

Full Length Research Paper

Ferric reducing antioxidant power and total phenols in *Cordia africana* fruit

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Antioxidants are beneficial compounds found in a lot of foods. *Cordia africana* (Lam.) is a small fruit eaten all over Tigray and other parts of Ethiopia. The fruit was tested for its antioxidant content using the ferric reducing antioxidant power (FRAP) assay and total phenols (TP) measured with Folin Ciocalteu's reagent, across four different agroecological zones and three land use classes in Tigray. The average FRAP value on dry weight basis was 30.8 ± 1.45 mg Trolox equivalent 100 g^{-1} fruit, and the average TP value on dry weight basis 2317.0 ± 104.0 mg gallic acid equivalent 100 g^{-1} fruit. Both FRAP and TP values were found to be significantly ($p < 0.05$) different across the agroecology with the lower altitude agroecology giving the highest value and the dry mid altitude agroecology giving the least value. The difference in land use showed no effect on the FRAP value; however the TP values were significantly ($p < 0.01$) different across the different land use. The highest value of TP was found in the wild and the lowest was found in the backyard land uses. *C. africana* is a fruit with good quantities of TP, and small amounts of antioxidants measured with FRAP. Both FRAP and TP values showed variation across agroecology, while only the TP content vary across land use. The fruit was also found to have $9.07 \text{ mg } 100 \text{ g}^{-1}$ fruit, which makes it a good source of the vitamin to meet part of the daily requirement. As antioxidants and vitamin C are highly beneficial to general health, the consumption of this fruit should thus be recommended and promoted.

Key words: *Cordia africana* fruit, ferric reducing antioxidant power, total phenol, agroecology, land use.

INTRODUCTION

The benefits of antioxidants have been studied and discussed by many scientists in food science, medical science and general health areas (Baumann, 2009; Cadenas and Packer, 2002; Packer et al., 2000; Sen et al., 2000; Tardif and Bourassa, 2006). These show the

multi-disciplinary nature of the studies and the multiple use and application of antioxidants. When it comes to *Cordia*, several species have been investigated for their antioxidant properties of the fruits, roots, barks and leaves. For example the leaves of *Cordia wallichii* and

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Abbreviations: ANOVA, Analysis of variance; FRAP, ferric reducing antioxidant power; TP, total phenol measured with Folin Ciocalteu's reagent; m.a.s.l., meters above sea level; DW, dry weight basis; TE, trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) equivalent; GAE, gallic acid equivalent.

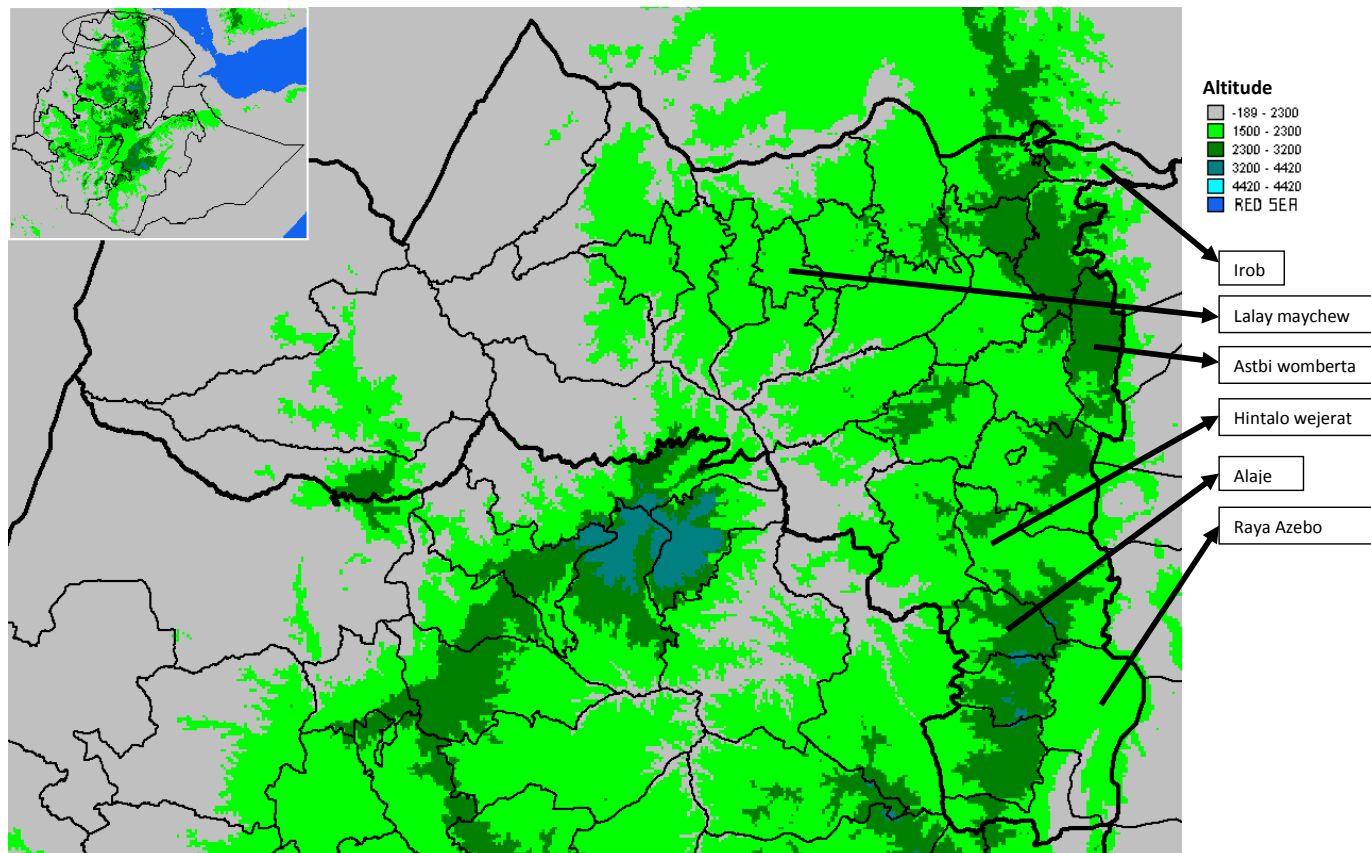


Figure 1. Map of selected woredas in Tigray, Ethiopia, East Africa, showing their relative location and altitude (Generated on DIVA-GIS software). Altitude: Irob; Lalay maychew; Astbi womberta; Hintalo wejerat; Raya Azebo; Alaje.

Cordia verbenacea were looked into by two different studies (Makari et al., 2008; Michielin et al., 2011).

The antioxidant content of fruits vary due to cultivar (genetic variance, provenance) (Cordenunsi et al., 2002; Howard et al., 2003; Kotíková et al., 2011; Wicklund et al., 2005), ripening stages (Gull et al., 2012; Kotíková et al., 2011; Vendramini and Trugo, 2000), various climate conditions such as season and production location (Howard et al., 2003; Iqbal and Bhangar, 2006), temperature (Howard et al., 2003; Wang and Zheng, 2001), altitude and ultraviolet radiation (Bhattacharya and Sen-Mandi, 2011) in addition to overall environmental conditions (Oh et al., 2009; Wang, 2006; Yuri et al., 2009).

Cordia africana is a fruit found wide spread in the Middle East, West, East, and Southern Africa. It is known by the name Sudan teak, East African Cordia, large-leafed Cordia, and Sebastian fruit (ICRAF, 2008). In Tigray, *C. africana* fruit is eaten by the local community during its fruiting season of April to June. It is collected and eaten by shepherds and children when found in the wild, and collected, and eaten or sold by women and children when grown in farms or backyards. Generally, the fruit is eaten fresh, however traditionally the fruit is also dried and kept for use during off season. The objective of this study was to determine the antioxidant levels of *C. africana* within

the context of the typical Tigrayan diet when consumed fresh.

MATERIALS AND METHODS

C. africana fruit sampling strategy

In Tigray, it is found that *C. africana* grows in the wild (natural forests, community afforestation sites, church forests), farm lands, grazing lands and people's backyards (home gardens) within the altitude range of 1500 to 2950 m.a.s.l. Thus, the present experimental design took into account the different agroecological and land use patterns. In two studies, the diversity of *C. africana* was observed by looking at genetic markers (Derero et al., 2011), seed physical characteristics and germination time (Loha et al., 2006; Loha et al., 2009). The results of these studies found that the populations of *C. africana* investigated had more genetic diversity within the populations rather than between populations. Thus, the present experimental design took into account both inter- and intra-population diversities, looking at variations across the different populations at the different agroecology and land uses.

The existing study areas where *C. africana* grows were divided into four agroecological zones. A woreda (second smallest level administrative body in Ethiopia) was randomly selected from each of the agroecological zone so as to represent the agroecological area. Figure 1 shows an altitudinal map of Tigray Regional State showing the three different agroecological zones based on altitude. Estimated rainfall data were added to this map in order to

determine the selection of the four woredas. The four randomly selected woredas were: Irob (Weyna dega, midland) which is mid altitude 1500-2300 m.a.s.l., moist with mean annual rainfall ranging from 316 to 823 ml year⁻¹; Laelay Maychew (Weyna dega) mid altitude 1500-2300 m.a.s.l., dry mean annual rainfall ranging from 639 to 673 ml year⁻¹; Atsbi Womberta (Dega, highland) higher altitude 2300- 3200 m.a.s.l., moist mean annual rainfall ranging from 577 to 608 ml year⁻¹; and Raya Azebo (Kolla, lowland) lower altitude 500-1500 m.a.s.l., dry mean annual rainfall ranging from 633 to 770 ml year⁻¹. These agroecological classifications follow the standard set for Ethiopia by the Soil Conservation Research Programme (Hurni, 1986). However, no adequate number of trees could be found in the three land use categories in Irob and Atsbi Womberta. As a result, Hintalo Wajerat (Weyna dega) mid altitude 1500-2300 m.a.s.l., moist mean annual rainfall ranging from 516 to 716 mL year⁻¹ and Alaje woredas (Dega) higher altitude 2300- 3200 m.a.s.l., moist mean annual rainfall ranging from 624 to 839 mL year⁻¹ were selected as substitutes. Within the selected woredas, a village was purposively selected where *C. africana* could be found growing in the wild, farm lands and backyards in consultation with the woreda level forestry experts. Ten trees were selected randomly from each site of the wild, farm and grazing land and backyards. From each tree, 250 to 450 g mature fruits were collected, labelled, and placed in a cooler (which had an average of 4°C) for transport. The transport from Laelay Maychew and Raya Azebo took 24 h from collection to placement in the laboratory, and that of Alaje and Hintalo Wajerat arrived in the laboratory 5 h after collection. On the same day of arrival, the size, colour and weight of 10 representative fruits from each tree was measured. Two representative fruits were taken in triplicate for the moisture level and ash content determination. The whole fruit and fruit stones were also separated out and ashed for ash content determination. The remainder of the fruit was placed in a refrigerator (4°C) until processed. Within two to three days (stored at 4°C), fruits from each tree were homogenized. For homogenization, initially the fruit cap was removed; then the fruit skin was removed. Following this, the sticky flesh was dissolved into a specified amount of water (50-150 mL depending on number of fruits collected and fruit flesh size) and by blending it with an egg whisk. When the stone and flesh are separated, the skin is placed into the dissolved fruit flesh and blended into a homogenized fruit pulp paste.

Analytical methods

The size, colour, weight, moisture and ash were determined on individual fruits, while TP, FRAP, and vitamin C values were determined from the homogenized samples. As the homogenisation process involved dilution, TP, FRAP and vitamin C values were calculated back to discount the dilution. The principles followed in the analytical measurements were the following:

1. Antioxidants: 3 g of the homogenate was extracted in 30 mL of methanol and the antioxidant levels were determined by ferric reducing activity power (FRAP), and total phenols (TP) using Folin Ciocalteu's reagent. For both analysis, the Konelab 30i outline and method was followed (Volden et al., 2008; Zargar et al., 2011).
2. Size: the size was measured on both diagonal and vertical directions of the fruit (Bertin et al., 2009). This was done using a micro-calliper.
3. Colour: the colour was initially measured using a colour chart from the Natural Colour Systems (Hård and Sivik, 1981). The Natural Colour Systems colours were then converted to the CIE L*a*b* colour (Osorio and Vorobyev, 1996; Özkan et al., 2003) reading using a Minolta colour meter.
4. Weight: the weight of a selected representative 10 fruits was measured in grams using a portable digital balance with a

sensitivity of 0.001 g (Ercisli and Orhan, 2007).

5. For vitamin C measurement 2,6-dichloroindophenol titrimetric method using oxalic acid as extractant was used, AOAC 967.21 was used (Hernández et al., 2006).

6. Ash: As separating the flesh and the stone without dilution was difficult, ashing was done on the whole fruit and on the separated out stones. The overall procedure followed the AOAC 940.26 standard (Horwitz and Latimer, 2005).

The overall experimental setup gave a nested or hierarchical design of the four woredas, with three land uses and ten replicas. Each laboratory analysis was measured with three parallels. The results were aggregated per tree. To test for land use (inter population), variances and agroecological (intra population) variances, the fully nested ANOVA was used (Minitab 16.1, USA). The FRAP and TP means were tested for grouping and ranking using Tukey's tests. Further investigation was done using a Principal Component Analysis (PCA, Unscrambler 10X, USA and PAST (Hammer et al., 2001)), to test the relationship between the FRAP and TP loadings and agroecology. Another set of PCA was applied to test the relationship between FRAP and TP across agroecology and land use. This relationship was further explored by making a correlation matrix (Minitab 16.1, USA).

RESULTS AND DISCUSSION

Fruit physical and chemical properties

The mean, standard error, minimum, median and maximum values of the FRAP and TP, both on fresh fruit (FW) and dry weight (DW) basis, are presented in Table 1.

The antioxidant levels in Table 1 show the measured values. There is a difference between the fresh and dry weight FRAP and TP values because the fresh fruit contained a lot of moisture with a mean of 56.89%. The FRAP average is comparable to that found from the bark extract in a similar species *Cordia dichotoma* bark, with 22.8 mg mL⁻¹ TE on a dry weight basis (Ganjare et al., 2011). The average total phenol values (DW) are at least 5.7 times higher than fruit extract found in a similar species, *Cordia myxa*, with a variation found in the literature of 373.9-400 mg 100 g⁻¹ GAE (Aberoumand and Deokule, 2009b; Aberoumand, 2011b; Souri et al., 2008). TP values in fresh fruits are comparable with that found in *Cordia exaltata* fruit with 190 mg 100 g⁻¹ on a FW basis (Silva et al., 2007). To date, there is no daily requirement set for the consumption of antioxidants and total phenols. The American average daily intake of total phenols has been set to 450 mg GAE (Chun et al., 2005), and the Mexican average daily intake of antioxidants is 170.2 - 240.2 mg trolox equivalents day⁻¹, which is stated to be similar to 222-1004 mg GAE per day as found in Spanish Mediterranean diets, which is used as a reference for European countries (Hervert-Hernández et al., 2011). Taking these figures as bench marks, on the one hand, these antioxidant levels are too low for daily intake levels to be reached as 5222 g would be needed to reach the American recommended values. On the other hand, the total phenol content is high enough to meet the American and Mexican/Spanish Mediterranean daily intake levels

Table 1. Basic statistical results of the FRAP and TP measurements on both fresh (FW) and dry weight (DM) basis, and other basic fruit parameters measured.

Variable	Mean	SE Mean	Minimum	Median	Maximum
FRAP FW (mg 100 g ⁻¹) TE	4.6	0.2	1.1	4.2	10.4
FRAP DW (mg 100 g ⁻¹)	30.8	1.45	8.7	27.8	78.5
TP FW (mg GAE 100 g ⁻¹)	264.1	12.2	70.5	248.5	687.9
TP DW (mg GAE 100 g ⁻¹)	2317.0	104.0	578.0	2215.0	4980.0
Ash whole fruit (%)	2.00	0.02	1.62	1.97	2.38
Average size (cm)	1.33	0.02	0.90	1.30	1.76
Moisture (%)	56.89	0.64	41.94	58.15	74.97
L (L scale)	31.48	0.22	25.08	31.42	43.71
a (a scale)	3.37	0.27	-5.97	3.43	9.87
b (b scale)	31.20	0.32	20.36	31.12	49.81
Weight (g)	15.34	0.50	6.60	14.00	33.70
Vitamin C (mg/100 g FW)	9.07	0.25	4.96	8.25	18.93
Vitamin C (mg/100 g DW)	20.20	0.31	14.15	19.77	30.38

FRAP = Ferric reducing activity power, TP = total phenols, TE = TROLOX equivalent, GAE = gallic acid equivalent.

Table 2. Nested ANOVA analysis of the FRAP and TP values of *C. africana* fruits.

Variable	Tested parameter	DF	Variance explained	F	P	Significance level
FRAP (DW)	Agroecological	3	10.83	4.54	0.04	*
	Land use	8	0.29	1.03	0.42	ns
TP (DW)	Agroecological	3	21.62	4.17	0.05	*
	Land use	8	13.99	3.17	0.00	**

FRAP = Ferric reducing activity power, TP = total phenols, dry weight (DW), * = significant at 95 %, ** = significant at 99% and ns = not significant.

by consuming 170.4 g. The fruiting season lasts on average for three months, as each tree has a different time of fruit maturation, and per tree fruits have different times of maturation. Assuming people consume the fruit during the fruiting season, it helps them to meet the daily recommended rates of total phenol need. On average people will eat about 100 g of the fruit at any given time, unless they are using it for treating gastro-intestinal illnesses, for which they would consume about 750 g at one time.

The ash content was $2 \pm 0.02\%$, lower than that reported by Murray et al. (2001) (5.1 to 7.8% for *Cordia sinensis*) Aberoumand and Deokule (2009a) (6.7 and $6.7 + 0.80\%$) and Aberoumand (2011a) for *Cordia myxa*. The average fruit diameter was 1.33 cm with an average moisture content of 56.89%. The average colour is light yellow (L 31.48, a 3.37 and b 31.2). The weight of the 10 representative fruits sampled was on average of 15.34 g. The fresh weight vitamin C was found to be 9.07 mg 100 g⁻¹ fruit. This value is similar to that found in banana and apples (Planchon et al., 2004; Wall, 2006). According to the FDA guidelines, this value meets 15.12% of the daily required vitamin C levels for an average adult and according to FAO/WHO guidelines, this value meets 30.23% of the daily requirements

(FAO/WHO, 2002; Food and Drug Administration, 2011).

Looking at the relatively good content of total phenols and vitamin C, it is obvious that this fruit is a nutritionally important fruit, with a limited range of use. Taking into account the benefits of antioxidants and vitamin C, it is clear that work is needed in the promotion and popularisation of this fruit. It is also clear that there is need for further study on its other nutritional benefits, processing and marketing potential to aid its promotion. The high variation in its size and weight also shows that there is great variation, which has implication on selection of the fruit source for promotion. As it grows in most parts of Africa and the Middle East its use and promotion has a wider significance than that of the studied area.

Fully nested ANOVA, principal component analysis (PCA) and correlation results

As the experimental setup had four agroecological zones and three land use systems, from which 10 trees were selected randomly, the experimental setup fits best to the nested or hierarchical design. ANOVA for a fully nested design was run and the results are presented in Table 2.

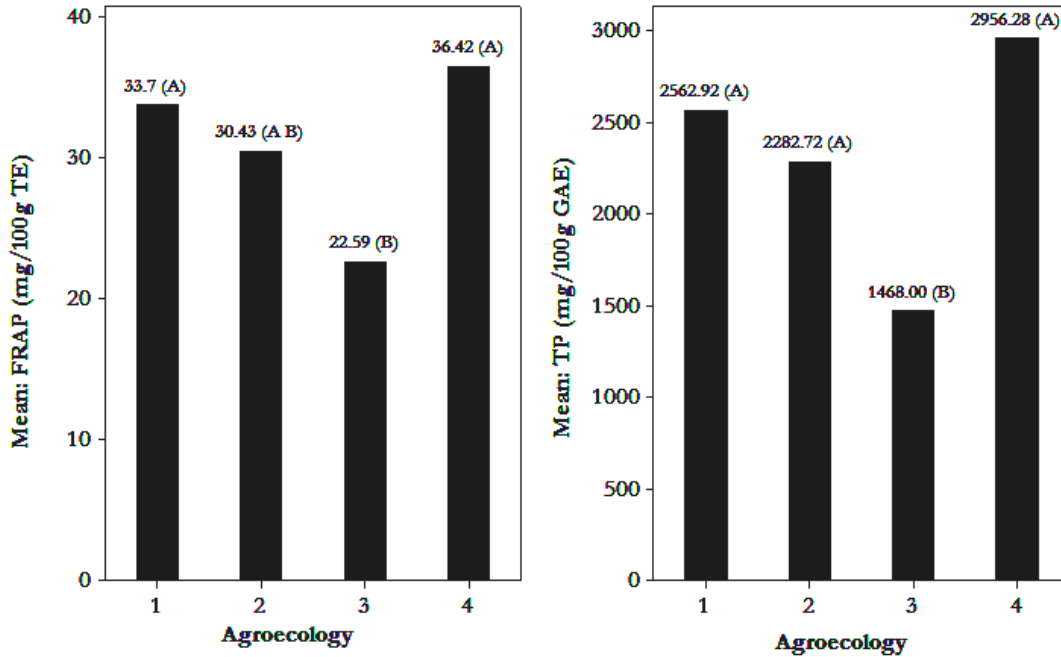


Figure 2. Tukey's ranking and grouping of mean values for FRAP and TP (based on dry weight (DW)) across agroecology. 1 = highland, 2 = moist mid altitude, 3 = dry mid altitude, 4 = lowland.

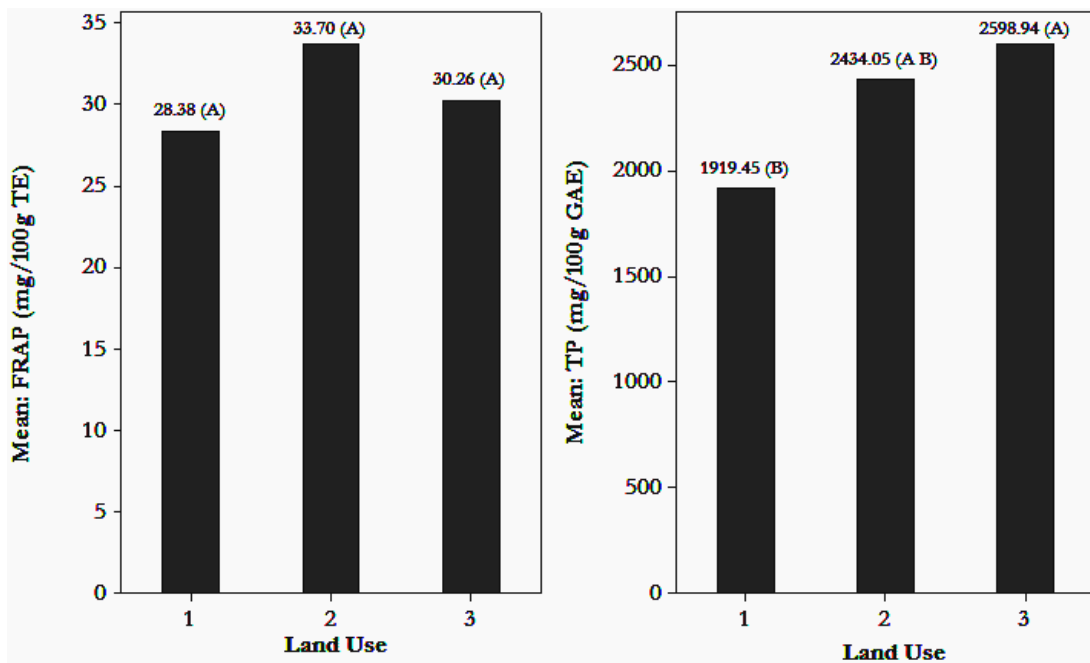


Figure 3. Tukey's ranking and grouping of mean values for FRAP and TP (based on dry weight (DW)) across land use. 1 = backyard, 2 = farm land, 3 = wild.

As can be seen in Table 2, both the FRAP and TP values were significantly different for the different agroecologies tested. The FRAP values were not significantly different from each other across the different land uses, while the

TP values were significantly different across the different land uses. All the parameters were also tested for ranking and grouping using Tukey's test. The results are summarised in Figures 2 and 3. As can be seen in Figure 2,

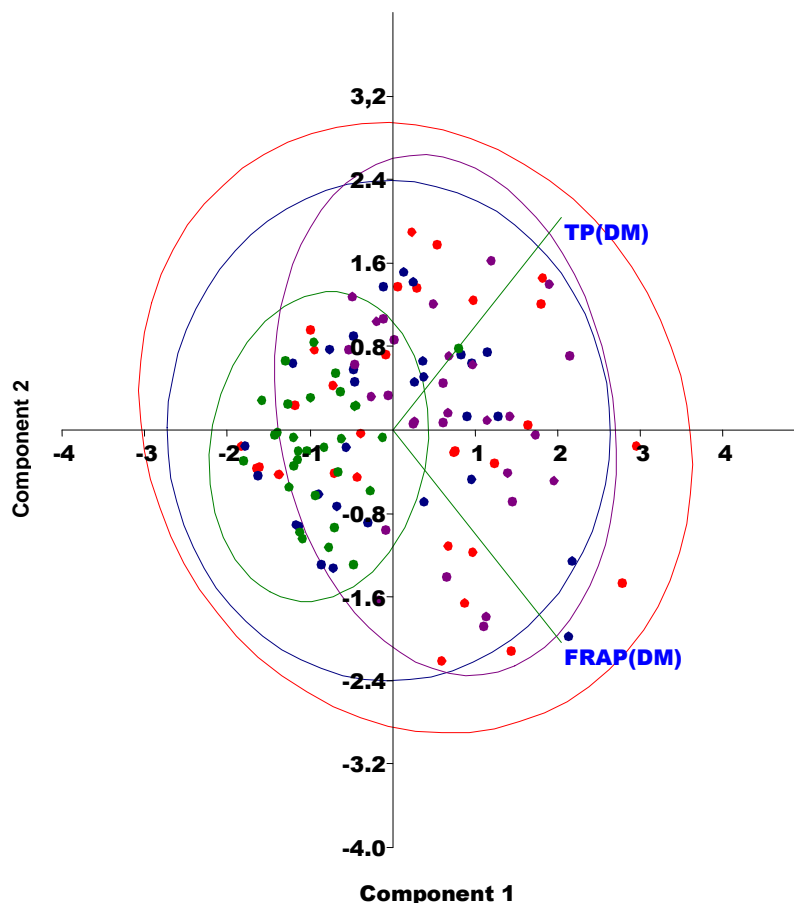


Figure 4. PCA of FRAP and TP values based on agroecology. Red = higher altitude, dark blue = moist mid altitude, green = dry mid altitude, purple = lower altitude.

the lower altitude site had the highest values for both FRAP and TP, while the mid altitude dry site had the lowest values with significant differences between these highest and lowest values. The lower altitude area has higher temperatures as compared to the other sites, and these results are in agreement with a strawberry study, where it was noted that both phenolic content and antioxidant content of the fruits increased with increasing growing temperature (Wang and Zheng, 2001).

The FRAP values in Figure 3 were not significantly different across land use in both the nested ANOVA and Tukey's tests. The TP values showed a significant difference with highest values measured for fruits from the wild and lowest values for fruits from the backyard, while that in the farm land was in between. As the trees in the backyards are purposefully selected, and those from the farm land are semi purposefully selected, one possible explanation for this difference could be a factor of selection resulting with trees with special traits. Breeding and cultivar (genetic variety, provenance) development starts with this, and several studies have shown that cultivars have a significant effect on the total

phenol values (Cordenunsi et al., 2002; Howard et al., 2003; Kotíková et al., 2011; Wicklund et al., 2005). Though there have not been studies showing that *C. arifcana* has specific cultivars, studies on its genetic variation within provenances (specific geographical location) have shown that there is a high variation (Derero et al., 2011; Loha et al., 2006, 2009). Another reason can be difference in the micro climate of these land uses, with the wild predominantly being marginal where environmental stress is highest, and the backyard being the most conducive with watering, organic matter and ash application creating a difference in the stress levels in the trees. The farm lands are flat and more fertile than the wild areas. In relation to this, several studies have shown that fruits grown under stressful conditions produce higher levels of phenolic compounds (Oh et al., 2009; Tomás-Barberán and Espín, 2001; Yuri et al., 2009).

A principal component analysis of the agroecological groups was run for both FRAP and TP values as presented in Figure 4. As can be seen with respect to the principal components (PC) 1 and 2, the score of the

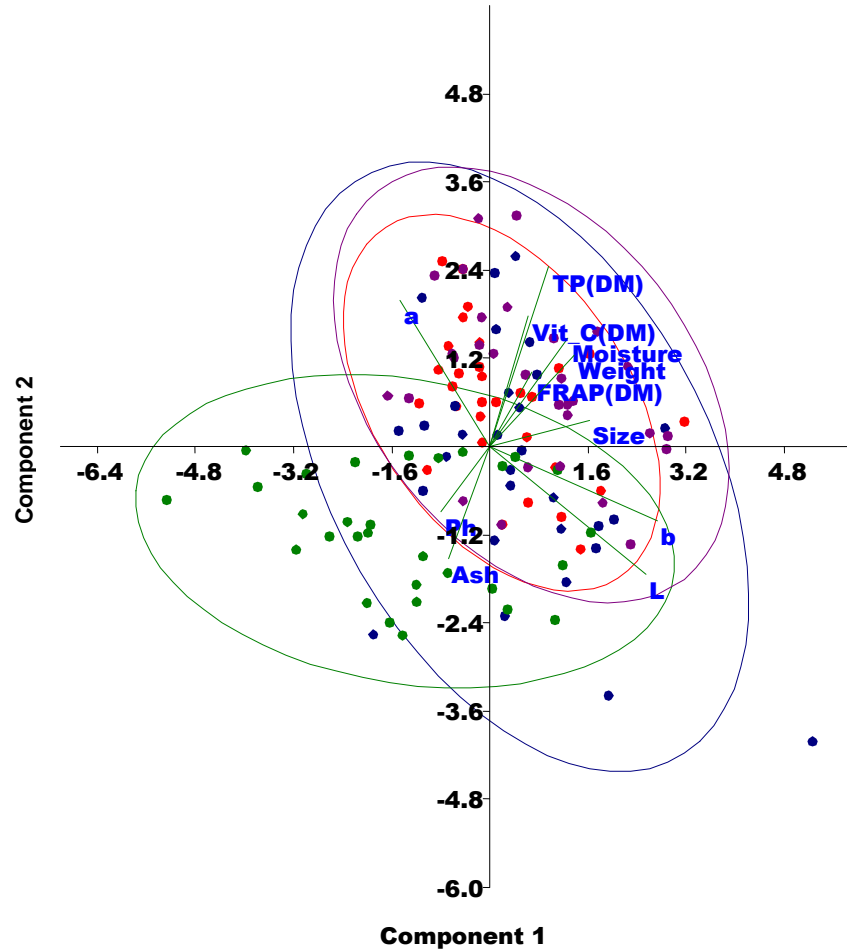


Figure 5. PCA of FRAP and TP and other related fruit parameters based on agroecology. Red = higher altitude, dark blue = moist mid altitude, green = dry mid altitude, purple = lower altitude.

higher altitude agroecology was more widely scattered, while they were narrowly scattered for the dry mid altitude agroecology. The loadings for FRAP and TP best explain the score of the lower altitude agroecology and least explain the score range of the dry mid altitude agroecology concurring with the ANOVA and Tukey's grouping results (Table 2, Figures 2 and 3). Another principal component analysis with agroecological and land use groups was run for both FRAP, TP and other related fruit parameters (Figures 5 and 6). The loadings for the first two principal components show that FRAP, TP, vitamin C, moisture content, weight and average size have a positive relationship, while whole fruit ash content have a negative relationship. The colour parameters L^*a^*b showed minimal relationship. With respect to the agroecological grouping (Figure 5), the scores of the higher altitude agroecology was the narrowest in scatter, yet the relationship it had with the loadings was similar with that of the moist mid altitude and lower altitude agroecologies. The scores of the dry mid altitude

agroecology were least explained once again by the loadings for FRAP, TP, vitamin C, moisture, weight and colour axis a. This result shows a similar pattern as that of ANOVA and Tukey's grouping; where the dry mid altitude agroecology is separate (Figures 2 and 3).

Looking at the land use groupings of the scores (Figure 6), the backyard and farm land showed similar patterns. On the other hand, the wild land use was slightly different in that it had more response to the colour axis of L^*a^*b values and was less explained by the other loading directions. This also shows a similar pattern as that of ANOVA and Tukey's grouping, with the wild score being separate (Table 2, Figures 2 and 3).

Following the PCA analysis, a correlation relationship with FRAP and TP of a few related fruit parameters was run to further investigate the observed relationships. The correlation matrix showed a similar picture as that of principal component analysis in Figures 5 and 6, with both positive and negative relationships with the selected fruit parameters except for that of $L^*a^*b^*$. The FRAP

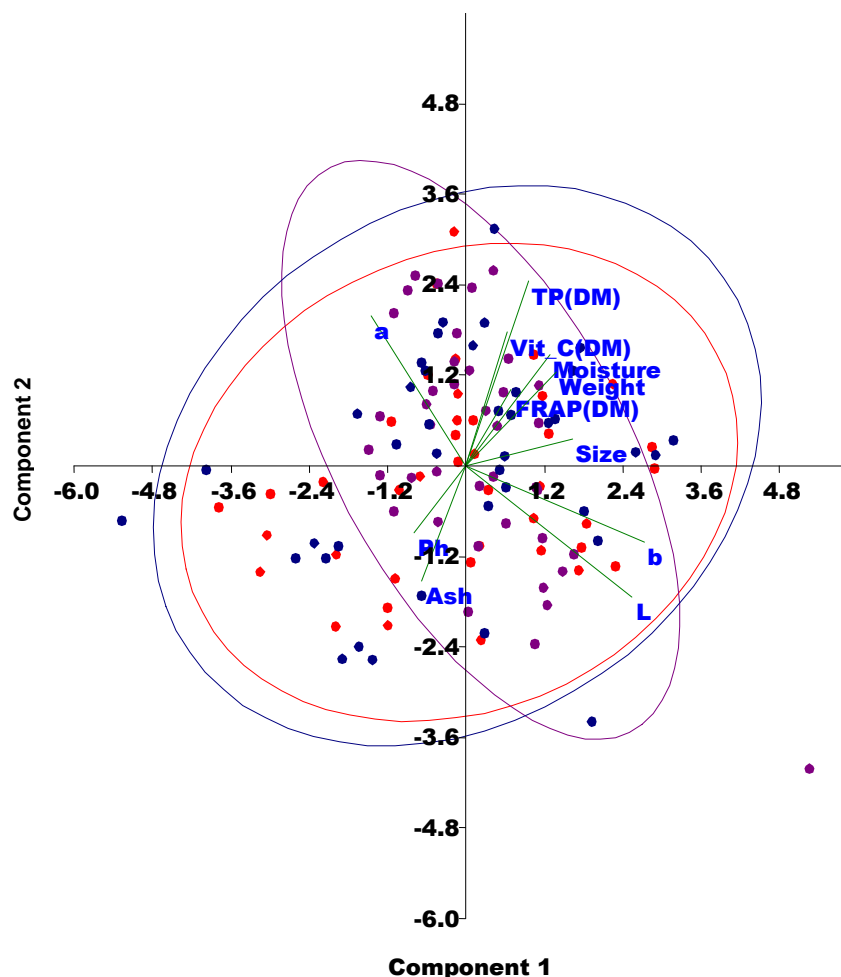


Figure 6. PCA of FRAP, TP and other related fruit parameters based on land use. Red = backyard, dark blue = farm land, purple = wild.

values were only correlated significantly with total phenol and fruit moisture levels, while the TP values are significantly correlated with the moisture, weight, vitamin C and fruit ash content. However, it needs to be noted that though there was a significant relationship considering the p values, the Pearson's correlation coefficient was very low. The correlation relationship of TP with fruit ash content was negative as indicated by the PCA in Figures 5 and 6. The positive relationships could be explained as vitamin C is an antioxidant and the weight and moisture content are indicators of better fruit growth. The negative relationship can be explained by the fact that phenols are acidic, and acidic compounds have lower pH. With respect to the relationship with the fruit ash content, the highest content of total phenols was found in the wild which are marginal lands. The soil fertility in these areas is lower, and previous studies have shown that soil fertility and mineral contents are related (Havlin et al., 2009; Lucas et al., 1942).

These results have implications for the use and promotion of this fruit. Currently, the fruits is consumed locally and sold in local markets (Demel and Abeje,

2005). Currently, in most parts of Ethiopia and Tigray there are massive afforestation efforts underway, that if managed properly have a great potential to contribute to improved food security, poverty alleviation and environmental rehabilitation (Egziabher, 2006; Fentahun and Hager, 2009; Gebrehiwot and Headquarters, 2004; Mengistu et al., 2005; Tewolde-Berhan et al., 2000; Yami et al., 2006). This study has shown that the fruit is useful and beneficial for health, and the TP values were highest in the wild. The promotion and wider use of the fruit can be achieved through its incorporation in the existing enclosure, communal forestry, and hillside distribution efforts. In addition to this, efforts need to be put in place to promote and market the fruit.

Conclusion

Cordia africana is a fruit with good quantities of TP-total phenol antioxidants. The antioxidant content tested with the FRAP, was not high. Both FRAP and TP values showed variation across agroecology, and only the

phenolic content with variation across land use. The phenolic content is also strongly related to the FRAP, moisture content, weight, vitamin C and fruit ash contents.

The significance of these results is in its implication for use. These fruits are consumed by the local community and are sold in local markets. As these antioxidants and vitamin C tested are known to have beneficial effects towards health, the continued consumption of these fruits in Tigray is highly recommended. As the fruit is known to grow in most parts of Africa and the Middle East, its use needs to be made known and the fruit needs to be promoted. Further study on its overall nutritional value, processing potential and marketability will help in the promotion of the fruit. As the size and weight was also found to have great variability, care needs to be taken to select appropriate seed sources for promotion. With the growing afforestation efforts in the region, this species should be given focus as its TP values where the highest in the wild, which represents the afforestation sites. Understanding their use will help in the up scaling of their use and marketing.

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