Antioxidant activities in *Vitex doniana* and *Saba comorensis* fruits from coastal forests of Tanzania

Dominic Charles and Clarence Mgina

Chemistry Department, University of Dar es Salaam, Dar es Salaam, Tanzania

**ABSTRACT**

Coastal forests of Tanzania are endowed with edible wild fruit plant species that can be used as dietary supplements to humans. The antioxidant activities of two common wild fruits *Vitex doniana* and *Saba comorensis* from two coastal regions (Tanga and Coastal) were determined using diphenyl-1-picrylhydrazyl (DPPH). The antioxidant activities for *V. doniana* ranged between 71.0 ± 0.4 and 49.11 ± 0.04 in fruit samples from Tanga and 65.6 ± 0.1 and 48.3 ± 0.1 in fruit samples from Coast region. The activity in *S. comorensis* ranged between 56.28 ± 0.02 and 44.22 ± 0.03 for fruit samples from Coast region and between 61.77 ± 0.00 and 51.8 ± 0.1 for fruit samples from Tanga. However, the differences in antioxidant activity between fruits from the two regions were not significant (*P > .05*) as they performed on the same latitudinal gradient influenced by the similar coastal habitat conditions. Data indicated a positive correlation between concentration of the fruit extracts and the free radical scavenging capacity where higher antioxidant activity was observed at 1000 µg/ml fruit extract concentration than in 62.5 µg/ml. The regression analysis indicated significant influence of the wild fruit extracts on antioxidant activity in fruit samples from Tanga than it was in fruit samples from Coast regions. The considerable antioxidant activity depicted by the fruits of both *V. doniana* and *S. comorensis* indicated their nutraceutical potential as natural antioxidants.

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**Introduction**

Coastal forests of Tanzania are part of the global natural plant communities that provide benefit to large number of local communities in many parts of the world. These forests harbor diverse plant species that provide beneficial goods and ecosystem services to humans including indigenous wild fruits that have been the source of food to local communities over the years. The wild fruit species are widely distributed across the latitudinal gradient from the south to the north–eastern part along the coastal forest strip in Tanzania and the local community obtains the same during dry seasons. However, the use of wild fruits as food has largely declined due to the increase in cultivated exotic fruit plants except for a few rural communities that continue utilizing these indigenous resources in their natural habitats. This may be caused by the lack of information on nutritional and pharmaceutical values of the wild fruits and hence their potential important contribution to human nutrition and health.

Wild fruits are rich in protein, carbohydrate, fat and nutrient elements, vitamins, and phytochemicals. Most of the wild fruits are eaten in unprocessed form by a number of local communities in the coastal regions of Tanzania; however, their nutritive value and contributions to the maintenance of human health have not been recognized and therefore not widely exploited. Wild fruits form part of the medicinal plants that largely helps in suppression of high blood pressure, cancer...
and inflammation, [8] and minimize the risk of developing various human diseases. [9,10] Wild fruits are potential source of antioxidants because they have high abundance of flavonoids carotenoids, vitamin C, phenolic compounds. [11–13] Physiological activities in the body result into a number of free radicals that are harmful because they increase the probability of occurrence of various nonpathogenic degenerative diseases. [14] Antioxidants are free radical scavengers [15] and the use of wild fruits as part of the human diet contributes to lowering risks associated with oxidative stresses caused by free radicals. [16]

The potential of wild fruits as a source of antioxidants and food supplements has ignited attention to various communities across the world. [17] A large number of fruit plants has been assessed for their antioxidant activities and their potential application to minimize health risks to human. [18] There are reports on the antioxidant potentials of Ficus carisa, [19] Ficus microcarpa, [20] Morus alba, [21] Punica granatum, [22] Ziziphus mauritiana. [23] However, limited information exists on the nutritive value of V. doniana and S. comorensis, which are among wild fruits that are frequently utilized by the local communities in the coastal regions of Tanzania. The Coast and Tanga regions were selected to represent parts in the coastal area where natural forest fragments both protected and unprotected are found. The main objective was to determine the antioxidant activities in V. doniana and S. comorensis which are among wild fruits from coastal forests of Tanzania. Data generated informs the local communities and the society at large on potential of wild fruits to human nutrition and health.

Material and methods

Collection of wild fruit samples and preparation of fruits extracts

The wild fruits were collected in March and April 2019 from their natural habitats and local markets in Tanga and Coast regions in Tanzania. They were identified by the botanist from the herbarium of Botany Department, University of Dar es Salaam, Tanzania. In the laboratory the fresh edible parts of the wild fruits were separated from the main fruit samples and dried for 3 weeks, then grinded using a blender to obtain a powder. Hundred milligram of the powdered sample were soaked in 300 mL of 96% ethanol to obtain the fruit extracts. The solvent was then evaporated in vacuum under reduced pressure to obtain the ethanol fruit extracts were stored at room temperature for subsequent analysis.

Antioxidant activity in wild fruit extracts

Diphenyl-1-picrylhydrazyl (DPPH) was used to determine antioxidant activity based on the procedures described by. [24] Different concentration of the fruit extracts (1000, 500, 250, 125, 62.5 μg/ml) were measured and then 5 ml of 96% ethanol and 1, 1-diphenyl-1-picrylhydrazyl (DPPH) were added to each sample and shaken thoroughly and the mixture was allowed to stand in the dark for 30 minutes. A control was prepared using ethanol as a base line correction to reduce an offset in the overall sample absorbance. The absorbances of the sample extracts were measured at 517 nm using a UV-VIS spectrophotometer (SPECORD 210/PLUS). Ascorbic acid was used as a standard that was prepared with the concentration range of 1000, 500, 250, 125, and 62.5 μg/ml and used to determine the antioxidant as shown in equation that follows:

\[
AA\% = \left(\frac{(ABS\text{control} - ABS\text{sample})}{ABS\text{control}}\right) \times 100
\]  

(1)
Table 1. The percentage antioxidant activity of *V. doniana* fruits from Tanga and Coast forests of Tanzania.

| Fruits extract concentration µg/ml | Antioxidant activity of *V. doniana* from Tanga | Antioxidant activity of *V. doniana* from Coast region | t-value | P-value | Conclusion |
|-----------------------------------|-----------------------------------------------|-----------------------------------------------|---------|---------|------------|
| 1000                              | 71.01 ± 0.39                                  | 65.61 ± 0.05                                  | 1.5516  | P > .05 | Not significant |
| 500                               | 65.43 ± 0.04                                  | 55.34 ± 0.25                                  | 1.6624  | P > .05 | Not significant |
| 250                               | 61.10 ± 0.01                                  | 54.27 ± 0.01                                  | 1.7038  | P > .05 | Not significant |
| 125                               | 57.83 ± 0.04                                  | 51.03 ± 0.00                                  | 0.6818  | P > .05 | Not significant |
| 62.5                              | 49.11 ± 0.04                                  | 48.27 ± 0.06                                  | 0.2221  | P > .05 | Not significant |

*Data analysis*

The antioxidant activities of *S. comorensis* and *V. doniana* fruits extracts were compared between samples from Tanga and those from Coast Regions using two sample *t*-test present in the graph pad software version 3.06 (Graphpad, 2003). The regression analysis was used to determine the influence of the concentration of fruit sample extracts on the antioxidant activity in each locality.

*Result*

*Antioxidant activity in *S. comorensis* and *V. doniana***

The results showed that *V. doniana* have higher antioxidant activity than *S. comorensis* (Tables 1 and 2). Both fruits from forests of Tanga region show higher activity than those from Pwani forests. This difference in antioxidant activities can be attributed to different amounts of phenols and flavonoids present in fruit samples which may be due to differences in environmental and climatic factors between the regions.

*Free radical scavenging activity*

The results on DPPH radical scavenging activity in *S. comorensis* and *V. doniana* are presented in Tables 1 and 2. It was observed at higher concentration of 1000 µg/ml, antioxidant activity ranged between 65.56% and 65.66% in *V. doniana* for the fruit samples from Pwani and 69.62% to 71.40% for the fruit samples from Tanga. This difference was, however, not statistically significant based on two sample *t*-test (*t* = 1.5516, *df* = 2, *P* > .05). The pattern was also the same even at lower concentration (62.5 µg/ml). *S. comorensis* at a concentration of 1000 µg/ml had antioxidant activity in a range between 56.26% and 56.30% for the fruit samples from forests of Pwani and 61.77% for the fruit

Table 2. The percentage antioxidant activity of *S. comorensis* fruits from Tanga and Coast forests of Tanzania.

| Fruit extract concentration µg/ml | Antioxidant activity of *S. comorensis* from Coast | Antioxidant activity of *S. comorensis* from Tanga | t-value | P-value | Conclusion |
|-----------------------------------|-----------------------------------------------|-----------------------------------------------|---------|---------|------------|
| 1000                              | 56.28 ± 0.02                                  | 61.77 ± 0.00                                  | 0.3997  | P > .05 | Not significant |
| 500                               | 51.57 ± 0.00                                  | 57.96 ± 0.00                                  | 0.0577  | P > .05 | Not significant |
| 250                               | 50.28 ± 0.00                                  | 56.18 ± 0.01                                  | 0.1424  | P > .05 | Not significant |
| 125                               | 48.13 ± 0.01                                  | 55.11 ± 0.03                                  | 0.0604  | P > .05 | Not significant |
| 62.5                              | 44.22 ± 0.03                                  | 51.82 ± 0.05                                  | 0.0572  | P > .05 | Not significant |
samples from forests of Tanga. As in the case of *V. doniana*, the differences between the two regions were not statistically significant. The data show that both *V. doniana* and *S. comorensis* have radical scavenging capacity with the former exhibiting a higher capacity than the later.

**Influence of extract concentrations on antioxidant activity**

It was observed that there was a positive correlation between the concentrations of the wild fruit extracts with free radical scavenging activity. The antioxidant activities increased with increasing concentration of wild fruit samples and standards. This observation is similar to those reported by Motalleb et al. (2005). Data on this correlation has been shown in Figure 1. The changes in the concentration as independent variable correlated significantly with shifts in the scavenging activities ($r = 0.811$, $df = 4$, $P < .05$) from the tabulated value.

**Discussion**

The determination of free radical scavenging effectiveness of the extracts from various plant parts based on diphenyl-1-picrylhydrazyl (DPPH) has been widely evaluated in a number of studies. [15,25,26] 1, 1-diphenyl-1-picrylhydrazyl (DPPH) was used to determine the capacity to scavenge free radicals from *S. comorensis* and *V. doniana* and the data has been presented in Tables 1 and 2 and Figure 1. It was observed in this study that *V. doniana* and *S. comorensis* fruit extracts to have good antioxidant activity (Tables 1 and 2). The results on antioxidant activity in this study using *V. doniana* fruits are different from the results reported by [18]. This difference is attributed to the differences in the kind of solvents and the DDPH stable radicals used. In this study, 1, 1 DPPH was used instead of 2, 2 DPPH stable radicals to determine the antioxidant activity in wild fruit extracts. This is the reasons why the
ethanol extracts used by Ref. [18] showed higher antioxidant activity based on the 2, 2 DPPH than 1, 1 DPPH used in this study. Various studies used 1, 1 DPPH \(^{[27,28]}\) showed lower results of antioxidant activities compared to those used 2, 2 DPPH such as. \(^{[15,29]}\) Although fruits from Tanga Region recorded higher antioxidant activity in both \(S. comorensis\) and \(V. doniana\) than those from Coast Region, the observed difference in fruit extracts between the two regions was not significant \((P > .05).^{[30]}\) pointed out that flavonoids and phenols are the most powerful free radical scavenger in the human body and their presence increased the antioxidant activity similar to the observation by Ref. \(^{[31,32]}\) in wild fruits. This suggests a similar conclusion based on the activity observed in the fruits of \(S. comorensis\) and \(V. doniana\) in this study. James et al. \(^{[33]}\) reported shoots and roots in \(V. doniana\) extract to have a very good therapeutic armamentarium and therefore used in the treatment of oxidative stress associated with hyperlipidemia. Although, Agbafor and \(^{[15]}\) observed lower capacity to inhibit DPPH in leaves extracts of \(V. doniana\) \((15 \pm 3\% )\) than it was in its fruits.

A number of studies showed that the strength of the activity for a particular extract depends on the solvent used. Shan (2019) reported higher percentage of DPPH activity in the acetone extracts \((95 \pm 9\% )\) than ethanol extracts from \(S. comorensis\) and \(V. doniana\) fruits recorded in this study. However, the antioxidant capacity in \(S. comorensis\) fruit extracts recorded in this study are close to the activity levels \((58.4 \text{ mg/100 g})\) of water extract reported by Anthony et al. (2017) in the same fruits from Zanzibar Islands. The \(S. comorensis\) fruits from Tanga Region had antioxidant activity that was comparable to the levels at \((23.22 \text{ to } –62.54\% )\) reported by \(^{[28]}\) based on the 100 \(\mu\text{g/ml}\) of the water extracts. In this study, the ethanol extract of \(V. doniana\) fruits at 1000 \(\mu\text{g/ml}\) had a very higher antioxidant capacity than those reported by \(^{[15]}\) \((20.4 \pm 2.6\% )\) for water extracts of \(V. doniana\) and \((14.5 \pm 2.7\% )\) of the ethanol extracts of \(V. doniana\). \(^{[28]}\) reported that 1 \(\mu\text{g/ml}\) of the leaf extracts from \(V. doniana\) and \(Adansonia digitata\) depicted lower activity \((\text{ranged from 0.00% to 26.70%})\) than 10 \(\mu\text{g/ml}\) of the methanolic and hydroethanolic extracts \((\text{which was at the range of 79.81% and 77.39%}, \text{respectively.})\). This indicates that the capacity to scavenge free radicals depends much on the concentration of the fruit extracts as observed in this study that as the concentration of the extract increases the scavenging activity also increases (Figure 1).

Author \(^{[34]}\) observed higher antioxidant activity in \(Pinus densiflora\) that 20% ethanol extracts \((266 \pm 4 \text{ mg/g})\) than in 20% acetone extracts \((258 \pm 6 \text{ mg/g})\) and 20% methanol extracts \((200 \pm 4 \text{ mg/g})\). This observation is generally higher than the antioxidant activity observed in both \(S. comorensis\) and \(V. doniana\) ethanol extract fruits. This was most probably caused by low phenolic contents and flavonoids as a result of enzymatic degradation during drying of the sample and preservation. However, the results from present study still suggesting that antioxidant activity in \(S. comorensis\) and \(V. doniana\) fruits are higher than those in the domesticated fruits based on a number of studies in domesticated fruits such as that by Ref. \(^{[18]}\) on \(Citrus limon\) \((42.75 \text{ \mu mol})\), \(Musa acuminata\) \((32.80 \text{ \mu mol})\), \(Citrus paradise\) \((24.66 \text{ \mu mol})\), \(Citrus sinensis\) \((31.48 \text{ \mu mol})\) and \(Ananas comosus\) \((16.93 \text{ \mu mol})\) which are lower than the data reported in this study. Patthamakanokporn \(^{[35]}\) also reported in \(Mangifera indica\) \((21.0 \pm 7.7 \text{ \mu mol})\) and \(Psidium guajava\) \((18.4 \pm 0.4 \text{ \mu mol})\) and that of \(^{[25]}\) in \(Musa acuminata\) \((\text{Banana})\), one of the most widely domesticated fruits that had lower antioxidant activity \((35.8 \pm 4.1\% )\) than those observed in \(S. comorensis\) and \(V. doniana\) fruits in this study. It is perceived that the stronger activity depicted by the ethanol extracts indicates the best of this solvent in the determination of antioxidant activity in wild fruits used in this study.

Conclusion

It has been observed in this study that the ethanol extract in fruits of \(S. comorensis\) and \(V. doniana\) have sufficient activity to inhibit free radicals and hence a good natural source of antioxidants. Both the \(S. comorensis\) and \(V. doniana\) fruits from Tanga Region had higher antioxidant activity than those obtained from Coast Region. The difference in antioxidant activity between these fruits may be caused by variation in habitat conditions and locations. Moreover, these wild fruits have
exhibited higher antioxidant activity compared to some domesticated fruits indicating their importance in human diet. Therefore, the presence of higher antioxidant activities in *S. comorensis* and *V. doniana* fruits indicates their potential pharmacological use value in the maintenance of human body health.

**List of abbreviations**

- DPPH: 1,1-Diphenyl-1-Picrylhydrazile
- UV-VIS: Ultraviolet visible spectroscopy
- WHO: World Health Organization

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