and AA were positively high ($0.61 \leq r \leq 0.97$, $P<0.05$), especially between AOAM based on FRAP assay and TPH ($r = 0.97$, $P<0.01$) and AA ($r = 0.92$, $P<0.01$) (Table 7). Most techniques, including these four, used for determining antioxidant activity, showed high correlation with TPH in different crops. It could be that phenolic compounds, which are known as hydrophilic antioxidants, are secondary metabolites that are most abundant in fruits (Macheix et al., 1990). Gil et al. (2002) found high correlation ($r > 0.9$, $P \leq 0.05$) between antioxidant activities as determined by DPPH or FRAP assays and TPH in nectarines, peaches and plums. Also, high correlation between TPH and antioxidant activity as determined by FRAP or electron spin resonance spectroscopy were reported in fruit juices (Gardner et al., 2000). Whereas, high correlation between antioxidant activity using any method and AA was likely to be found in only fruits that contain high AA such as orange (Gardner et al., 2000) and guava (Table 6). Gil et al. (2002) demonstrated that there was no correlation between AA and antioxidant activity as determined by DPPH or FRAP assays in nectarines, peaches and plums. The AA in nectarines (4.8–13.2 mg/100 g), peaches (3.6–12.6 mg/100 g), and plums (2.5–10.2 mg/100 g) were very low compared to guavas (174.2–396.7 mg/100 g). The high correlation between any of AOAM with TPH or AA in guava suggested that it was feasible to use TPH or AA to screen for AOAM. Both AA and TPH showed high positive correlation with AOAM as determined by all assays, which indicates that AA and TPH are important contributors to antioxidant activity in guava extracts. Although high phenolics may cause problems with browning in fruits, Ozoglu and Bayindirli (2002) reported that ascorbic acid can inhibit enzymatic browning in apple juice. Therefore, guava fruits containing high amount of AOAM are less likely to experience flesh browning problem.

### Table 4
Antioxidant activity of guava fruit methanol extracts as determined by the ABTS, DPPH, FRAP, and ORAC assays from four guava genotypes

<table>
<thead>
<tr>
<th>Genotype</th>
<th>ABTS</th>
<th>DPPH</th>
<th>FRAP</th>
<th>ORAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allahabad Safeda</td>
<td>37.9±3.4</td>
<td>32.0±5.1</td>
<td>33.3±1.4</td>
<td>25.3±1.6</td>
</tr>
<tr>
<td>Fan Retief</td>
<td>34.4±2.1</td>
<td>27.7±1.7</td>
<td>30.4±1.2</td>
<td>21.0±2.4</td>
</tr>
<tr>
<td>Ruby Supreme</td>
<td>22.3±0.9</td>
<td>16.2±1.0</td>
<td>15.5±1.4</td>
<td>18.2±2.3</td>
</tr>
<tr>
<td>Advanced selection</td>
<td>29.6±2.3</td>
<td>24.9±0.5</td>
<td>25.3±1.1</td>
<td>20.5±1.8</td>
</tr>
<tr>
<td>Assay mean ($P=0.01$)</td>
<td>31.1±6.8 a</td>
<td>25.2±6.7 b</td>
<td>26.1±7.8 b</td>
<td>21.3±3.1 c</td>
</tr>
</tbody>
</table>

### Table 5
ANOVA for antioxidant activity by the ABTS, DPPH, and FRAP assays based on dichloromethane extraction from four guava genotypes

<table>
<thead>
<tr>
<th>Source</th>
<th>Df</th>
<th>MS</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guava</td>
<td>3</td>
<td>0.032</td>
<td>0.11</td>
</tr>
<tr>
<td>Assay</td>
<td>2</td>
<td>0.238</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Guava x Assay</td>
<td>6</td>
<td>0.023</td>
<td>0.18</td>
</tr>
<tr>
<td>Error</td>
<td>24</td>
<td>0.014</td>
<td></td>
</tr>
</tbody>
</table>
The AOAM based on ABTS, DPPH, and FRAP assays were negatively correlated with BET. Correlation between all pairs of AOAM assays were positively high (0.68 ≤ r ≤ 0.97, P < 0.01) indicating that guava extracts had comparable activities in all four assays. High correlation between these four techniques was also found in other crops. Connor et al. (2002) found high correlation among ORAC, FRAP, and methyl linoleate oxidation assays in blueberries. Awika et al. (2003) also found high correlation between ORAC, ABTS, and DPPH in sorghum and its products. There were no consistent correlations between assays to measure AOAM and AOAD nor were AOAD assays correlated among themselves. Correlations among AOAM based on ABTS, DPPH, FRAP, and ORAC assays were positively high and ranged between 0.68 and 0.97: the highest correlation was between ABTS and FRAP (0.97) and the lowest correlation was between DPPH and ORAC (0.68).

4. Conclusion

The ABTS, DPPH, FRAP, and ORAC assays gave comparable results for the antioxidant activity measured in methanol extract of guava fruit extracts. The FRAP technique showed high reproducibility, was simple, rapidly performed and showed the highest correlation with both ascorbic acid and total phenolics. Therefore, it would be an appropriate technique for determining antioxidant in guava fruit extract. Antioxidant activity measured in methanol extract may also be estimated indirectly by using ascorbic acid or total phenolics since they showed high correlation with all assays. Antioxidant activity measured in dichloromethane extract in guava fruit extract was low (2% of total) compared to antioxidant activity measured in methanol extract. Ascorbic acid and phenolics are the major contributors to antioxidant activity in guava fruit.

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Table 6

Antioxidant activities of guava fruit extracts as determined by the ABTS, DPPH, and FRAP assays based on dichloromethane extraction from four guava genotypes

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Antioxidant activity (µM TE/g FM)</th>
<th>Genotypic mean (P = 0.11)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ABTS</td>
<td>DPPH</td>
</tr>
<tr>
<td>Allahabad Safeda</td>
<td>0.38 ± 0.10</td>
<td>0.20 ± 0.06</td>
</tr>
<tr>
<td>Fan Retief</td>
<td>0.42 ± 0.06</td>
<td>0.35 ± 0.28</td>
</tr>
<tr>
<td>Ruby Supreme</td>
<td>0.58 ± 0.15</td>
<td>0.39 ± 0.19</td>
</tr>
<tr>
<td>Advanced selection</td>
<td>0.38 ± 0.10</td>
<td>0.15 ± 0.04</td>
</tr>
<tr>
<td>Assay mean (P = 0.01)</td>
<td>0.44 ± 0.10 a</td>
<td>0.27 ± 0.12 b</td>
</tr>
</tbody>
</table>

Table 7

Pearson’s correlation coefficients of antioxidant activities, ascorbic acid, total phenolics, and total carotenoids content

<table>
<thead>
<tr>
<th>Trait*</th>
<th>TPH</th>
<th>BET</th>
<th>AA</th>
<th>ABM</th>
<th>ABD</th>
<th>DPM</th>
<th>DPD</th>
<th>FRM</th>
<th>FRD</th>
</tr>
</thead>
<tbody>
<tr>
<td>BET</td>
<td>−0.79**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>0.89**</td>
<td>−0.50ns</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>ABM</td>
<td>0.97**</td>
<td>−0.67*</td>
<td>0.88**</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>ABD</td>
<td>−0.58*</td>
<td>−0.53**</td>
<td>−0.44ns</td>
<td>−0.55**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DPM</td>
<td>0.86**</td>
<td>−0.81**</td>
<td>0.81**</td>
<td>0.85**</td>
<td>−0.66*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DPD</td>
<td>−0.26ns</td>
<td>0.52**</td>
<td>−0.12ns</td>
<td>−0.26ns</td>
<td>0.22ns</td>
<td>−0.38ns</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FRM</td>
<td>0.97**</td>
<td>−0.73*</td>
<td>0.92**</td>
<td>0.97**</td>
<td>−0.60*</td>
<td>0.92**</td>
<td>−0.32ns</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FRD</td>
<td>0.56**</td>
<td>−0.05ns</td>
<td>0.36ns</td>
<td>0.57ns</td>
<td>−0.29ns</td>
<td>0.62*</td>
<td>−0.32ns</td>
<td>0.54**</td>
<td></td>
</tr>
<tr>
<td>ORM</td>
<td>0.81**</td>
<td>−0.57ns</td>
<td>0.61*</td>
<td>0.82**</td>
<td>−0.21ns</td>
<td>0.68*</td>
<td>−0.18ns</td>
<td>0.74**</td>
<td>0.74**</td>
</tr>
</tbody>
</table>

*TPH = total phenolics, BET = β-carotene, AA = ascorbic acid, ABM = antioxidant activity measured in methanol extract based on ABTS assay, ABD = antioxidant activity measured in dichloromethane extract based on ABTS assay, DPM = antioxidant activity measured in methanol extract based on DPPH assay, DPD = antioxidant activity measured in dichloromethane extract based on DPPH assay, FRM = antioxidant activity measured in methanol extract based on FRAP assay, FRD = antioxidant activity measured in dichloromethane extract based on FRAP assay, and ORM = antioxidant activity measured in methanol extract based on ORAC assay. ns = non significant and *, ** = significant at P < 0.05 or 0.01, respectively.
References


