EXPLORATORY EVALUATION OF ANTHOCYANIN CONTENTS AND ANTIOXIDANT ACTIVITY OF SECOND GENERATION PURPLE TEA AGAINST PIGMENTED FRUITS AND VEGETABLES

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ABSTRACT
Anthocyanins are an important group of highly hydro-soluble pigments in most species of plants that are produced by secondary metabolism of plants through the pentose phosphate, shikimate and flavonoid pathways and are accumulated in cell vacuoles. They are polyphenols with strong antioxidant activity, which is largely responsible for some biological activities including prevention or lowering the risk of cardiovascular diseases, diabetes, arthritis and cancer. In fruits and vegetables anthocyanins are present in the form of glycosides. Anthocyanins have also been enhanced in tea through conventional breeding with the first generation purple tea cultivars already released for commercial utilization. Further purple tea improvement programme in Kenya has led to development of second generation purple tea plants focusing on further enhancement of anthocyanins content. A study involving second generation tea cultivars established in four experimental fields in Kenya namely; Timbilil, Kangaita, Nandi and Chesumot, compared levels of production of anthocyanins in the tea cultivars with purple-pigmented fruits and vegetables purchased from Kericho market. Total anthocyanin content was quantified by pH differential method using a UV- spectrophotometer, while radical scavenging activity of the tea extracts was assessed using 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH). There were significant ($p \leq 0.05$) differences, in the anthocyanins content among the tea varieties, fruits and vegetables. The anthocyanin contents in the tea cultivars ranged from 38.6 to 1037.0 mg/l, 169.7 to 501.0 mg/l, 78.3 to 682.1 mg/l and 19.7 to 876.9 mg/l, in Timbilil, Kangaita, Nandi and Chesumot, respectively. In the pigmented fruits and vegetables, the anthocyanin content was 1.5 and 425.3 mg/l for beetroot and red cabbage, respectively. The radical scavenging activity of second generation purple tea ranged from 82.8 to 91.5 % compared the pigmented fruits and vegetables that exhibited lower radical scavenging capacity of 1.14 to 45.3%. The observed enhanced level in the anthocyanins content and radical scavenging activity within some of the second generation purple teas have demonstrated the availability of improved cultivars with greater potential in antioxidant capacity and suitable alternative as super food for health conscious populations.

INTRODUCTION
Tea (*Camellia sinensis* (L.) O. Kuntze) is the most popular and commonly consumed beverage worldwide. Consumers have become more health conscious leading to an increase in the demand for drinking healthy tea [10]. Currently, there is considerable interest in anthocyanins owing to their potential health benefit in human, which is associated with their antioxidant potential and reported positive effects on blood vessel walls [23]. This has necessitated the need to develop novel tea cultivars for processing high value tea products enriched with bioactive molecules [21]. One of the objectives of tea improvement programme in Kenya is to develop tea cultivars with high contents of biomolecules with health enhancing properties. Thus, a purple-pigmented tea variety was eventually selected from the Tea Research Institute (TRI) Crop Improvement Programme and released for commercial use by the tea industry in 2009 [15]. This is referred to as the first (1^{st}) generation. The variety, together with its siblings and other purple-leafed tea varieties, were subjected to investigation and were found to be rich in anthocyanins [17], which are strong antioxidants [25]. Anthocyanins are also found in high value vegetable crops and fruits such as blue berries, grapes, red cabbage and eggplant [8]. Thus, the entry of purple tea variety into cultivated tea fields resulted into injection of a unique tea cultivar to be a source of

high value tea product with additional health benefits [15,17]. Volunteer plants with darker purple leaf colouration have been collected from plantations of the first generation purple teas. These are referred to as the second (2^{nd}) generation.

Purple tea offers great promise in transforming the tea industry through boosting the sub-sector's competitiveness and empowering small-scale Kenyan tea farmers with a specialty product that is attracting niche market in the global arena. It has been speculated that consumption of reasonable amounts of anthocyanin rich teas, containing both anthocyanins and catechins, is likely to attenuate the adverse effects of oxidative stress in the body by decreasing oxidation of essential cellular proteins, lipids and nucleic acids [17,25], thereby protecting the body against degenerative diseases [1]. Thus, an explorative study was conducted to compare levels of total anthocyanins and antioxidant activity in 2^{nd} generation purple tea varieties to pigmented fruits and vegetables with the aim of providing readily available alternative super food for promoting human health.

MATERIALS AND METHODS

Sample collection and processing

Purple tea samples comprising two leaves and a bud were harvested in triplicates from 2^{nd} generation tea bushes appearing in four purple clonal tea fields at Timbilil (0˚ 22'S, 35˚ 21'E, 2180 amsl), Nandi (0.5°S, 37.3°E, 1980 amsl), Chesumot (0°18˙ 60˙S, 4.4'E, 2096 amsl) and Kangaita (0˚30'S, 37˚16'E, 2180 amsl), while the green leafed varieties (TRFK 6/8 and Yabukita) were sourced from Timbilil whereas pigmented fruits (Blue berries – *Vaccinium sp.*, Purple Pomegranate - *Punica granatum* and Purple Tree tomato–*Solanum betaceum*) and vegetables (Red cabbage-*Brassica oleracea*, Beetroot- *Beta vulgaries* and eggplant- *Solanum melongena*) were procured from Kericho town main market, and transported to the laboratory using cooler boxes. The purple tea samples were only collected from the 2^{nd} generation volunteer plants with the 1^{st} generation as a check controls from all the four sites.

The samples were steamed for one minute and dried using a microwave oven (Samsung, GE 109 MST,Malaysia) at 100°C, then pulverized with a grinder (Type AR11, Moulinex, China) to fine powder and stored in Aluminium sachets. The pigmented fruits and vegetables were chopped into small pieces and dried in an oven at 80°C (UNE- 400, Memmert, Germany) then milled to fine powder and stored in Aluminium sachets. Thereafter, the stored samples were used for anthocyanins quantitation and antioxidant activity measurements.

Anthocyanin extraction and purification

Five grams of each of pulverized samples were accurately weighed into 125 ml conical flasks covered with aluminium foil, then mixed with 50 ml MeOH/formic acid mixture; (99/1v/v) and magnetically stirred at 900 rpm (MHK-4, MRC, Israel) for 4 hours at room temperature (25°C) [12]. The resultant solution was filtered using cotton wool, into 250 mls round bottom flasks and evaporated to dryness using a Rotavapour (Rotavapor Buchi R-3000, Switzerland) under reduced pressure at 35°C. The extract was reconstituted using 10 mls purified water and filtered using a 0.45 µm cellulose nitrate membrane filters under vacuum, then passed through reverse phase (RP) C18 solid phase extraction (SPE) cartridges (Sigma-Aldrich, USA) with the aid of manifold, previously activated with MeOH/HCl (90:10) v/v. Anthocyanins were adsorbed onto the column, while sugars, acids and other water-soluble compounds were eluted from the column with 0.01 % HCl in distilled water. Anthocyanins were then recovered into test tubes using methanol acidified with formic acid (90:10) v/v to a volume of 20 mls, and covered with aluminium foil to prevent photo degradation and stored at 4°C prior until quantification. The cartridges were subsequently eluted with ethyl acetate (Fischer Scientific, UK) to remove phenolic compounds other than anthocyanins for use in the subsequent extraction.

Determination of Anthocyanins content

The pH differential method as described by Kerio *et al*. [17] with slight modifications was used to quantify the total anthocyanins content in various samples. After extraction, 0.4 mls of each of the extracts was mixed with 3.6 mls of each of two different buffers at different pH (0.025M, potassium chloride at pH 1.0 and 0.4M sodium acetate at pH 4.5), then left to stand for 15 minutes in the dark. Absorbance readings were then taken at wavelengths of 520 nm and 700 nm using a double beam grating (UV-Spectrophotometer, 1800 series Japan). Anthocyanin content was calculated using the equation below:

$$\text{Total monomeric anthocyanin } (mg\ /l) = \frac{A}{£*L} * mw * DF * 1000$$

$$A = (A_{520\,nm} - A_{700\,nm})pH\ 1 - (A_{520nm} - A_{700nm})pH\ 4.5$$

Where;
A = Absorbance difference between 520 nm and 700 nm at pH of 1.0 and 4.5
DF = Dilution factor
L = Cell path length (1 cm)
10^3= Factor for conversion from g to mg,

The total anthocyanin content was expressed as mg cyanidin-3-glucoside equivalents per litre of the extract.

Antioxidant activity measurements

The stable DPPH: 2, 2-diphenyl-1-picrylhydrazyl was used for determination of free radical scavenging of the pigmented samples using a modified method of Brand-Williams [7] in which 5 g of pulverized samples was infused in 100 mls boiling distilled water and stirred for 10 minutes using magnetic stirrer (Model-MHK-4, Israel). The extract was filtered through a nylon mesh followed by filter paper (Whatman no.2). Ten milliliters of the infusion was then dried to constant weight in pre-weighed ashing tube in an oven at 103˚C for 12 hours, the weight of the soluble solids was measured and expressed as mg/ml. The soluble solids of the extracts were standardized to give stock solutions of 50 mg/100 ml. Methanolic solution 100 µl of the antioxidant was mixed with 4 ml DPPH solution (6×10^{-5}M made with 80% methanol) in a test tube and absorbance measured at wavelength of 517 nm using UV-Spectrophotometer (1800 series Shimadzu Japan). The percentage (%) inhibition against DPPH was obtained by the formula = $\frac{A_B - A_A}{A_B} \times 100$ where A_B is the absorbance of the blank sample and A_A is the absorbance of tested sample after 15 min.

Data analysis

Data generated from each site of the study were subjected to separate analysis of variance assuming a completely randomized design using SAS statistical software with least significance differences at p≤0.05 used to separate the means. Summarized data was also presented in figures and principal component analysis.

RESULTS AND DISCUSSION

Total Anthocyanins and antioxidant activity

Kangaita site

Total anthocyanins (TA) content varied significantly (p≤0.05) between 1st and 2nd generations of purple tea varieties, fresh unprocessed pigmented fruits and vegetables (Table 1). The TA content in purple tea cultivars in Kangaita site ranged from 167.3 mg/l to 500.8 mg/l for TRFK 306/1044/2 and TRFK 306/OP, respectively. The best performing 1st generation commercial cultivar, TRFK 306/3, had 14.6 % lower TA content than the best 2nd generation variety, TRFK 306/OP. Compared to the fresh unprocessed fruits and vegetables, TRFK 306/OP had 17.8 % and 81.6 % higher TA content than red cabbage and blue berries by respectively. Additionally, majority of first generation purple tea cultivars outperformed some second generation purple tea cultivars and fresh unprocessed pigmented fruits and vegetables used in this study. The variation of TA in both 1st and 2nd generation purple tea cultivars might be as a result of considerable segregation associated with random inter-mating among the purple tea bushes of TRFK 306 variety comprising 4 cultivars. The antioxidant activity varied significantly (p≤0.05) between purple tea

cultivars, fresh unprocessed pigmented fruits and vegetables. First generation purple tea cultivar TRFK 306/4 had antioxidant activity of 91.0 % which was marginally higher compared to the best 2nd generation purple tea cultivars, but significantly higher than that of fresh unprocessed pigmented fruits and vegetables. It is notable that there was no significant ($p \leq 0.05$) difference between 1st and 2nd generation purple tea cultivars. The high antioxidant activity of purple tea cultivars reported is attributed to hydroxyl groups necessary for free radical-scavenging activity which is in line with previous study by Kerio *et al*. [18]. However, low antioxidant activities exhibited in fresh unprocessed fruits and vegetables was due to low total anthocyanins content. Since these fruits and vegetables are renowned superfood, it is apparent that some of the 1st and 2nd generation purple tea varieties provide better alternative category of superfood in form of novel beverage for health conscious populations.

TABLE 1: *Total anthocyanins and antioxidant activity of 1st and 2nd generation purple teas at Kangaita site compared to fresh unprocessed fruits and vegetables*

Purple tea	TA(mg/l)	% AA
*TRFK 306/OP	500.8	89.6
TRFK 306/1	285.6	89.7
TRFK 306/2	423.5	90.8
TRFK 306/3	427.7	90.4
TRFK 306/4	404.2	91.0
*TRFK 306/1044/1	197.8	84.9
*TRFK 306/1044/2	167.3	84.1
*TRFK 306/1044/3	170.3	85.2
*TRFK 306/1044/4	170.3	84.8
Mean	305.3	87.8
Fresh unprocessed fruits and vegetables		
Beetroot	1.5	1.1
Blue berries	275.8	6.0
Egg plant	102.1	6.0
Pomegranate	13.2	3.1
Red cabbage	425.3	45.7
Tomato tree	5.7	1.3
Mean	137.3	10.5
Grand Mean	221.3	49.2
CV (%)	2.3	0.6
LSD ($p \leq 0.05$)	107.78	1.26

NB: TA- Total anthocyanins, AA- Antioxidant activity
**Second generation purple tea varieties*

Chesumot site

Results for Chesumot site presented in Table 2 show that the TA content in purple tea ranged from 19.3 mg/l to 875.8 mg/l for bushes Chesumot 31 and Chesumot 17, respectively. However, majority of 2nd generation purple tea bushes significantly ($p \leq 0.05$) outperformed the fresh unprocessed pigmented fruits and vegetables used in this study. The antioxidant activity similarly varied significantly ($p \leq 0.05$) among the purple tea cultivars, and the fresh unprocessed pigmented fruits and vegetables. The purple tea bush, Chesumot 12 had the highest AA of 91.3 % which was comparable to that of Chesumot 17, but significantly higher than for fresh unprocessed pigmented fruits and vegetables. These results were however higher than those reported by Karori *et al*. [16].

TABLE 2: *Total anthocyanins (TA) and antioxidant activity (AA) for second generation purple tea at Chesumot site compared to fresh unprocessed fruits and vegetables*

Purple tea	TA (mg/l)	% AA	*Purple tea*	TA (mg/l)	% AA
Chesumot 1	210.0	90.1	Chesumot 24	146.5	85.5
Chesumot 2	159.2	89.1	Chesumot 25	120.4	85.8
Chesumot 3	636.5	90.6	Chesumot 26	115.5	84.4
Chesumot 4	174.1	89.5	Chesumot 27	162.9	87.3
Chesumot 5	574.7	90.8	Chesumot 28	122.0	82.8
Chesumot 6	174.4	86.5	Chesumot 29	128.9	84.9
Chesumot 7	149.6	89.1	Chesumot 30	126.0	86.3
Chesumot 8	703.5	90.9	Chesumot 31	19.3	84.1
Chesumot 9	181.0	85.5	Chesumot 32	143.3	85.8
Chesumot 10	136.0	87.4	Chesumot 33	801.0	91.2
Chesumot 11	146.3	90.7	Mean	251.3	87.4
Chesumot 12	242.3	91.3	*Fresh unprocessed fruits and vegetables*		
Chesumot 13	126.8	86.3	Beetroot	1.5	1.1
Chesumot 14	194.8	89.2	Blue berries	275.8	6.0
Chesumot 15	118.6	85.3	Egg plant	102.1	6.0
Chesumot 16	601.7	89.9	Pomegranate	13.2	3.1
Chesumot 17	875.8	90.5	Red cabbage	425.3	45.7
Chesumot 18	71.8	84.4	Tomato tree	5.7	1.3
Chesumot 19	56.5	82.8	Mean	137.3	10.5
Chesumot 20	50.1	82.1	Grand Mean	194.3	48.9
Chesumot 21	87.7	84.0	CV (%)	99.4	3.6
Chesumot 22	55.6	82.8	LSD ($p \leq 0.05$)	114.0	1.3
Chesumot 23	51.9	81.0			

TA- Total anthocyanins; AA- Antioxidant activity

Nandi site

In Nandi TA ranged from 77.7 mg/l to 682.5 mg/l, for bushes Nandi 6 and Nandi 8, respectively (Table 3). Majority of the 2nd generation purple teas in Nandi had significantly higher content of TA than the fresh unprocessed pigmented fruits and vegetables. The AA varied significantly ($p \leq 0.05$) between the purple tea bushes, fresh unprocessed pigmented fruits and vegetables. The overall AA mean for the Nandi 2nd generation purple teas of 87.9% was significantly higher than that of fresh unprocessed pigmented fruits and vegetables at 10.5 % revealing great room for alternative superfood with rich antioxidant capacity.

TABLE 3: *Total anthocyanins and antioxidant activity for second generation purple teas at Nandi site compared to fresh unprocessed fruits and vegetables*

Purple tea	TA(mg/l)	% AA
Nandi 1	237.1	88.7
Nandi 2	326.1	90.9
Nandi 3	243.2	88.1
Nandi 4	322.0	87.7
Nandi 5	409.1	89.8
Nandi 6	77.7	82.4
Nandi 7	477.7	87.8
Nandi 8	682.5	90.9
Nandi 9	82.0	84.9

Nandi 10	566.0	90.1
Nandi 11	311.2	88.8
Nandi 12	177.5	84.4
Nandi 13	648.1	91.1
Nandi 14	156.1	84.5
Nandi 15	255.7	86.3
Nandi 16	129.1	88.3
Nandi 17	579.1	88.7
Nandi 18	522.9	89.1
Mean	344.6	87.9
Fresh unprocessed fruits and vegetables		
Beetroot	1.5	1.1
Blue berries	275.8	6.0
Egg plant	102.1	6.0
Pomegranate	13.2	3.1
Red cabbage	425.3	45.7
Tomato tree	5.7	1.3
Mean	137.3	10.5
Grand Mean	240.9	49.2
CV (%)	55.5	2.9
LSD ($p \leq 0.05$)	130.5	1.6

Timbilil site

The TA content in Timbilil site ranged from 38.6 mg/l to 1037.0 mg/l, for 2nd generation purple tea bushes TRFK 306Fld16/6 and TRFK 306/Fld17/10, respectively (Table 4). Besides, 24 varieties of 2nd generation purple tea bushes significantly ($p \leq 0.05$) surpassed 1st generation purple tea cultivars and fresh unprocessed pigmented fruits and vegetables used in this study. The antioxidant activity (AA) similarly varied significantly ($p \leq 0.05$) between purple tea cultivars, fresh unprocessed pigmented fruits and vegetables. Bush TRFK 306 Gb/13 recorded the highest radical scavenging activity of 91.4%, which was significantly higher than first generation purple tea cultivars with AA ranging from 83.3% to 85.8%, for TRFK 306/4 and TRFK 306/2, respectively. The least antioxidant activity of 81.8% was recorded for TRFK 306 Gb/7, which, however, was higher than for fresh unprocessed pigmented fruits and vegetables.

TABLE 4: *Total anthocyanins and antioxidant activity of purple and green teas at Timbilil site compared to fresh unprocessed fruits and vegetables*

Purple tea	TA	%AA	*Purple tea*	TA	%AA
*TRFK306/1	347.5	84.8	TRFK 306/Gb/11	444.0	88.4
*TRFK 306/2	337.1	85.4	TRFK 306/Gb/12	309.9	84.2
*TRFK 306/3	277.0	85.8	TRFK 306/Gb/14	317.0	89.4
*TRFK 306/4	248.6	83.3	TRFK 306Fld16/1	350.7	85.4
TRFK 306/Fld/17/4	291.5	90.4	TRFK 306Fld16/2	179.6	85.2
TRFK 306/Fld/17/9	151.6	91.3	TRFK 306Fld16/3	122.1	83.7
TRFK 306/Fld/17/11	368.4	88.1	TRFK 306Fld16/4	175.7	83.9
TRFK 306/Fld/17/14	572.6	89.9	TRFK 306Fld16/5	311.0	84.2
TRFK 306/Gb/2	583.1	91.0	TRFK 306Fld16/6	38.6	83.1
TRFK 306/Gb/6	784.8	89.2	TRFK 306Fld16/7	200.9	85.6
TRFK 306/Gb/9	406.3	90.6	TRFK 306Fld16/8	308.7	88.2
TRFK 306/Gb/13	640.6	91.4	TRFK 306Fld16/9	280.4	86.1

TRFK 306/Fld/11/1	53.3	87.3	TRFK 306Fld16/10	292.7	85.2
TRFK 306/Fld/11/2	130.1	89.7	TRFK 306Fld16/11	411.1	91.2
TRFK 306/Fld/11/3	96.4	83.7	TRFK 306Fld16/12	214.5	87.7
TRFK 306/Fld/11/4	348.2	88.6	TRFK 306Fld16/13	167.6	88.2
TRFK 306/Fld/11/5	73.1	85.8	TRFK 306Fld16/14	327.7	85.3
TRFK 306/Fld/11/6	126.8	84.5	TRFK 306Fld16/15	106.4	87.9
TRFK 306/Fld/8/1	135.2	85.4	TRFK 306Fld16/16	87.1	82.3
TRFK 306/Fld/8/2	471.9	89.0	TRFK 306Fld16/17	216.4	88.2
TRFK 306/Fld/8/3	465.3	90.4	TRFK 306Fld16/18	123.4	84.5
TRFK 306/Fld/8/4	173.1	89.6	TRFK 306Fld16/19	138.8	85.8
TRFK 306/Fld/8/5	189.8	84.9	TRFK 306Fld16/20	741.4	91.2
TRFK 306/Fld/17/1	639.9	90.8	TRFK 306Fld16/21	91.0	85.7
TRFK 306/Fld/17/2	117.7	85.1	TRFK 306Fld16/22	818.6	91.3
TRFK 306/Fld/17/3	137.7	84.6	TRFK 306Fld16/23	114.6	82.7
TRFK 306/Fld/17/5	372.3	87.1	TRFK 306Fld16/24	145.9	85.9
TRFK 306/Fld/17/6	211.4	91.0	TRFK 306Fld16/25	98.5	82.3
TRFK 306/Fld/17/7	448.5	85.9	TRFK 306Fld16/26	125.5	88.3
TRFK 306/Fld/17/8	132.0	89.4	**TRFK 6/8	55.8	89.8
TRFK 306/Fld/17/10	1037.0	91.0	**Yabukita (TRFK St. 536)	40.3	87.2
TRFK 306/Fld/17/12	717.5	90.1	Mean	353.2	88.0
TRFK 306/Fld/17/13	249.2	87.0	*Fresh unprocessed fruits and vegetables*		
TRFK 306/Fld/17/15	305.9	90.6	Beetroot	1.5	1.1
TRFK 306/Fld/17/16	386.9	90.1	Blue berries	275.8	6.0
TRFK 306/Fld/17/17	145.3	85.4	Egg plant	102.1	6.0
TRFK 306/Gb/1	299.1	89.1	Pomegranate	13.2	3.1
TRFK 306/Gb/3	574.7	88.3	Red cabbage	425.3	45.7
TRFK 306/Gb/4	582.2	90.9	Tree tomato	5.7	1.3
TRFK 306/Gb/5	734.3	91.2	Mean	137.3	10.5
TRFK 306/Gb/7	85.1	81.8	Grand Mean	245.2	81.6
TRFK 306/Gb/8	472.9	91.1	CV (%)	73.9	25.6
TRFK 306/Gb/10	265.2	85.1	LSD($p \leq 0.05$)	64.3	6.2

NB: *TA- Total anthocyanins; AA- Antioxidant activity*

** First generation purple tea varieties*

*** Green leaf coloured varieties which are normally high in catechins content*

Fifteen bushes of the 2nd generation purple tea plants had at least twice as much total anthocyanin content as the already released variety, in Kangaita and Timbilil sites. This demonstrates heterosis in anthocyanin enhancement giving opportunities for selection of novel 2nd generation varieties. Thus, improved second generation varieties with total anthocyanin content above 600 mg/l should be considered for cloning for further evaluation in replicated clonal field trials in various tea growing areas for validation and possible commercialization.

Principal component analysis

The principal component analysis in Figure 1 showed that the majority of 2nd generation purple tea bushes with relatively higher content of total anthocyanins than the 1st generation cultivars are found in Timbilil site, followed by Chesumot site, while those from Nandi and Kangaita were average. Apparently, the pigmented fruits and vegetables (controls) were generally lower in total anthocyanin content than the colored teas.

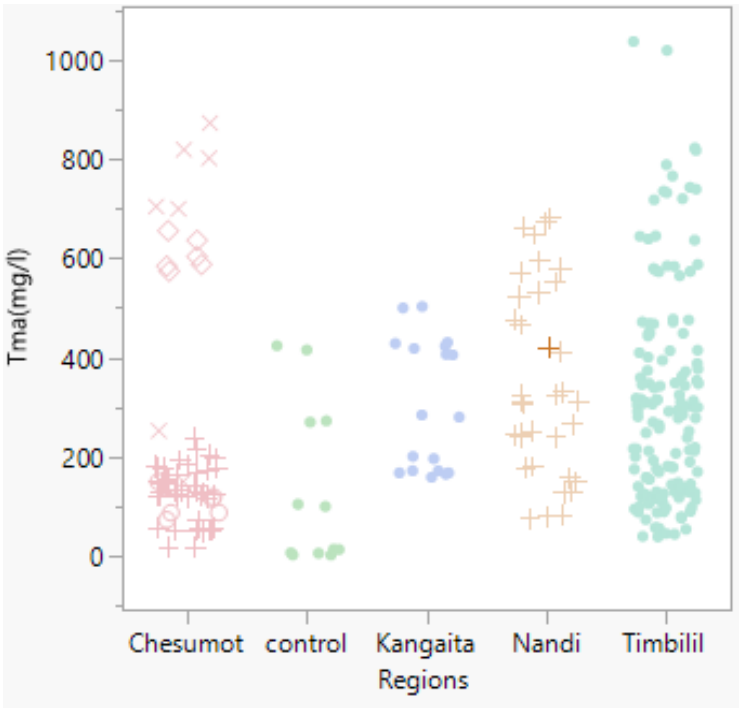

FIGURE 1: *Variations in anthocyanin contents for various tea samples*

Although there may be inherent genetic differences among the 2nd generation purple tea materials, the effect due to agro-ecological differences might have contributed to variation in total anthocyanin contents [11,16,18]. Among the pigmented fruits and vegetables, red cabbage, blue berries and eggplant had higher anthocyanins contents than some 2nd generation tea cultivars, unlike tree tomato, beetroot and pomegranate, which had relatively low content of total anthocyanin. Beetroot had the lowest content since, instead of accumulating anthocyanin, it accumulates higher levels of betalain pigment [6] Many plant phenolic compounds have stronger antioxidant properties than vitamins E and C, which are predominant in fruits and vegetables [9]. The current findings corroborate reported in a previous study [17], with differences in clonal plants being a major contributor of variation in plant metabolites even when subjected to similar agronomic practices [11] The varying ability of different cultivars in absorbing nutrients from the soils have also been recorded in different agronomic regions [3]. These may affect the ability of clones to synthesize and accumulate the bioactive molecules such as catechins and anthocyanins [12] resulting in the observed variation in the levels of total anthocyanins amongst the studied varieties.

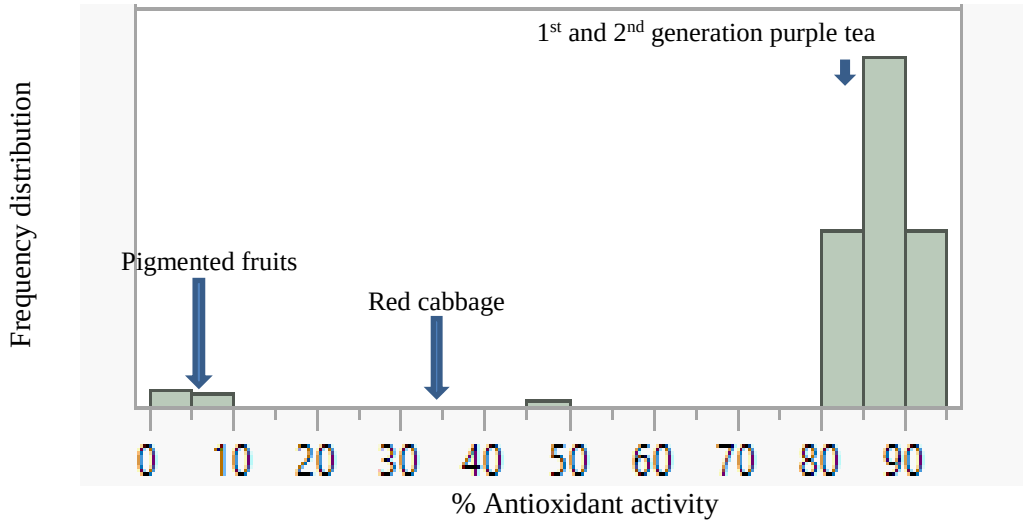

FIGURE 2: *Variations in antioxidant activities of the studied plants*

Antioxidant activity (AA) for studied plants and sites

The results of AA exhibited significant differences ($p \leq 0.05$) among all the entries in all study sites with beetroot recording the lowest radical scavenging capacity, while pigmented fruits registered higher than that reported by other workers [13,16] (Tables 1 to 4). The observed higher AA in purple tea samples could be as a result of presence of anthocyanins and catechins which are potent antioxidants [18] unlike pigmented fruits and vegetables which have low catechin content [6]. Catechins and anthocyanins contains hydroxyl groups at the third carbon atom, which stabilizes the catechin phenoxyl radical through participation in the electron delocalization [9, 21], an important phenomenon in the anti - radical potential. Further, the availability of protonated oxygen within anthocyanin structure provides electrons to reactive oxygen species [11]. The scavenging activity is entirely reliant on the chemical constitution of anthocyanins, which depends on the basic structural orientation of the compound as the ring orientation will determine the ease by which a hydrogen atom from a hydroxyl group can be donated to a free radical as well as the capacity of the anthocyanin to support an unpaired electron [2].

CONCLUSION

Second generation purple tea varieties are rich in anthocyanins and antioxidants which can be utilized as raw materials in processing of specialty teas. Such products can serve as functional foods and natural remedy against various diseases and disorders related to oxidative stress and free radical effects. They can further serve as new potential nutraceutical sources.

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