

Quantification of phenolics, flavonoids and antioxidant activity of *Tamarindus indica* from selected areas in Tanzania

MOURICE MBUNDE, ROBINSON H. MDEGELA[✉], H.S. LASWAI, F.P. MABIKI

Department Veterinary Medicine and Public Health, Faculty of Veterinary Medicine, Sokoine University of Agriculture. P. O. Box 3021, Morogoro, Tanzania. Tel.: +255 23 260 45 42; Fax.: +255 23 260 46 47, [✉]email: mdegela@suanet.ac.tz, rmdegela@yahoo.com

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Abstract. Mbunde M, Mdegela RH, Laswai HS, Mabiki FP. 2018. *Quantification of phenolics, flavonoids and antioxidant activity of Tamarindus indica from selected areas in Tanzania. Biofarmasi J Nat Prod Biochem* 16: 22-28. The objective of this study was to establish the quantities and antioxidant activity in fruits and leaves of *Tamarindus indica* L. collected from three agroecological zones of Tanzania represented by the Morogoro, Tanga, and Dodoma regions. Samples were examined for their total phenolic and flavonoid contents as well as their antioxidant activity. The total phenolic content showed a significant difference in all fruit and leaves extracts and ranged from 1994.4 ± 530.77 to 17874.67 ± 5234 mg GAE/100 g. Similarly, the total flavonoid content in tamarind leaf and fruit extracts ranged from 880 ± 609.45 to 11483.11 ± 2559.67 mg CE /100 g dry weight. There was a significant difference between the antioxidant activity in the leaf ($54.39 \pm 0.13\%$) and fruit extracts ($40.11 \pm 0.03\%$). Tamarind leaf extracts exhibited significantly higher radical scavenging activity than fruit extracts. The antioxidant activity in fruit extracts expressed in percentage ranged between $29.27 \pm 0.06\%$ and $40.11 \pm 0.03\%$, while in leaf extracts, the activity ranged from $22.33 \pm 0.08\%$ to $54.39 \pm 0.13\%$. The radical scavenging activity from Coastal leaf extracts had the highest activity, followed by Eastern leaf extracts and lastly, Central leaf extracts. The highest activity was shown in the fruit samples by Coastal fruit extracts, followed by Central fruit extracts, and Eastern fruit extracts were the least active. The values in the Ferric reducing power (FRAP) assay ranged between 6968 ± 3655.91 μ M Fe (II)/g and 76822.67 ± 23259.9 μ M Fe (II)/g for leaves and fruits dry mass, respectively. These values correspond to the antioxidant activity, positively correlated with the total phenolic and flavonoid contents. Geographical location and climatic conditions have been reported to have enormous effects on the amount and activity of antioxidants available in both tamarind leaves and fruits. Findings from the study indicated that tamarind could be utilized as a cheap source of antioxidants. However, more agronomic studies should be considered to confirm the effects of agroecological differences on antioxidant activity.

Keywords: Antioxidant activity, flavonoids, phenolics, *Tamarindus indica*

INTRODUCTION

Oxidation reactions that occur mainly in the human body are likely to produce free radicals, bringing about various disorders, including atherosclerosis, ischemia, arthritis, reperfusion injury of many tissues, gastritis, and cancer (Seal 2011). To protect the cells, organs, and systems of the body against the deleterious effects of free radicals, humans have a highly sophisticated and complex antioxidant protection system that functions interactively and synergistically to neutralize free radicals (Percival, 1996). Antioxidants prevent the oxidative damage caused by free radicals in the body, as they can react with free radicals, chelate catalytic metals, and act as oxygen scavengers. The antioxidant compounds in the body are primarily obtained from external sources, mainly through the consumption of fruits and vegetables. The need for a supply of antioxidants becomes even more critical with increased exposure to free radicals originating from external sources, such as exposure to x-rays, cigarette smoking, ozone, air pollutants, and industrial chemicals (Dimitrios 2006; Kumar 2011).

Since ancient times, humans have depended on natural sources, especially plants, to protect against the effects of various diseases and improve their lifestyles. With

technological advancement and recent research findings, it has been revealed that certain non-nutritive chemicals produced by plants, such as terpenoids, flavonoids, and other phenolic compounds, which were initially thought to be of no importance to human health, possess antioxidant properties (Seal 2011).

Antioxidant compounds have been searched in several types of plant materials, such as vegetables, fruits, leaves, barks, and roots, in the form of crude plant drugs. Polyphenolic compounds, which are dominant in antioxidant activity, are then found to be common in leaves, fruits, stems, and barks. In plants, these compounds are essential for normal growth development and defense against infection and injury (Seal 2012; Aires et al. 2013). Epidemiological reports proposed that dietary intake of natural products has proved to have a strong inverse correlation with the risk of developing cancers and coronary heart disease (Lako et al. 2007; Zidenberg-Cherr and Heneman 2008). Antioxidants in natural sources, especially fruits and vegetables, have created a high demand for natural products to control and treat various infections and diseases. Some chemically synthesized drugs claim to have undesirable side effects (Mayunzu et al., 2011).

Tamarindus indica L., commonly known as tamarind, has a long history in traditional medicine throughout Africa and Asia (El-Siddig et al., 2006; Lourith et al., 2009). In Tanzania, this species is increasingly being used by society for juice making or as a vegetable. Modern medical science has also confirmed its laxative and diuretic properties. All morphological parts of *T. indica* can be used, from the fruit pulp and seed to the leaves, bark, and flowers. Ailments such as diarrhea, ulcers, jaundice, eye infections, and digestive problems can be treated with infusions, pastes, and powders from *T. indica* (Khairunnuur et al., 2009; De Caluwé et al., 2010). Herbal practices are still widely used wherever *T. indica* is accessible (Rudrappa 2009).

Numerous studies have reported tamarind as having high levels of vitamins; A, B, and C and organic acids like citric, ascorbic acids, tartaric and malic, and polyphenols flavonoids. These compounds are primarily responsible for potent antioxidant, hepatoprotective, and antimicrobial activity (Lamien-Meda et al., 2008; Lourith et al., 2009; Rodríguez-Amado et al., 2012).

Many wild fruits and leaves contain significant amounts of antioxidant compounds, essential in preventing various diseases (Javanmardi et al., 2003). The properties of antioxidants are mainly brought about by polyphenolic compounds such as flavonoids, anthocyanins, phenolic acids, and phenolic diterpenes. *Tamarindus indica* L. is reported to contain many polyphenolic compounds with the potential for antioxidant activity (Pieta 1998). Nevertheless, the quantities of antioxidants may vary with geographical location (Aires et al., 2011; Mahmood et al., 2012). Despite the extensive utilization and availability of *T. indica* in most parts of Tanzania, little is known about the amount and activity of antioxidants from this plant.

Furthermore, there is limited information on comparative analysis of antioxidant compounds available in the wild tamarind from different agroecological zones of Tanzania. Thereby, this study was designed to fill the existing knowledge gap. Findings from the study would be useful in providing baseline information about the antioxidant and antioxidant capacity of *T. indica*.

The objectives of this research were: (i) To quantify the number of phenolics and flavonoid contents in leaves and fruit extracts of *T. indica* from the Coastal, Eastern, and Central zones of Tanzania. (ii) To determine the antioxidant activities of extracts from leaves and fruits of *T. indica* from the zones above Tanzania.

MATERIALS AND METHODS

Description of areas where samples were collected

This study involved sample collection from three different locations that fall in agroecological zones. Coastal zone (Tanga), Eastern Plateau and Mountain Blocks (Morogoro), and Central Plateau (Dodoma). The Coastal zone (Tanga) lies 500-1200 meters above sea level and has been developed over gneissic rocks. The region has poorly

drained, flat, broad topographical depressions developed on young alluvium and strongly dissected areas of pronounced slopes, often rocky and severely eroded. Two main types of soils are available: sandy clay loams and sandy clays, and sands and loamy sands. The region is mostly infertile and lacks moisture acceptance properties due to a tendency for surface sealing. It experiences bimodal rainfall ranging from 700-1200 mm per annum (USDA 2005; Handeni 2008).

The Eastern Plateau and Mountain block, which encompasses the Morogoro region (Mvomero district), exhibits undulating plains to dissected hills and mountains and moderately fertile clay soil. The area experiences unimodal rainfall ranging from 800 to 1400 mm (USDA 2005; Mbogoni and Ley 2008).

The Central Plateau (Dodoma region) has undulating plains with rocky hills and low scarps. Its soil is drained with low fertility. The rainfall is unimodal and unreliable, ranging from 500 to 800 mm (USDA 2005).

Study design

This observational study design was adopted whereby samples were collected and taken to the laboratory to extract and analyze antioxidants. Samples were collected from three villages, namely Misima (Tanga), Doma (Morogoro), and Ntyuka (Dodoma), purposively selected from the three zones. The collection of the samples was done purposively based on the availability of tamarind species with mature fruits in certain areas. In each region, five samples (leaves and fruits from five tamarind trees) were taken from one village. The basis for the selection of each area was the climatic condition, i.e., semi-arid, woodland, and coastal climatic conditions.

Materials

Equipment and apparatus

Whatman no. 1 filter paper, UV-visible spectrophotometer (UNICO VIS1200 Version SS-1.24, United Products and Instruments, Inc.), bench centrifuge, Buchner funnel, separating funnel (250 mL), beakers (250 mL), volumetric flask (5, 10, 25, 50 mL), measuring cylinder (5, 10, 25, 50 mL), conical flasks (25 mL), cuvettes, Eppendorf tips, micropipettes.

Chemicals and reagents

Methanol (CH_3OH), ethanol ($\text{C}_2\text{H}_5\text{OH}$), hydrochloric acid (HCl), Folin Ciocalteu Reagent (FCR), TPTZ (2, 4, 6-tripyridyl-s-triazine), iron sulphate heptahydrate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$), iron chloride (FeCl_3), sodium carbonate (Na_2CO_3), sodium hydroxide (NaOH), sodium acetate (CH_3COONa), sodium acetate buffer ($\text{C}_2\text{H}_3\text{NaO}_2 \cdot 3\text{H}_2\text{O}$), sodium nitrite (NaNO_2), aluminium trichloride (AlCl_3), standard Gallic acid, butylated hydroxytoluene (BHT), 1, 1-diphenyl-2-picryl-hydrazyl (DPPH), vitamin C (ascorbic acid) and catechin. All the chemicals used, including the solvents, were analytical grade and purchased from the University Suppliers.



Figure 1. Map of the study area where samples were collected

Methods

Collection of plant materials

Fresh tamarind leaves and ripened fruits were collected from several populations of tamarind species in the selected agroecological zones (Plate 1). The Global Positioning System (GPS) was used to mark the coordinates and photographs of the plant taken at each location.

Extract preparations

The separated leaves were air-dried under the shade at room temperature (30°C) and ground to a powder using a grinding machine. Ten grams of leaf powder (made from 2 g of individual sample) was extracted in 99.9% methanol for 48 hrs at 30-33°C. The fruits were peeled, and the pulps were separated from the seed (Ashafa et al., 2010). Ten percent pulp extract was prepared by soaking 10 g (made from 2 g of individual sample) of the fresh pulp in 100 mL of 99.9% methanol, then mixed thoroughly (Khairunnuur et al. 2009). The mixture was homogenized and allowed to stand for 48 hrs. Both fruit and leaf extracts were filtered through Whatman filter paper No.1. The filtrate was evaporated under reduced pressure at 40°C using a rotary evaporator. The crude extract obtained was stored at -20°C until further analysis (Ashafa et al. 2010).

Coding of samples

The samples collected from three different zones were coded, as shown in Table 1.

Table 1. Coding of samples collected from three zones

Code	Agro-ecological zone	Source of tamarind extract
LVMR	Eastern zone	Leaves
FRMR	Eastern zone	Fruits
LVDM	Central zone	Leaves
FRDM	Central zone	Fruits
LVTA	Coastal zone	Leaves
FRTA	Coastal zone	Fruits

Note: LVMR = Morogoro leaf extract; FRMR = Morogoro fruit extract; LVDM = Dodoma leaf extract; FRDM = Dodoma leaf extract; LVTA = Tanga leaf extract; FRTA = Tanga fruit extract

Determination of total phenolics content

The concentration of total phenolics was measured according to the previously described method (Velioglu et al. 1998) with some modification whereby the diluted aqueous solution of each extract (0.5 mL) was mixed with Folin Ciocalteu reagent (0.2 N, 2.5 mL). The mixture was incubated at room temperature for 5 minutes and then added with sodium carbonate solution (75 g/L in water, two mL). After incubation for 2 hours, the absorbance was read at 760 nm. A standard calibration curve was plotted using Gallic acid (0, 25, 50, 75, 100, 125, 150, 175, 200, 225, 250 mg/L). The measurements were expressed as mg of Gallic Acid Equivalents (GAE)/100 g of fruit weight.

Determination of total flavonoid contents

Total flavonoid content was measured using a colorimetric assay (Zhishen et al. 1999). One mL aliquot of catechin standard solutions (5, 10, 20, 40, 60, 80, 100, 120, 140, 160, 180, 200 mg/L) was placed in a test tube, then added with 4 mL of ddH₂O and 0.3 mL (5%) NaNO₂. After 5 min, 1.5 mL (2%) AlCl₃ was added to the test tube and shaken to homogenize. Five minutes later, 2mL of 1 M NaOH was added to the mixture and shaken well. The absorbance of the mixture, pink in color, was read by spectrophotometer at 510 nm versus the prepared standard. Total flavonoid content in the fruit extract was expressed as mg/100 g catechin equivalents (CE) (fresh weight basis). All samples were analyzed in triplicate.

Determination of 1, 1-diphenyl-1-picrylhydrazyl scavenging activity

Antioxidant activity of tamarind leaves and fruit extracts was determined using the stable DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging as described elsewhere with some modification (Mensor et al. 2001). A four mL of 0.1 mM DPPH solution was added into 2 mL of the solutions of Butylated hydroxyl toluene (BHT) in methanol at different concentrations (25, 50, 75, 100, 125, 150 mg/L). The mixtures were shaken vigorously and incubated at room temperature for 30 min. Next, 4 mL

of DPPH was added to 1 mL of sample diluted with 2 mL of methanol, and the mixture was shaken vigorously and allowed to stand for 30 min at room temperature. The absorbance was read at 517 nm wavelength using a UV-VIS spectrophotometer (UNICO VIS1200 Version SS-1.24) with Butylated hydroxyl toluene (BHT) was used as the reference. Lower absorbance values of the reaction mixture indicated a higher free radical scavenging activity. The solution was measured spectrophotometrically at 518 nm. The antioxidant activity (AA) was calculated as below: $AA\% = 100 - [(Absorbance\ of\ the\ sample - Absorbance\ of\ the\ blank) / (Absorbance\ of\ the\ control)] \times 100$ (Mensor et al. 2001).

Determination of ferric reducing antioxidant power (FRAP)

Ferric reducing antioxidant power (FRAP assay) was modified from the earlier study by Benzie and Strain (1996). The stock solutions included 300 mM acetate buffer (3.1 g CH_3COONa and 16 mL CH_3OOH), pH 3.6, 10 mM TPTZ (2, 4, 6-tripyridyl-s- triazine) solution in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ solution. The fresh working solution was used in a ratio of (10:1:1) by mixing acetate buffer, TPTZ, and $FeCl_3 \cdot 6H_2O$. The temperature of the solution was increased to 37°C before use. A volume of 100 μ L extracts/standard was placed in a test tube and diluted with 300 μ L of distilled H_2O , then 2.85 mL of the FRAP solution was added and incubated for 30 min. Readings of the colored product (ferrous tripyridyltriazine complex) were taken at 593 nm. The standard curve lies between 100 and 700 μ L $FeSO_4 \cdot 7H_2O$. Results were expressed in μ M Fe (II)/g dried mass and compared with that of catechin.

Data analysis

Results were obtained from three replicate experiments. For each variable, treatment means were subjected to Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (DMRT) of the CoHort CoStat software version 6.33. Significant differences were reported at $p < 0.05$.

RESULTS AND DISCUSSION

The antioxidant capacity of the different extracts derived from fruit and leaf and the ranking order for each assay are presented in Tables 2, 3, and 4 and Figures 2 and 3. The variation observed in the number of polyphenolic contents in fruits harvested from three different agroecological zones might be attributed to the series of complex biochemical reactions during fruit ripening. The complex reactions affected the formation of phenolics, anthocyanins, flavonoids, carotenoids, and other volatile compounds leading to the development of final characteristics and distinct flavors of mature fruit (Gull et al. 2012).

Total phenolics content

The underlying mechanism of the method used in the phenolic determination (Folin-Ciocalteu assay) is an oxidation/reduction reaction according to the redox

properties of antioxidant compounds that can react with the Folin-Ciocalteu Reagent (FCR), enhancing the measurement of phenolic concentration (Norshazila et al. 2010). Table 2 shows the phenolic content of tamarind extract and the ranking order for each extract.

This study demonstrated that phenolic compounds level in tamarind extracts from the three different agroecological zones varied significantly in both leaves and fruit extracts. In general, among the six sample extracts, the tamarind leaf and fruit extracts from the Coastal zone demonstrated the highest levels of phenolic content compared to the other zones. There was a high variation in phenolic content between tamarind morphological parts (i.e., fruits and leaves) in all cases. For instance, a tamarind leaf extract from the Coastal zone had a significantly higher concentration of phenolics (Table 2) than fruits from the same zone. The higher level of phenolics in tamarind fruits and leaves has not been reported as in the case of the present study except in seed extracts, as reported by Lourith et al. (2009). Depending on the solvent used, they found the contents to be approximately 713.24 mg GAE/100 g to 63,691 mg GAE/100 g. Compared to the present study, other groups also reported a low phenolics content in tamarind pulp (957.33 ± 13.20 g of GAE/100 g of fruit) (Lamien-Meda et al. 2008). Meanwhile, a study on the tamarind fruit done by Khairunnuur et al. (2009) reported lower levels of phenolic contents compared to the findings of this study, ranging from 19.21 ± 0.29 g GAE/100 g in seed and 2.14 ± 0.05 g GAE/100 g in fruit.

This study further showed a significant variation in the total phenolic content in the tamarind leaf extract from the Coastal and Central zones (Table 2). No significant difference was observed in the total phenolic content of the tamarind leaf extract from the Eastern (Morogoro) and Coastal (Tanga) zone, likewise for fruit extracts from samples collected in the Central (Dodoma) and Eastern zones. These findings suggest that tamarind leaves and fruits growing in Tanzania contain more phenolic contents than those reported elsewhere, suggesting that Tanzania *T. indica* could have potent antioxidant activity.

The presence of higher concentrations of phenolic compounds in leaves could be explained by the change in the biochemical composition in the later stages of fruit ripening, i.e., different phenolic acids condense to form complex phenolic compounds such as tannins and lignin (Gull et al., 2012). Tamarind trees sampled in this study mostly had only younger leaves, thus supporting the argument by Rodríguez-Amado et al. (2012). They reported that younger leaves of tamarind bear higher phenolic compounds since the plant needs to protect itself from predators' attacks. Plant extract containing high levels of phenolic compounds may scavenge free radicals such as superoxide anion radicals and peroxy radicals in the human body and protect human cells or tissues against oxidative stress (Norshazila et al. 2010).

Total flavonoid content

The distribution of total flavonoid content (TFC) in tamarind leaves and fruit extracts concerning geographical

regions is presented in Table 3. The level of flavonoids in tamarind leaf extracts except those samples collected from the Central zone were significantly higher than those of fruit extracts from all zones. In all extracts, fruits harvested from the Coastal zone had the lowest flavonoid content (Tables 3). Tamarind fruits extract from samples collected from the Central and Coastal zone did not show any significant differences in concentration of flavonoids among them. Likewise, the leaf extracts from samples collected from the Coastal and Eastern zones showed no significant difference in their flavonoid contents. However, a substantial difference in flavonoid content was found between the leaf and fruit extracts from the Coastal zone region, as demonstrated in Table 3.

The flavonoid content in the present study was higher than the values reported in other reports. Lamien-Meda et al. (2008) reported lower levels of flavonoids (2.18 ± 0.21 mg QE/100 g) in fruit methanolic extracts and (5.68 ± 0.10 mg QE/100 g) in fruit acetone extract tamarind growing in Burkina Faso. The presence of higher concentrations of flavonoid compounds in younger leaves compared to those in fully ripe fruits could be attributed to the fact that the later stages of fruit ripening exhibit different phenolic acids that may condense to form complex phenolic compounds such as tannins and lignin. Hence, due to changes in phenolic compounds with maturity, fully-ripe fruit possessed relatively lower amounts of total flavonoid contents (Gull et al. 2012). Concentrations of flavonoid compounds in younger leaves compared to the fruits may be attributed to the fact that flavonoids are responsible for color formation; therefore, most samples were found to have yellow and red colors (Plate 6), suggesting a higher level of flavonoids. The growing conditions could also influence differences in flavonoid contents of different samples of tamarind from different locations, the genetic make-up of the species, amount of precipitation, altitude, temperature, soil conditions, and availability of nutrients (Jaffery et al. 2003; Mahmood et al. 2012; Rodríguez-Amado et al. 2012). The above factors might have affected the concentration of flavonoids in fruit and leaf extracts tested by altering the composition of their phytochemicals.

On the other hand, it was detected that tamarind has a higher concentration of phenolics than flavonoids in both leaf and fruit extracts tested. Tamarind leaf extracts had significantly higher polyphenolic compounds (phenolics) and flavonoids than fruit extracts.

Radical scavenging activity

The DPPH test determines the antioxidant activity, which is based on the ability of the stable free radical 2,2-diphenyl-1-picrylhydrazyl to react with hydrogen donors, including phenols (Lamien-Meda et al. 2008). The bleaching of 2,2-diphenyl-1-picrylhydrazyl by a test compound represents its capacity to scavenge free radicals generated independently from any enzymatic or transition metal-based system. Antioxidant compounds available in sample extracts react with DPPH, a stable free radical, to convert it to 1,1-diphenyl-2-(2,4,6-trinitrophenyl)hydrazine (Ali et al. 2010).

The bleaching of DPPH solution regularly increases with increasing sample fruit and leaf extracts in a given volume, as shown in Figure 3. The bleaching action of antioxidant compounds like the solution (Lamien-Meda et al. 2008). The antioxidant activity of the samples tested showed variation over the tested samples (Figure 3). The results showed that scavenging activity decreased in the following order: LVTA<LVMR<LVDM<FRTA <FRDM <FRMR. This trend implied that tamarind growing in the Coastal zone had the highest reduction potential than in other zones. This observation reflected the concentration of phenolics and flavonoids observed in the sample extracts from the Coastal Zone.

There was a significant difference in the antioxidant activity between the extracts from the leaf ($54.39 \pm 0.13\%$) and that from the fruit ($40.11 \pm 0.03\%$). Radical scavenging activity observed in leaf and fruit extracts was correlated with the concentration of phenolics and flavonoids found in all the extracts, with a strong positive correlation between total phenolic content and radical scavenging activity ($R^2 = 0.923$). This correlation suggested that polyphenols are responsible for antioxidant activity. Lamien-Meda et al. (2008) underscored that variation in radical scavenging ability among the tamarind extracts over the regions can be brought by the difference in climate and solvent used.

Table 2. Total phenolics content (mg GAE/100 g) of tamarind leaf and fruit extracts collected from different agro-ecological zones in Tanzania (n = 6)

² Code	Agro-ecological zone	¹ Total phenolics content (mg GAE/100 g)
LVMR	Eastern zone	17874.67±5234 ^a
LVTA	Coastal zone	17799.25±4825.05 ^a
LVDM	Central zone	6144.6±2205.23 ^b
FRTA	Coastal zone	4755±1699.25 ^{bc}
FRMR	Eastern zone	2073.33±287.39 ^c
FRDM	Central zone	1994.4±530.77 ^c

Note: ¹Values are mean ± SD of 6 samples analyzed individually in triplicates. Values with superscripts significantly different at ($p < 0.05$) in leaf and fruits extracts. ²LVMR = Morogoro leaf extract; FRMR = Morogoro fruit extract; LVDM = Dodoma leaf extract; FRDM = Dodoma fruit extract; LVTA = Tanga leaf extract; FRTA = Tanga fruit extract

Table 3. Total flavonoid contents of tamarind leaves and fruit extracts from different agro-ecological zones in Tanzania (n = 6)

² Code	Agro-ecological zones	¹ Total flavonoid content (mg Ce 100/G Dry Wt)
LVTA	Coastal zone	11483.11±2559.67 ^a
LVMR	Eastern zone	9853.33±6588.47 ^a
LVDM	Central zone	3957.33±390.82 ^b
FRMR	Eastern zone	2146.67±107.7 ^{bc}
FRDM	Central zone	1088±294.23 ^c
FRTA	Coastal zone	880±609.45 ^c

Note: ¹Values are mean ± SD of 6 samples analyzed individually in triplicates. Values with superscripts significantly different at ($p < 0.05$) in leaf and fruits extracts. ²LVMR = Morogoro leaf extract; FRMR = Morogoro fruit extract; LVDM = Dodoma leaf extract; FRDM = Dodoma fruit extract; LVTA = Tanga leaf extract; FRTA = Tanga fruit extract

Ferric reducing activity

The ferric reducing antioxidant potential (FRAP) assay was applied to examine the free radical scavenging capacities and the lowering possibilities of the antioxidant constituents of the tamarind extracts. This assay is usually based on the reducing power of a compound (antioxidant) and measures the reduction of Fe^{3+} (ferric iron) to Fe^{2+} (ferrous iron). As the ferric ion is reduced to a ferrous ion, the values in the FRAP assay express the corresponding concentration of electron-donating antioxidants (Ali et al., 2010).

Table 4 displays the FRAP values of tamarind leaf and fruit extracts. The antioxidant activity was found to vary among the extracts from different agroecological zones of Tanzania. The trend for the decrease in FRAP values or reduction potential among the extracts was $\text{LVTA} < \text{LVMR} < \text{LVDM} < \text{FRTA} < \text{FRMR} < \text{FRDM}$. When comparing the FRAP values among the extracts, it was found that tamarind leaf extracts collected from the Coastal zone had the highest FRAP values ($p < 0.05$), followed by leaf extracts from the Eastern zone (Table 4).

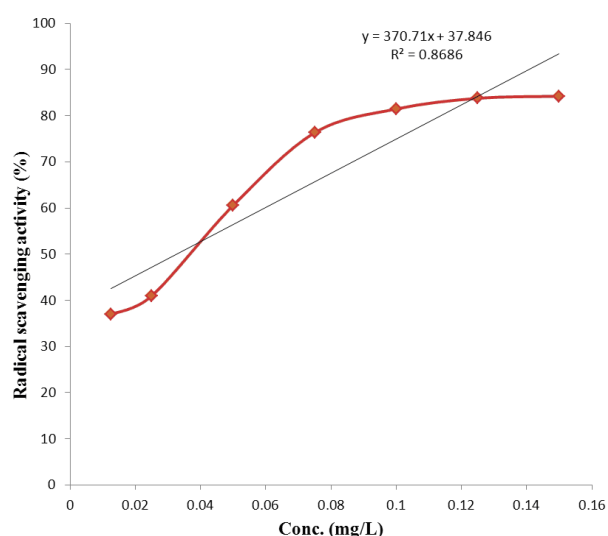


Figure 2. DPPH-free radical scavenging activity (RS %) of butylated hydroxytoluene (BHT) used as standard

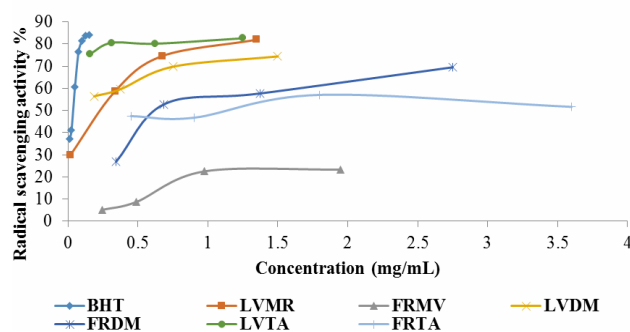


Figure 3. Effect of concentration of tamarind extracts in free radical scavenging activity. BHT = Positive Control; LVMR = Morogoro leaf extract; FRMR = Morogoro fruit extract; LVDM = Dodoma leaf extract; FRDM = Dodoma leaf extract; LVTA = Tanga leaf extract; FRTA = Tanga fruit extract

Table 4. Ferric Reducing Antioxidant Power (FRAP) values of tamarind leaf and fruit extracts from different Agro-ecological zones in Tanzania (n = 6)

² Code	Agro-ecological zones	¹ $\mu\text{M Fe (II)}/\text{g dry mass}$
LVTA	Coastal zone	76822.67 ± 23259.9^a
LVMR	Eastern zone	32776 ± 24506.66^b
LVDM	Central zone	8199.33 ± 2929.49^c
FRTA	Coastal zone	6968 ± 3655.91^c
FRMR	Eastern zone	5328 ± 2945.96^c
FRDM	Central zone	3860 ± 2377.57^c

¹Values are mean \pm SD of six samples analyzed individually in triplicates. Values with the superscripts significantly different at ($p < 0.05$) in leaf and fruits extracts. ²LVMR = Morogoro leaf extract; FRMR = Morogoro fruit extract; LVDM = Dodoma leaf extract; FRDM = Dodoma leaf extract; LVTA = Tanga leaf extract; FRTA = Tanga fruit extract

Leaf and fruit extracts obtained from the Central zone did not show any difference between them but showed a significant difference ($p < 0.05$) with the leaves from the Coastal and Eastern zone. There was a considerable difference in FRAP values ($76822.67 \pm 23259.9 \mu\text{M Fe (II)}/\text{g dry mass}$) in leaf extracts from the Coastal zone compared with FRAP values of fruit extracts from the same location. Total phenolic and flavonoid content correlates with ferric reducing antioxidant activity. These results agreed with Khairunnuur et al. (2009), who found a significant difference between FRAP values of tamarind fruit and seed extracts. The impact of geographical factors on the antioxidant property has been shown by variations in FRAP values observed among tamarind extracts obtained from the three agroecological zones.

This study showed that the amount and activity of antioxidants were higher for samples from the Coastal zone (moderate temperature) than for samples from the Central zone (extreme temperature). This indicated the effect of the agroecological zone on the amount and activity of antioxidants available in both tamarind fruits and leaves. The Coastal zone (Tanga) and Eastern zone (Morogoro) were observed to possess favorable factors that promote the production of phytochemicals in tamarind as compared to the Central zone (Dodoma). These two zones (Tanga and Morogoro region) share the same geographical factors: temperature, soil type, and rainfall (USDA 2005). According to Gull et al. (2012), moderate temperature conditions ($25/30^\circ\text{C}$) are suitable for increasing antioxidant content. The authors further argued that plants growing in extreme cold ($18/12^\circ\text{C}$) or hot (above 35°C) temperatures produce fruits and leaves with lower antioxidant content (Gull et al. 2012).

In conclusion, from the results, the amount of phenolics and flavonoid content of leaf extracts is higher than that of fruit extracts. Furthermore, there is variation in phenolics and flavonoid content amongst the three agroecological zones in which sample extracts obtained from the Coastal zone contained the highest amount of these polyphenols. Leaf extract from the Eastern and Coastal zone exhibited significantly higher antioxidant activity levels than extracts from the Central zone. Also, the leaf extracts showed higher radical scavenging activity than fruit extracts in the

following orders LVTA<LVMR<LVDM <FRTA<FRDM<FRMR, from high to low amounts, respectively. Antioxidant activity was positively correlated with the total phenolics and flavonoid contents. The polyphenols content and the mean DPPH and FRAP in all sample extracts differed significantly. This behavior is frequent in natural products due to variation that relates to climate, soil characteristics, and phenological stages of the plant, at the fructification stage. Phenolics and flavonoid compounds in leaves of the plants are higher in earlier growth stages, probably as a strategy of the plant to protect itself from the insects and predators' attacks.

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