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Comparative study of the antioxidant and antibacterial activities of two guava (*Psidium guajava*) fruit varieties cultivated in Andasa Horticulture Site, Ethiopia

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A R T I C L E I N F O	A B S T R A C T
	This investigation was carried out to evaluate the levels of phytochemicals and antioxidant potential as well as antibacterial activity of the two varieties of guava (*Psidium guajava*) fruit collected from Andasa horticulture site, Ethiopia. The antioxidant potential for guava fruit extracts was assessed using different in-vitro antioxidant assays, namely reducing power, Folin-Ciocalteu (F-C) assay and aluminum chloride methods. Overall, the white flesh guava fruit exhibited the highest levels of TPC, TFC and reducing power than the pink flesh guava. The mg/100g of AEAC (FRAP assay), AEAC (PMA), GAE and QE of pink and white flesh guava fruits determined in this study were 125.73, 260.28, 262.78, 377.18, 21.25, 24.61, 7.05 and 22.10, respectively. Antibacterial property of the two guava fruit variety extracts was evaluated against three gram-positive and two gram-negative bacteria using agar well diffusion technique. Ethanol extracts from both pink and white flesh guava exhibited antibacterial activity with zone of inhibition ranging from 29.50 to 30.33 and 24.33 to 29.33 mm respectively. On the other hand, only the chloroform extract from pink flesh guava showed poor antibacterial activity against MRSA and Shigella pathogens among the five tested organisms and the zone of inhibition measured was 11 and 10 mm respectively.

Capsule Summary: The findings of this study support the view that *Psidium guajava* fruit is promising sources of potential antioxidants and may be effective as preventive agents in the pathogenesis of some diseases.

INTRODUCTION

Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources. Free radicals can be generated by metabolic pathways to body tissues, introduced by external sources, from food, drugs and polluted environments. These free radicals may oxidize nucleic acids, proteins, lipids or DNA and can initiate degenerative disease. Antioxidants scavenge free radicals such as peroxide, hydro peroxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases (Miller and Rice-Evans, 1999).

Plant antioxidants include a variety of structural types of a wide range of antioxidant activity. The chemo-protective effects of plants are related to dietary antioxidants (vitamin C, vitamin E, beta carotene, polyphenols, e.g., flavonoids, flavones, flavonols) and are sources of exogenous natural antioxidants. Interest in natural antioxidants, especially of plant origin, has greatly increased from recent

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years. Hence consumer attention has recently been focused on the health –promoting properties of fruits and vegetables, which reduce the risk of several serious chronic diseases (Kevers et al., 2007).

Experimental and epidemiological studies indicate that consumption of grains, fruits and vegetables is related to lower incidence of disease. These foods contain a wide variety of phytonutrients, including antioxidants, which occur to all parts of higher plants. It is generally suggested that antioxidants in fruits, vegetables, tea and red wine are key to explaining how these foods function to reduce the incidence of chronic disease. These beneficial effects have been partly attributed to the consistent compounds which possess antioxidant activity (Miller et al., 200).

Particularly fruits may be more effective and economical than supplements in protecting the body against oxidative damage under different conditions. According to the literature, on the basis of the wet weight of fruit (edible portion); strawberry, guava, grapes, mango, avocado, orange, papaya, mango, lemon, pineapple, apple, banana, tomato and other fruits have higher antioxidant level (Blomhoff, 2005; Phipps, 2007)

Thus, fruits of many plant species contain a variety of antioxidant compounds which can act as decomposers of peroxides, scavengers of free radicals, quenchers of singlet and triplet oxygen, synergists and inhibitors of enzymes. Besides, these antioxidant compounds possess a multitude of health functions including protection against cancer and coronary heart disease. The protective effects of natural antioxidants are due to their considerable ability to retard or alleviate the extent of oxidative damage by reacting with free radicals (Khala et al., 2013; Kriengsak et al., 2005).

Guava (Psidium guajava)

Guava (Psidium guajava) is valued as a potential source of various antioxidant components including, pectin, ascorbic acid (vitamin C), tannins, phenols, triterpenes, flavonoids, essential oils, saponins, carotenoids, fibre, sugars and minerals (including, potassium, calcium and iron) but low in fat and calories (Kriengsak et al., 2005). So, like other fruits and vegetables, guava is also a rich source of antioxidants and thus can help to prevent degenerative diseases. The leaves have been extensively used for the treatment of diarrhea, bacterial infection, pain and inflammation. An essential oil isolated from the leaves has shown anti-cancer properties. The bark extract has long been used for the treatment of diabetes (Javaria et al., 2012; Joseph and Priya, 2011; Dev and Ramica, 2012). Now days, the white and pink fruit variety of this plant are cultivated in the different parts of Ethiopia. So far, present investigation was carried out to evaluate the levels of phytochemicals and antioxidant potential as well as antibacterial activity of the two varieties of guava (*Psidium guajava*) fruit collected from Andasa horticulture site, Ethiopia. The antioxidant potential for guava fruit extracts was assessed using different in-vitro antioxidant assays, namely reducing power, Folin-Ciocalteu (F-C) assay and aluminum chloride methods.

MATERIAL AND METHODS

Sampling collection and extraction

The two varieties of fresh guava fruits were collected from Andasa horticulture site and washed with tap water to remove any dust. Then the edible portions with their skin were chopped and cut in to smaller pieces and 20 g of this portion of each fruit was mixed with 200 mL of distill water. The mixture was transferred in to the blender and blended for 1 min. The resulting juices were transferred to 250 mL conical flasks and manually shaken for 10 min at room temperature to increase the solubility. The juices were filtered using sieve to separate the extracts from the residues. The non- particulate juices were filtered and centrifuged at 3000 rpm for 10 min. Then, the clear supernatant solutions were covered with aluminum foil and placed in a dark place for further analysis. The standard samples for antibacterial activity test were obtained from Department of Biotechnology, University of Gondar and the clinical resistant pathogenic bacterial isolates were collected from Gondar College of Medicine and Health Sciences Hospital.

Estimation of total phenolic contents

The total polyphenol content (TPC) was determined by Folin-Ciocalteu method (Amin et al., 2006). A standard gallic acid solution with varying concentrations in water was prepared for calibration purposes. Then crude extracts (1 mL) were mixed with Folin-Ciocalteu reagent (1 mL) and distilled water (5 mL). After the mixtures were allowed to stand for 5 min at room temperature, 7% aqueous sodium carbonate (W/V, 1 mL) was added. The volume was made up to 10 mL by adding more distill water. After 1 h, the absorbances of the resulting blue colored solutions were measured at 760 nm with a UV-vi spectrophotometer. So, the total phenolic contents were determined as mg GAE/100 g of fruit extracts using an equation obtained from the standard gallic acid calibration graph. All determinations were performed in triplicate.

Estimation of total flavonoid content (TFC)

The TFC were estimated by using aluminum chloride method with a little modification (Chang et al., 2002). 1 mL fruit extracts were mixed with water (4 mL) in a 10 mL volumetric flask. In the beginning, aqueous 5% $NaNO_2$ (0.3 mL) was added to the volumetric flask then at 5 min, 10% $AlCl_3$ (0.3 ml) and at 6 min, 1.0 *M* NaOH (2 mL) were added sequentially. Finally, the volume was made up to 10 mL by adding more distilled water and the reaction mixture in the flask was thoroughly mixed. The absorbance was noted at 510 nm using a UV-visible spectrophotometer. TFC were calculated using a standard calibration curve, prepared from quercetin solution within the concentration range of 0.5–5.5 ppm (μg/ml). The amounts were expressed as quercetin

equivalents (mg QE/100 g of fruit extracts). All measurements were performed in triplicate.

Determination of antioxidant activity by ferric reducing antioxidant power method

The ferric reducing power of each fruit extract was investigated by using the potassium ferricyanide-ferric chloride method with slight modifications (Oyaizu,1986). Ascorbic acid solution was used as a standard. Based up on the yield obtained, different concentrations of the extracts 2.5, 5.0, 7.5 and 10.0 % v/v were prepared by series dilution. From each portions of concentration, 1 mL was mixed with sodium phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and then after 2.5 mL, 1.0% potassium ferricyanide was added. The mixture was incubated at 50 °C for 20 min followed by addition of 2.5 mL of 10% trichloroacetic acid to terminate the reaction and the mixture was centrifuged at 3000 rpt for 10 min. The upper layer of the solution (2.5 mL) was decanted and diluted with distilled water (2.5 mL) and 0.1% ferric chloride (0.5 mL,) and the absorbance was recorded at 700 nm using UV-vis spectrophotometer. All samples were analyzed thrice and results were averaged.

Preparation of fruit extracts for antibacterial activity

Antibacterial activity of the white and pink flesh guava fruit extracts were investigated by using agar diffusion assay (Sudhir et al., 2012). The guava fruit varieties were washed with water and cut in to smaller pieces, allowed to air dry to reduce the microbial load on the sample due to handling and transportation. Then, the dried pieces were ground using a grinder in to a fine powder. 50 g of each dried and powdered samples of fruit varieties were soaked in to 230 mL ethanol for 24 h at ambient temperature. The solution was stirred for 24 h using a magnetic stirrer. The mixture was then filtered and kept at 37⁰C. Stock solutions of crude ethanol extracts were prepared by diluting with a 1:1 proportion of distill water. The same procedure was used to prepare the CHCl₃ extracts [Mahfuzul Hoque et al., 2007].

Collection of the tested organisms

Five different bacterial strains; three strains of gram-positive (*Staphylococcus aures* (ATCC 25923), *Staphylococcus aures* and *Streptococcus pneumonia)* and two strains of gram-negative (*Escherichia coli*, and *Shigella flexneri* (ATCC 12022)) were used. The bacterial cultures were maintained in their appropriate agar slants at 4⁰C until use.

Preparation of inoculums

The tested microorganisms were separately cultured on nutrient agar at 37 ⁰C for 24 h. This was achieved by streaking the inoculating loop containing the bacteria at the top end of the agar plate moving in a zig-zag horizontal pattern until 1/3 of the plate was covered. Then, three to five well-isolated overnight cultured colonies of the same morphological type were selected from an agar plate culture. The top of each colony was touched with a sterile bent wire-loop and the growth was transferred into a screw-capped

tube containing 5 mL of a suitable broth medium, which is tryptic soy broth (TSB). The broth culture (test tubes) was incubated without agitation for 24 h at 37 ⁰C until it achieves or exceeds the turbidity of the 0.5 McFarland standards. The turbidity of the actively growing broth culture was adjusted with sterile saline to obtain a turbidity optically comparable to that of the 0.5 McFarland turbidity standard 1.5×10^8 colony-forming units (CFU)/mL (Murray et al., 2004).

Inoculation of test plates

Optimally, within 15 min after adjusting the turbidity of the inoculum suspension, a small volume about 0.1 mL of the bacterial suspension was inoculated onto the dried surface of Mueller-Hinton agar plate and streaked by the sterile cotton swab over the entire sterile agar surface. This procedure was repeated by streaking two more times, rotating the plate approximately 60° each time to ensure an even distribution of inoculum and finally the rim of the agar was swabbed. The lid was left ajar for 3 to 5 min, but no more than 15 min, to allow for any excess surface moisture to be absorbed before applying the crude extracts on the respective well.

Preparation of media

Mueller-Hinton agar was prepared from a commercially available dehydrated base according to the manufacturer's instructions. 19 g of Mueller-Hinton agar powder was weighed using a clean electronic weighing balance; 500 mL of sterile distilled water was poured into a conical flask containing 19 g of Mueller-Hinton agar. The mixture was stirred with a sterilized glass rod and covered with a cotton wool, over which an aluminum foil was tightly wrapped and then autoclaved for 15 min at 121 ⁰C. Soon after autoclaving, the agar was allowed to cool and placed inside a water bath at about 50 ⁰C to maintain the media in a molten stage (to minimize the amount of condensation that forms).

Then, the agar medium was allowed to cool to room temperature in the laminar flow hood prior to pouring it into the petri-plate. Plates were dried faster in lower humidity by keeping them in a laminar flow hood. The freshly prepared and cooled medium was poured into flat-bottomed petri dishes on a level, horizontal surface to give a uniform depth of approximately 4 mm. This was achieved by pouring 20 mL of the medium for plates with diameters of 100 mm.

Preparation of 0.5 McFarland turbidity standards

A BaSO₄ 0.5 McFarland turbidity standards (Lalitha, 2004) was prepared by adding 50 μL (0.05 mL) of a 1.175% (wt/v) barium chloride dehydrate (BaCl₂.2H₂O) solution to 9.95 mLof 1% (v/v) sulfuric acid. McFarland standard screw-capped glass bottle was entirely sealed with aluminum foil and the barium sulfate suspension was transferred in 6 ml aliquots into screw-capped tube of the same size as those used in growing and diluting the bacterial inoculums. The tube was tightly sealed with paraffin to prevent loss by evaporation and stored in dark at room temperature until use. The correct density of a prepared McFarland turbidity standard was verified by using a spectrophotometer with a 1

cm light path length. For the 0.5 McFarland standards, the absorbance was adjusted at a wavelength of 625 nm and water was used as a blank. The absorbance at 625 nm was 0.05 for the 0.5 McFarland standards. The barium sulfate turbidity standard was vigorously agitated on a mechanical vortex mixer before each use and inspected for a uniformly turbid appearance. As with the barium sulfate standards, a 0.5 McFarland standard was comparable to a bacterial suspension of 1.5×10^8 colony-forming units (CFU)/mL (Andrews, J.M. 2006).
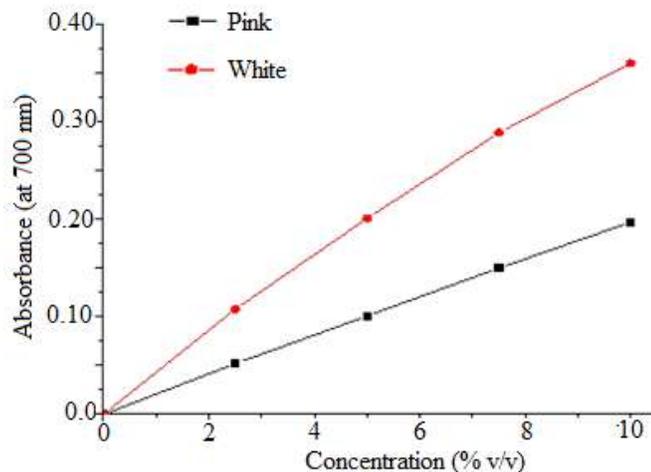
Fig. 1*: Ferric reducing power of pink and white flesh guava fruits
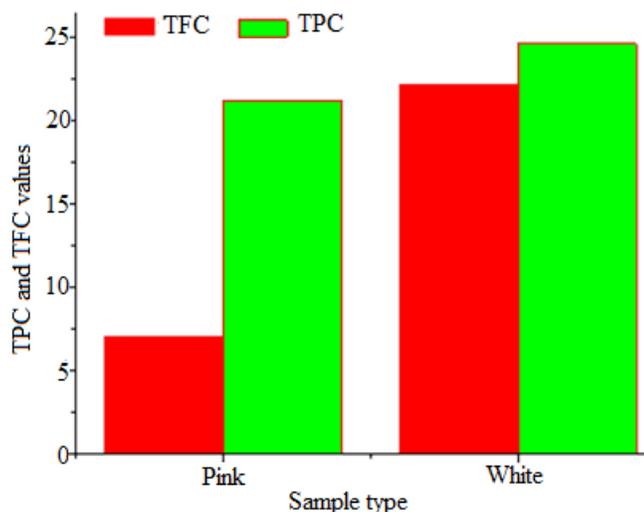
Fig. 2*: Total polyphenol and flavonoid content for both pink and white flesh guava fruits

Antibacterial activity determination of the fruit extracts

The selected strains of bacteria were inoculated into 6 mL of sterile nutrient broth, and incubated at 37 ^{0}C for 24 h. The cultures were aseptically swabbed on the surface of sterile nutrient agar plates using a sterile cotton swab. Suspensions

of the bacterial isolates were made in sterile normal saline and adjusted to the 0.5 McFarland's standard. Small volume (100 µL) of bacterial suspensions were added to each Mueller Hinton (MH) agar plate and then evenly seeded and streaked by means of sterile swab stick on the agar plate surface, and the plates were left on the laminar flow hood at room temperature for excess fluid to be absorbed. Agar wells were prepared by using a sterilized cork borer with 6 mm diameter, 4 mm deep and about 2.5 cm apart to minimize overlapping of zones (Srinivasan et al., 2001). By using a micropipette, 100µl of both chloroform and ethanol extracts from pink and white flesh guava were carefully added to the respective wells in the plate in triplicate and the antibiotic discs were dispensed with a dispensing apparatus (sterile pair of forceps) onto the surface of the inoculated agar plate and pressed down to ensure complete contact with the agar surface. Crude extracts and antibiotic discs were allowed to diffuse for about 40 min before incubation and then the plates were incubated in an upright position at 37 ^{0}C for 24 h. After overnight incubation, the diameters of inhibition zones were measured in mm using a plastic ruler, which was held on the back of the inverted Petri plate and the results were recorded. Antibiotic disc (Amoxycillin 25 µg) was served as positive control while sterile, distilled water was served as negative control.

RESULTS AND DISCUSSION

Ferric reducing power

For the reducing power analysis, calibration curve of ascorbic acid (AA) as standard was used and the curve was constructed by plotting the mean absorbance of AA versus the corresponding concentrations (2.5, 10, 17.5 and 25 ppm of AA. The equation obtained from the calibration curve was $y = 0.01435x + 0.01023$.where "y" is the absorbance measured at 700 nm and "x" is the concentration of AA in µg/mL. The linear regression coefficient (R^2) value obtained was 0.9998.

The results from the reducing potential of two verities of guava fruit extracts are shown in Table 1 and Fig. 3. The reducing power (RP) of the pink and white flesh guava fruit extracts was determined by direct electron donation from the reduction of ferricyanide $[Fe(CN)_6]^{3-}$ to ferrocyanide $[Fe(CN)_6]^{4-}$ complex. The resulting ferrous complex then reacts with ferric chloride to form Fe^{3+}/Fe^{2+} complex that has an absorption maximum at 700 nm. When the samples extracted were mixed with yellow colored $K_3[Fe(CN)_6]$ complex in the presence of phosphate buffer, the color of the mixture was remained the same. After incubation at 50 °C for 20 min using water bath, ferric chloride was added to 2.5 mL of the solution. During this, the Prussian blue color was appeared from the yellow color. This change of color was an indication of the reduction of Fe^{3+} to Fe^{2+} and the amount of Fe^{2+} complex was then monitored by measuring absorbance of the blue solution at 700 nm.

When the concentrations of the extracts increases from 2.5 to 10 %v/v, the color of the resulting Fe^{3+}/Fe^{2+}

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Table 1: Absorbance of ascorbic acid at 700 nm at different concentrations for FRAP assay

	Concentration (ppm)			
	2.50	10.00	17.50	25.00
Absorbance	0.048±0.004	0.150±0.007	0.263±0.009	0.369±0.009

Table 2: Reducing potential of extracts from guava fruit varieties

		Concentration (% V/V)			
		2.50	5.00	7.50	10.00
Absorbance, 700 nm	Pink flesh guava	0.052±0.001	0.101±0.004	0.150±0.004	0.197±0.003
	White flesh guava	0.108±0.002	0.201±0.005	0.289±0.008	0.360±0.001

Table 3: Percent reducing power of white and pink flesh guava fruit varieties

Varieties of guava fruit	Percent reduction power (% RP)
Pink flesh guava	53.30±0.779
White flesh guava	97.65±0. 414

Table 4: Absorbance of gallic acid at different concentrations at 760 nm

	Concentration, ppm			
	0.10	0.50	1.00	2.50
Absorbance	0.015±0.008	0.066±0.003	0.125±0.002	0.275±0.003

complexes varied from pale blue Prussian to deep blue. These reducing potential of guava fruit extracts measured at the concentrations of 2.5, 5.0, 7.5 and 10 % v/v were found to be increased in a concentration dependent manner (Table 2). At a concentration of 10% v/v, the maximum average absorbance of pink and white flesh guava fruit extracts obtained were 0.197 and 0.360, respectively. These absorbance values indicated that the white flesh guava has higher reducing power than the pink.

The AEAC values obtained for both pink and white flesh guava fruits were 125.73 and 260.28 mg of AA equivalent per 100 g of edible portion of the fruits, respectively. The highest antioxidant activity observed for white guava may be due to the presence of high content of vitamin C and other compounds having antioxidant activity.

The values of antioxidant activity observed in the present study are lower than the reported values (Thaipong et al., 2006; Dev and Ramica, 2012). Different factors may contribute for the difference. Temperatures, soil type, availability of nutrients in the soil and other environmental factors have a significant effect on the concentration of vitamin C and other phyto-nutrients. In addition, (Thaipong et al., 2006) reported that the antioxidant capacity of white flesh guava fruit had higher activity than pink flesh guava (Dev and Ramica, 2012). They reported 378.6 mg/100 g for white flesh guava and 174.2 to 396.7 mg/100 g for pink flesh

guava. Where as in the present investigation, the values of AEAC obtained for both pink and white flesh guava varieties were 125.73 and 260.28 mg/100g, respectively. Luximon-Ramma et al., 2003. also reported that white guava had higher antioxidant activity than pink guava in which the antioxidant activity values were 142.6 and 72.2 mg/100 g for white and pink flesh, respectively (Luximon-Ramma et al., 2003. As shown in Table 3, the percent reduction power of guava fruit extracts were also determined as 97.65% and 53.30 % from white and pink flesh guava extracts, respectively.

Total polyphenol content determination

The content of phenolic compounds was determined using gallic acid. The absorbance of GA was measured at 760 nm for the concentrations 0.1, 0.5, 1.0 and 2.5 ppm (Table 4).

The GA calibration curve which was plotted using mean absorbance against the corresponding concentrations gave an equation and a linear regression coefficient of $y = 0.10702x + 0.01055$ and $R^2 = 0.998$, respectively.

The values determined at 760 nm in terms of GAE in mg/100 g of both the pink and white flesh guava fruit extracts were 21.25 and 24.62, respectively. Therefore, in this study, the white flesh guava showed remarkable polyphenol content and consequently will show high power

Table 5: Total polyphenols and flavonoid contents of guava fruit extracts

Sample extracts	Total polyphenol content GAE (mg/100g)	Total flavonoid content QE (mg/100g)
Pink flesh guava	21.25±0.647	7.05±2.935
White flesh guava	24.62±0.674	22.10±2.351

Table 6: Absorbance of quercetin at different concentrations

Concentration (ppm)	0.50	1.50	2.50	3.50	4.50	5.50
Absorbance (510 nm)	0.059±0.006	0.076±0.007	0.09±0.000	0.11±0.010	0.126±0.005	0.149±0.006

Table 7: Antibacterial activity of ethanol and chloroform extracts of pink and white flesh guava fruits

Test organisms	mean zone of Inhibition (in mm) ± SD				
	Ethanol extracts		Chloroform extracts		Standard drug
	Pink	White	Pink	White	AML-25 µg
S. aures	29.50±0.87	26.67±0.58	0.00±0.00	0.00±0.00	14.00
MRSA	29.67±0.58	24.33±0.58	11.00±0.00	0.00±0.00	12.00
Strep	30.33±0.58	29.33±0.58	0.00±0.00	0.00±0.00	10.00
E. coli	30.33±0.58	25.67±0.58	0.00±0.00	0.00±0.00	11.00

against scavenging of free radicals. The Folin-Ciocalteu reagent reacts preferably with phenolic compounds; however, it can be reduced by some non-phenolic compounds, example Cu (II), vitamin C, etc. (Vitaly and Eduardo, 2005).

The contents in phenolic and flavonoid compounds quantified and AOA investigated in this study are within the range of those reported by G. Javaria *et al*. who evaluated variation in antioxidant attributes to three ripening stages of guava fruit from different geographical regions of Pakistan (Kriengsak et al., 2005). The values of TPC determined were ranged from, 24.81 to 33.16 (unripen), 18.45 to 26.51 (semi-ripen) and 11.47 to 20.54 mg GAE 100 g (fully ripen) guava fruit extracts, Luximon-Ramma et al. (2003) had also reported that white guava had TPC (247.3) than pink guava (126.4 mg GAE/100 g) (Luximon-Ramma, et al., 2003). In our investigation, the TPC for white flesh guava was 344.9 mg GAE/100 g and ranged from 170.0 to 300.8 mgGAE/100 g for the pink flesh guava.

Total flavonoid content determination

The content of flavonoid compounds (quercetin equivalent/100 g of fruits) was determined from the calibration curve using quercetin (QT) as the standard. The results the absorbance of QT measured at 510 nm for the concentrations of 0.5, 1.5, 2.5, 3.5, 4.5 and 5.5 ppm were shown in Table 6. The QT calibration curve which was plotted using mean absorbance against the corresponding concentrations gave an equation and linear regression value of Y = 0.01771x + 0.04852 and R = 0.9976, respectively.

Antioxidant activity of flavonoids depends on the structure and substitution pattern of hydroxyl groups (Milan, 2011). The Al^{3+} from $AlCl_3$ will bind with the ketone and hydroxyl group of the flavonoids as a Lewis acid and produced intense yellow colour (*Scheme* 1) (Tian et al., 2006). After addition of 1 M of 2 mL NaOH in to this solution, the intense yellow color was changed in to pink. This is an indication of the presence of flavonoid compounds in the extract. The data is summarized in Table 6*.* It was observed that the amount of total flavonoid content of white flesh guava was higher than the pink flesh variety.

The total flavonoid content determined at 510 nm in terms of QE in mg/100 g of both the pink and white flesh guava fruit extracts were 7.05 and 22.10, respectively. The result of the white flesh guava in the present study is in agreement to those reported by G. Javaria *et al*. who evaluated variation in antioxidant attributes to three ripening guava fruits at different geographical regions of Pakistan (Kriengsak, 2005). The values of TFC determined were ranged from 28.82 to 46.08, 24.61 to 43.10 and 18.65 to 31.09 mg QE 100 g of unripen, semi-ripen and fully ripen guava fruit extracts, respectively. In addition to this Alothman et al., 2009; El Sohafy et al., 2009) reported a concentration of 24.05 and 39.5 mg/100 g expressed as quercetin which is within the range of those values of white flesh guava estimated in the present study. Moreover, the values of TFC of pink and white flesh guavas determined in this study were in agreement with the values reported by Correa et al., 2011, varied from 10.67 to 46.82 mg/100 g (Aliyu et al., 2012). However, the vales were expressed in terms rutin standard.

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Fig. 3: Antibacterial activity of ethanolic extracts of pink and white flesh guavas towards some selected pathogens

The total antioxidant capacity of flavonoids and polyphenols can be affected by the concentration of atmospheric oxygen because of their easy autoxidation, mostly catalyzed by transition metals (Chen and Yen, 2007).

Antibacterial activities of fruit extracts

As it was shown in Table 7 and Fig. 1, the antibiotic standard drug (positive control) used in this study was amoxicillin (AML). It shows inhibitory effect on the five tested pathogens. Whereas water was used as a negative control and showed no inhibition zone against the tested organisms.

Significant variation was observed in the antibacterial activities (inhibition zones) of different extracts. Table 7 shows the inhibitory effect against the five pathogens of ethanol extracts from pink and white flesh guava. Inhibition zone of ethanol extracts from pink flesh guava against all tested pathogens was significantly greater (29.50-30.67 mm) than the ethanolic extract from white flesh guava (24.33-29.33 mm).

However, chloroform extract of pink flesh guava was not significant against *S. aures, strep, and E. coli.* Whereas the chloroform extract from white guava had no any inhibitory effect on the whole tested microorganisms. The result of this study showed that the antibacterial activity of ethanolic fruit extracts from pink and white flesh guava were effective against *S. aures, MRSA, Strep , E. coli* and *Shigella* compared with the standard.

In general, the results demonstrated in Table 7 showed that findings of the present study exhibited a considerably broader antimicrobial activity for ethanolic crude extracts compared to chloroform crude extracts. This may be due to the fact that the ethanolic extracts contain more biologically active phytochemicals than the chloroform extract. Overall, results revealed that extracts are potential source of bioactive compounds and these findings are in line with previous studies (Bajalan et al., 2017; Fakhfakh et al., 2017; Giacomini et al., 2017; Meng et al., 2017; Nair et al., 2017; Palanisamy et al., 2017; Paliwal et al., 2017; Sagbo et al., 2017).

CONCLUSIONS

The findings of this study support the view that guava fruits are promising sources of potential antioxidants and may be effective as preventive agents in the pathogenesis of some diseases. It was observed that the white flesh guava possesses higher antioxidant property, total polyphenolic and flavonoids contents compared to the pink flesh guava. This suggests that the phenolics and flavonoid compounds are present in higher concentrations in the white flesh guava than in the pink flesh guava. The ethanolic extracts from both pink and white flesh guavas were also exhibited a significant antibacterial activity against the bacterial strains but not the chloroform extract. Ethanol extract showed the highest degree of antibacterial activity against four bacterial strains and found to be comparative with standard. This might due to the presence of polar bioactive compounds in the ethanol guava fruits extracts. Contrary to antioxidant activity, the pink flesh guava demonstrated better antibacterial activity than the white guava. This proved that phytochemicls contributing to both AOA and antimicrobial activity may not be the same. These findings provide scientific evidence and confirmed the promising potential of these fruits to use for medicinal purposes and support their traditional uses in folk medicine. Moreover, guava can serve as an alternative natural antimicrobial supplements for the treatment of different pathogenic bacterial infections. According to our survey, guava fruits cultivated at Andasa Horticultural site demonstrated higher activities than most guava fruits grown in different parts of the world. However, more agricultural practice will be needed to improve quality and management of the plant.

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