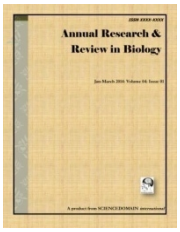

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Total Polyphenols, Flavonoids and Antioxidant Properties of Different Parts of *Tamarindus indica* Linn of Nigerian Origin

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Authors' contributions

Author SEA designed the study, wrote the protocol and interpreted the data. Authors JOO and UDI carried out the laboratory investigations, while author MLL managed the literature searches, managed the analysis of the study and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aim: To evaluate the polyphenols, flavonoids and the *in vitro* antioxidant activity of different parts of *Tamarindus indica* Linn.
Study Design: Methanolic extracts of the leaves, stem bark, root bark, fruit pulp, fruit bark and seeds of *Tamarindus indica* Linn were analyzed for their total polyphenol contents, flavonoid concentration and antioxidant activities in reference to Gallic acid equivalent (GAE), quercetin equivalent (QE) and Trolox equivalent (TE) respectively.
Place and Duration of Study: The study was done at the Department of Biochemistry, Faculty of Science, Ahmadu Bello University, Zaria, Kaduna State, Nigeria between September 2010 and December 2011.
Methodology: Total polyphenols and flavonoids concentration of the methanolic extracts were evaluated with the aid of a spectrophotometer using the Folin-ciocalteau's reagent and aluminum chloride reagent methods, respectively, while the antioxidant activity was evaluated by determining the radical scavenging activities of the extracts on 1, 1-diphenyl-2-picrylhydrazine (DPPH).
Results: The equivalent phenolics and flavonoids contents of the stem, fruit pulp and fruit bark (94±2.1 - 158±2.5 µg GAE /g and 27±1.0 - 39±0.7 µg QE /g respectively) were

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significantly (*P* = 0.05) higher than those of the seed, root and leaf (55±0.0 - 66±0.7 µg GAE /g and 21±0.7 - 17±1.0 µg QE /g respectively). The antioxidant activity of the stem, fruit pulp, fruit bark, seed, root and leaf were found to be 168±3.5, 143±3.5, 101±1.4, 83±3.5, 63±3.5 and 40±1.0 µg TE /g respectively. There was a strong positive correlation (r^2 = 0.97) between the polyphenols contents and the *in vitro* antioxidant activity.

Conclusions: These results suggest that different parts of *T. indica* possess high levels of polyphenols with significant antioxidant capacities to warrant further detailed studies on the possible roles of this property in their nutritional and health effects.

Keywords: Tamarindus indica; Polyphenols; Flavonoids; Antioxidant activity.

1. INTRODUCTION

Plants have been known to be an integral part of human food for centuries in the same way that early systems of medicine have largely depended on plant and plant products for the management of many diseases and ailments [1]. Even in the world today, there is renewed interest, not only in the nutritional importance of foods, but also in their health benefits [2]. This led to the opening up of the field of nutraceutical research; elucidating the health enhancing potentials of foods and the role constituents of foodstuffs play in disease prevention and management [3,4]. Plants owe their therapeutic potentials to the bioactive components, including, alkaloids, tannins, flavonoids and other compounds that are inherent in them [5], and hence, it not surprising that many of these health effects are also associated with various foods of plant origin. This is probably because plants have an almost limitless ability to synthesize aromatic substances, many of which are phenols or their oxygen substituted derivatives [3] that have been found very useful in combating oxidative – stress related diseases. Thus, a systematic search for useful bioactivities from medicinal plants is now considered to be a rational approach in nutraceutical and drug research.

Reactive oxygen species (ROS), which may be caused by exogenous agents or endogenously by products of normal cellular metabolism, at low to moderate concentrations, perform various physiological functions ranging from cellular signal transduction to defense against pathogens. However, during oxidative stress, there is an overproduction of ROS on one side and a deficiency of enzymatic and non-enzymatic antioxidant defense system on the other; resulting in degradation of cellular components, DNA, carbohydrates, proteins and lipids. This will eventually lead to cellular dysfunction and cell death. The involvement of free radicals in biological systems can lead to oxidative stress which plays a cardinal role in the pathogenesis of many degenerative diseases and also in the ageing process [2, 3]. Therefore, there is need to combat or mitigate this pathological condition using dietary constituents belonging to the general group, antioxidants.

Generally, antioxidants are antidotes for oxidative stress, and hence, nutritional antioxidants like ascorbic acid and tocopherols are known to complement the action of endogenous antioxidant enzymes. Similarly, polyphenols which are large family of natural compounds that are widely distributed in plant foods have been reported to have profound antioxidant abilities [2,4,6], because their considerable diversities make them different from other antioxidants. They include flavanoids, tannins, lignins and stilbenes among the major classes. Their antioxidant abilities contribute to the beneficial health effects of many vegetables and fruits [7]. One popular Nigerian and indeed tropical plant reputed for both its medicinal and culinary importance is *Tamarindus indica* Linn.

Tamarindus indicia Linn belongs to the dicotyledonous family, Leguminosae, sub-family Caesalpiniaceae, which is the third largest family of flowering plants with a total of 727 genera and 19,327 species [8]. *Tamarindus indica* fruit pulp is used for the preparation of beverages in different parts of the world, including the Northern parts of Nigeria and other West African countries. *Tamarindus indica* fruit contains high levels of carbohydrate, which provides energy and has good content of protein with many essential amino acids that help to build strong and efficient muscles. It is also rich in the minerals: potassium, phosphorus, calcium, magnesium and can provide small amounts of iron as well as vitamin A [8].

Tamarindus indica, Linn plant is widely used in traditional medicine in Africa and Asia for treatment of many diseases such as fever, dysentery, jaundice, gonococci and gastrointestinal disorders [4]. Pharmacological investigations on *T. Indica* fruit pulp, demonstrated them to have antibacterial, antifungal [9] and, cytotoxic [10] abilities. Antioxidant properties of the fruit pulp and the seeds of *T. indica* from Asia have also been reported [4,11]. Neither a concise data on the polyphenols contents of the other parts of *T. indica*, nor the relationship between the antioxidant potentials with the polyphenol contents, especially of the Nigerian variety is known, although the antioxidant potential of many natural products have been widely related to their polyphenols contents [4,6,12]. Thus, this study aims at wholesomely determining the total polyphenol and flavonoids content of the fruit pulp, the fruit bark, the stem bark, the roots, the seeds and the leaves of *T. indica* and establishes their respective *in vitro* antioxidant potential.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Gallic acid, Folin-Ciocalticeau phenol reagent and 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH), were purchased from Sigma Chemical Co. Ltd (USA). All other chemicals and solvents used in this study were of analytical grade and were acquired from BDH, Poole, England.

2.2 Collection of *Tamarindus indica* Linn

The various parts of *Tamarindus indica, Linn*, which include the leaves, stem bark, root bark, and whole fruit, were harvested from Zaria Local Government Area of Kaduna State, Nigeria. The plant was authenticated at the Herbarium Section, Biological Science Department, Ahmadu Bello University, Zaria where a Voucher No. 900265 was assigned for the sample deposited.

2.3 Preparation *of Tamarindus indica* Linn extracts

The ripened mature whole fruit was carefully peeled off to get the fruit bark and subsequently the seeds separated from the pulp. All the samples (fruit pulp, the fruit bark, the seeds, the stem bark, the roots, and the leaves) were separately dried at room temperature and pulverized using laboratory mortar and pestle. The pulverized samples were first defatted with petroleum ether for 8h and then extracted with methanol (4h x 2 times) using Soxhlex extractor. The methanolic extracts were then concentrated on water bath at 50ºC and further dried in air-tight containers containing desiccants to obtain the dry extracts. The extracts obtained were then stored in dark air tight bottles stored at 4ºC until analysis.

2.4 Determination of Total Polyphenol Content

Total polyphenol content was determined using the Folin reagent as described by Ayoola and coworkers [13]. Concentrations of 12.5, 25, 50, and 100 µg/ml of gallic acid were prepared in methanol for preparation of standard calibration curve. Concentrations of 0.1g/ml of plant extracts were also prepared in methanol and 0.5 ml of each sample was mixed with 2.5 ml of a ten-fold diluted Folin-Ciocalteau's reagent and 2 ml of 7.5% sodium carbonate. The mixture was allowed to stand for 30 min at room temperature before the absorbance was read at 760nm using a spectrophotometer. All determinations were performed in triplicates, and the total phenolic content was expressed in terms of gallic acid equivalent (GAE).

2.5 Determination of Total Flavonoids Content

The total flavonoids content (TFC) was estimated spectrophotometrically by the aluminum chloride method based on the formation of flavonoids-aluminum complex as described by Pharm and colleagues [14]. The sample (1 ml) was mixed with 1 ml of $AlCl_3$ in methanol (2% w/v) and incubated at room temperature for 15 min. The absorbance was then read at 430 nm, and the amounts of TFC were estimated from the standard calibration curve of 12.5-100 µg ml^{-1} quercetin, and hence, expressed in terms of quercetin equivalents (QE).

2.6 Determination of *in vitro* Antioxidant Activity

The scavenging activity of the extracts on 1, 1-diphenyl-2-picrylhydrazine (DPPH) was determined at 517nm using Trolox as standard following the procedure described by Atawodi and co-workers [15]. Briefly, an aliquot (50 µl) of either the sample extract or standard Trolox solution was added to 2 ml of methanolic DPPH solution (0.1 mM), 0.95 mL of 0.05 M Tris-HCl buffer (pH 7.4) and wrapped in aluminum foil to reduce influence of light. The absorbance was measured at 517 nm exactly 30 seconds after adding each of the extracts. A loss of absorbance at this wavelength was taken as a measure of the radical scavenging capacity of the extract added. The antioxidant capacity based on the DPPH free radical scavenging ability of the extract was expressed in terms of Trolox equivalent (TE) after extrapolation from a Trolox standard calibration curve of 20 to 200 µg g^{-1}.

2.7 Statistical analysis

All experiments were conducted in triplicates and results were expressed as mean ± SD. Analysis of variance (ANOVA) was used for statistical analysis using the Statistical Package for Social Sciences (SPSS) version 14 software. A value of P =0.05 was used to denote statistical significance.

3. RESULTS AND DISCUSSION

Figures 1-3 show the total polyphenol and flavoniods contents, as well as the antioxidant activities of the leaves, stem bark, root bark, fruit bark, seeds and pulp of *Tamarindus indica,* expressed as gallic acid, Quercetin, and Trolox equivalents respectively. Figure 1 shows the comparative polyphenols contents in the various parts analyzed. The stem bark, the pulp and the fruit bark had statistically significantly (P= .05) higher levels of total polyphenols contents; 158±2.5, 152±2.2 and 94±2.1 µg GAE /g respectively. These were followed by the

seeds (66± 0.7 µg GAE /g), root (60± 0.7 µg GAE /g), and the leaves (55±0.0 µg GAE /g) which had the least contents (Figure 1).

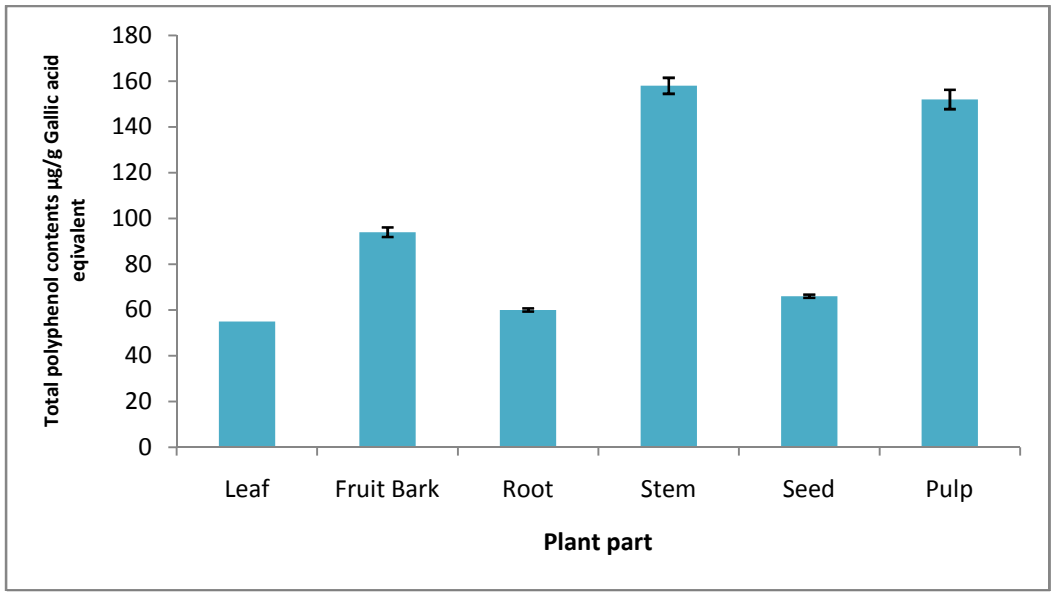

Figure 1. Total Polyphenol contents of various parts of *Tamarindus indica*
(Values presented are data from triplicate analyses expressed as Mean ± SD)

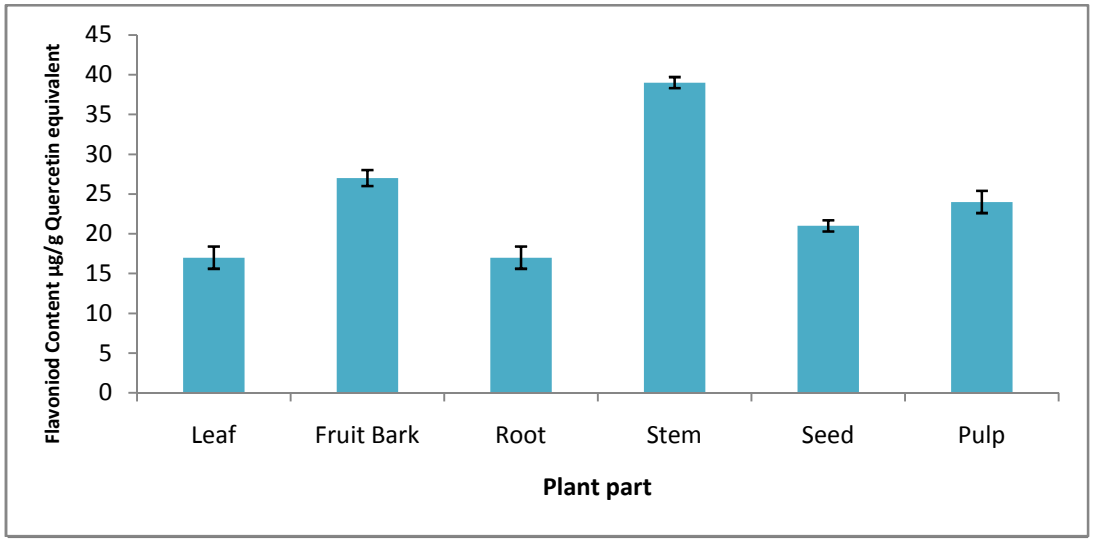

Figure 2. Total Flavonoids content of different parts of *Tamarindus indica*
(Values presented are data from triplicate analyses expressed as Mean ± SD)

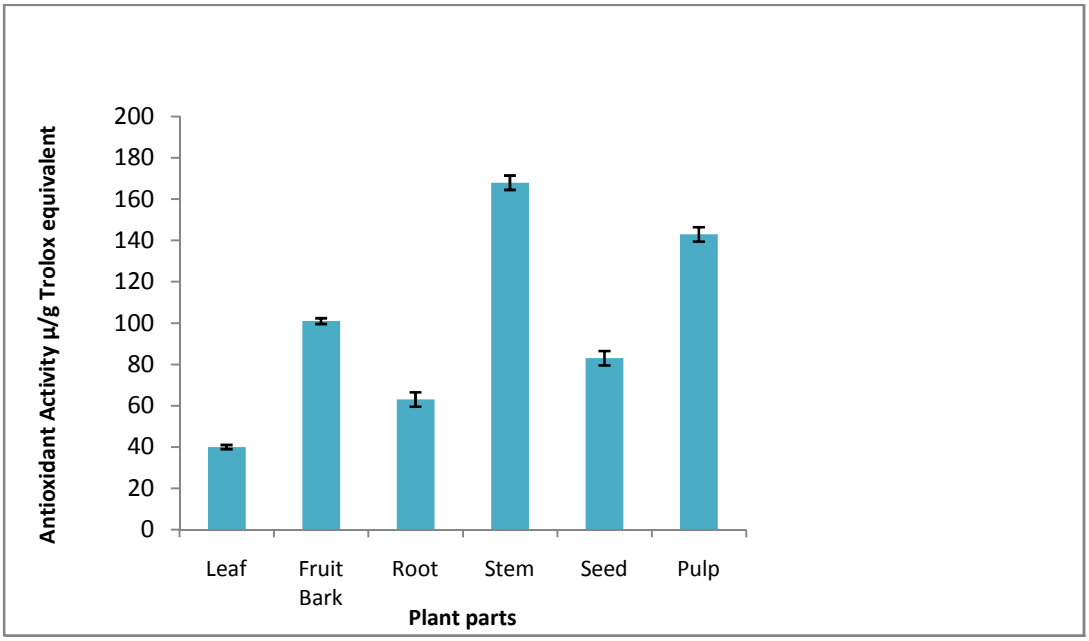

Figure 3. DPPH free radical scavenging activity of various parts of *Tamarindus indica*
(Values presented are data from triplicate analyses expressed as Mean ± SD)

The concentration of flavonoids on the other hand was highest in the stem bark (39±0.7 µg QE /g), followed by the fruit bark (27± 1.0 µg QE /g), then the pulp (24± 1.4 µg QE /g) and then the seed (21± 0.7 µg QE /g). The root bark and the leaves had the least amount of total flavonoids of 17±1.4 µg QE /g and 17± 1.0 µg QE /g respectively (Figure 2) and this level was statistically different (*P* = 0.05) from the levels in the seed and pulp, which in turn was statistically different (*P* =0.05) from levels in the fruit bark and the stem bark.

The *in vitro* antioxidant activity of the parts showed similar trends with that of the total polyphenol and the total flavonoids. Thus, the antioxidant activity of the stem extract (168±3.5 µg TE /g) and the fruit pulp (143±3.5 µg TE /g) were statistically (*P* =0.05) different from those of the seed (83± 3.5 µg TE /g), the root (63± 3.5 µg TE /g) and the leaf (40±0.0 µg TE /g) which had the least radical scavenging activity (Figure 3).

To further establish the relationship between the total polyphenols, the flavonoids contents and the antioxidant activity, a correlation analysis was carried out. Figures 4 and 5 clearly show a strong positive correlation between the antioxidant activity and the polyphenols contents (r^2 = 0.97) and between total flavonoids contents and *in vitro* antioxidant activity (r^2 = 0.77).

Generally, the analysis showed that the leaves, fruit pulp, fruit bark, seeds, roots and stems of the plant are rich in polyphenols and flavonoids (Figures 1 and 2). Although earlier workers have reported the presence of polyphenols, particularly flavonoids in some parts of the plant, particularly, the seeds [11] and the fruit pulp [4], this to the best of our knowledge, is the first comprehensive study comparing the presence of these antioxidant compounds in different parts of *Tamarindus indica* of Nigerian origin. Moreover, the values of polyphenols

found in the fruit pulp are in agreement with the values earlier reported by Khairunnur and coworkers [4].

The presence of polyphenols (Figure 1) and particularly, flavonoids (Figure 2), in the various parts of *T. indica* is of nutritional and medicinal significance. Polyphenols have been recognized as antioxidant agents, acting as free radical terminators [16] with therapeutic and physiological functions [12]. It has been reported that compounds such as flavonoids, which contain phenolic hydroxyl groups, are responsible for the radical scavenging and chelating effects [14] of many plants used in food and medicine [1]. The demonstrated capacity of *T. indica* to scavenge free radicals, thereby acting as an antioxidant is in line with similar reports on many polyphenol-rich foodstuffs and medicinal plants [17,18,19,20,21]. Such free radical scavenging potentials could chemoprevent oxidative stress–related diseases such as cancer, diabetes, hypertension, and other age-related disorders [22,23,24,25]. Hence *T. indica* which is widely available and used throughout the tropics for culinary and medicinal purposes could be utilized as a valuable food resource for natural antioxidant production.

The study reveals that the polyphenol and flavonoid contents varied between the different parts of *T. indica* (Figures 1 and 2). This variation of bioactive components of different parts of the same plant underscore the need to study all parts of a plant before any generalization is made on the plant's pharmacological and therapeutic potentials [26]. Nevertheless, the variation in the level of these antioxidant compounds has wide ranging medicinal implications. For instance, *T. indica* fruit pulp is widely used traditionally for management of jaundice and gastrointestinal diseases [4] which are known to progress with oxidative stress [27,28]. Thus, the high levels of polyphenols and flavonoids in the fruit pulp of the plant suggest that the therapeutic efficacy of this pulp in traditional medicine might be through free radical scavenging mechanism. This is consistent with the high polyphenol and flavonoid contents of the fruit pulp.

Nevertheless, the stem bark extract and the fruit pulp extract demonstrated the highest radical scavenging activity (Figure 3) while the leaves and the roots which had the lowest contents of polyphenols and flavonoids showed the least radical scavenging activity (Figure 3). Correlation analysis further reveals that a good correlation existed between polyphenol content (Figure 4) and antioxidant activity (r^2 = 0.97), and between total flavonoids contents (Figure 5) and antioxidant activity (r^2 = 0.77), suggesting strongly that the free radical scavenging capacity of *T. indica* extracts is directly related to the presence of polyphenols and related compounds. This positive correlation between the polyphenol content and the antioxidant activity is collaborated by similar findings on the phenolic contents of the fruit pulp and its ferric reducing antioxidant power (FRAP) [4], and between the seed coat and its strong antioxidant activity [10,11]. Experimental evidence has indeed proven that the main polyphenol components of *T. indica* seed pericarp is dominated by proanthcyanidins (73.4%) in various forms, namely (+)-catechin (2.0%), procyanidin B2 (8.2%), (-)-epicatechin (9.4%), procyanidin trimer (11.3%), procyanidin tetramer (22.2%), procyanidin pentamer (11.6%), procyanidin hexamer (12.8%) along with taxifolin (7.4%), apigenin (2.0%), eriodictyol (6.9%), luteolin (5.0%) and naringenin (1.4%). It was also reported that the polyphenol content of Tamarind seeds comprised only procyanidins, represented mainly by oligomeric procyanidin tetramer (30.2%), procyanidin hexamer (23.8%), procyanidin trimer (18.1%), procyanidin pentamer (17.6%) with lower amounts of procyanidin B2 (5.5%) and (-)-epicatechin (4.8%) [11,29].

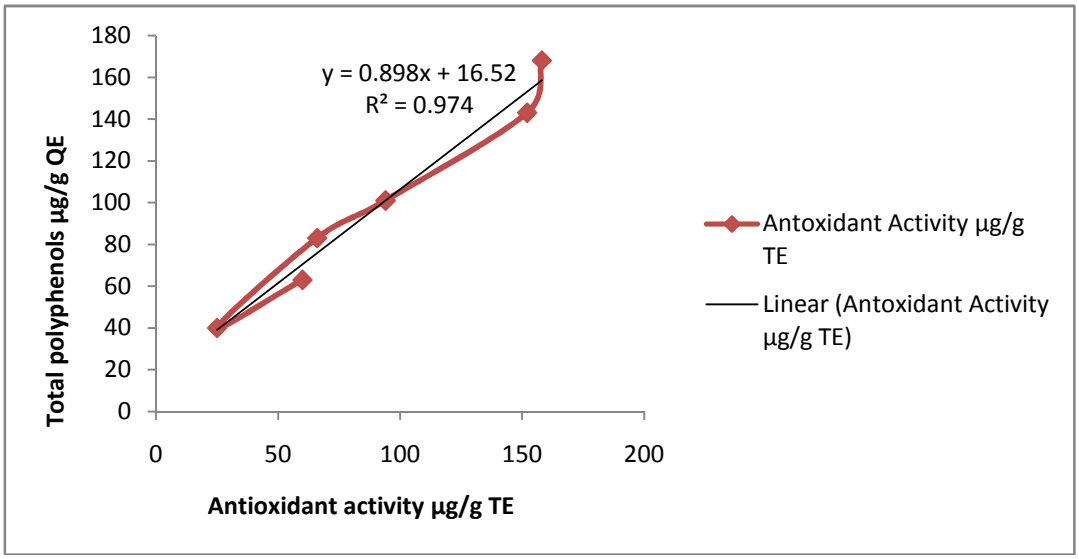

Figure 4. Relationship between total polyphenols and antioxidant activity of different parts of *Tamarindus indica*.

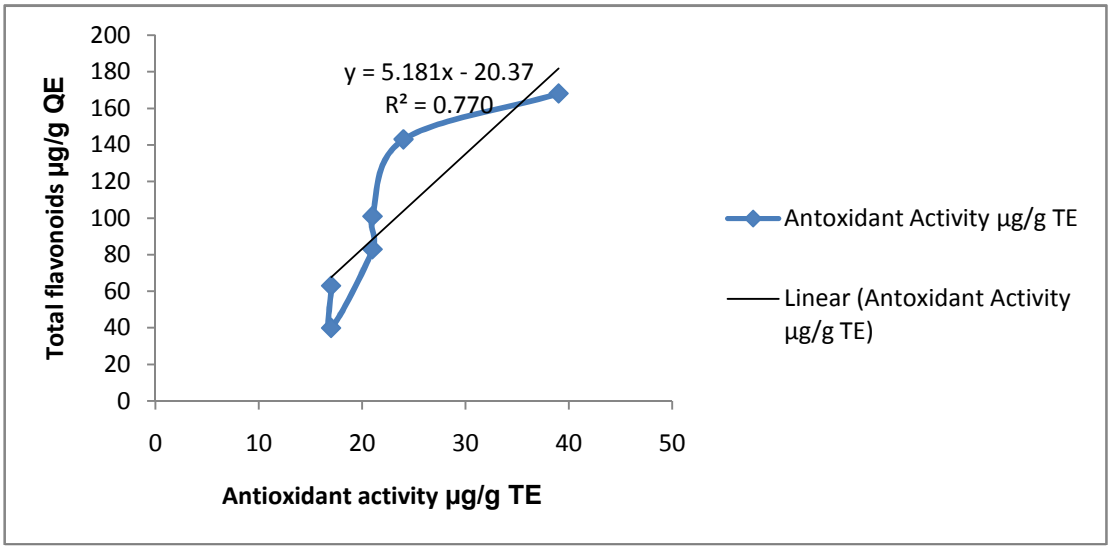

Figure 5. Relationship between flavonoids content and antioxidant activity of different parts of *Tamarindus indica.*

4. CONCLUSION

In conclusion, the high levels of total polyphenols and flavonoids in many parts of *T. indica* also suggest that this plant might potentially be an important source of cheap naturally occurring antioxidants that could be economically deployed as nutraceutical and health supplement. In addition, the various culinary applications of *T. indica* through routine food habits implies an increased antioxidant intake, thus contributing to improved nutritional and

health status of the populace. However, further investigations are required to establish the polyphenol profile of these parts and furthermore confirm the *in vivo* antioxidant effect of different parts of *Tamarindus indica* in animal models.

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COMPETING INTERESTS

Authors have declare that no competing interests exist.

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