PHYTOCHEMICAL EVALUATION OF *Terminalia catappa* Linn. FOR BIOMEDICINAL AND NUTRITIVE PURPOSES

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ABSTRACT

Terminalia catappa is a perennial tree species that has been introduced in gardens, parks and farmlands as an ornamental and shade tree, sand-dune stabilizer and source of fuelwood. This study assessed the qualitative and quantitative characteristics of phytochemicals present in the leaves and bark of T. catappa. It discussed the medicinal and nutritive potentials of the leaves and bark. Nine metabolites (alkaloids, flavonoids, saponins, tannins, anthraquinones, terpenoids, cardiac glycosides, phenols and steroids) were identified in the leaves while two of the metabolites were absent in the bark (anthraquinones and steroids). The alkaloids content (36.65% and 35.15%) was highest, followed by saponins (2.90% and 2.25%) and terpenoids (0.25% and 0.60%) in leaves and bark. Similarly, flavonoids (19.69 µg and 15.72 µg) were highest, followed by tannins (4.23 µg and 2.83 µg) and phenols (2.07 µg and 1.76 µg) in leaves and bark, respectively. The phytochemicals are capable of fighting oxidative stress and possess therapeutic properties that could be potentially explored in drug discovery.

Keywords: Plant metabolites, Phytochemicals, Medicinal plant, Tropical Almond

INTRODUCTION

Terminalia catappa Linn. is a member of the family Combretaceae, commonly grown in tropical and subtropical climes. It grows naturally in East and West Africa, West indies and across tropical and subtropical America (Orwa *et al.*, 2009; Brown and Cooprider, 2013). The species has been massively introduced to forests, gardens and parks as an ornamental plant, sand-dune stabilizer and shade-tree (Orwa *et al.*, 2009). *Terminalia catappa* is fast growing at the juvenile stage, flowering and fruiting within 2 - 3 years after planting (Brown and Cooprider, 2013). It is well adapted to areas with mean annual rainfall ranging from 1000 – 3500 mm and mean annual temperature from 13 - 36^{o}C. It grows in soil types ranging from saline to alkaline sand (Orwa *et al.*, 2009).

Terminalia catappa produces fruits containing tannic acid, corilagin, brevifolin-carboxylic-acid, beta-carotene, cyanidin-3-glucoside, ellagic-acid, gallic-acid, glucose and pentosans. At mature fruits are usually transformed from green through yellow to bright red or dark-purplish red (Jensen, 1995).

The leaves of *T. catappa* are used in the treatment of ailments such as hepatitis, diarrhea, pyresis, gastritis, urinary infection and dermatitis (Germosen- Robinean, 2014). The leaves were also used for treatment of eye problems, leprosy and different categories of cancer, as they exhibit anti-clastogenic, antioxidant and anti-cancer

properties (Allyn *et al.,* 2018; Silalahi, 2022). Extracts from different parts of the tree exhibit biological activities such as antifungal, antimicrobial, antioxidant, antimetastatic, hepatoprotective, antidiabetic, mutagenic and aphrodisiac properties (Pandya *et al.,* 2013; Mininel *et al.,* 2014).

The negative consequences of synthetic antioxidants, such as toxicity and carcinogenicity, have led to a high demand for natural antioxidants for nutraceutical, biomedicinal, and food additives (Mariod *et al.,* 2008). Tree species such as *T. catappa* could provide alternative sources of phytochemicals. This study determined the qualitative and quantitative characteristics of phytochemicals in leaves and bark of the species.

MATERIALS AND METHODS

Collection of Plant Materials

The leaves and bark of *T. catappa* were collected in May, 2021 from the Arboretum of Forestry Research Institute of Nigeria, Ibadan (FRIN). The specimens were identified at the Forestry Herbarium Unit of Forestry Research Institute of Nigeria, Ibadan, Nigeria. Fresh leaves and bark were cut into pieces and air-dried separately for 10 day.

Preparation of Extracts

Air-dried leaves and bark of *T. catappa* were milled and passed through a size 60 mesh screen sieve. In a Soxhlet apparatus, hot extraction method was used to obtain extracts, with methanol as solvent, at 55 - 85°C for 6 – 8 hours. Aqueous methanol is a good solvent for extraction of antioxidants because of the large range of phenolic compounds that can dissolve in it (Mbengul *et al*., 2013).

Qualitative Phytochemical Screening

Alkaloids

Extract (1 g) was diluted in 20 ml of ethanol, boiled and filtered. In 5 ml of the solution, 2 ml of dilute ammonia was added followed by 5 ml of chloroform. The mixture was gently shaken to extract the alkaloid base, and then the chloroform layer with 10 ml acetic acid. The alkaloid base was divided into three portions. Mayer's reagent was added to the first portion and a cream colour formation confirmed the presence of alkaloids. To the second portion, Dragendorff's reagent was added and a reddish brown precipitate, confirmed the presence of alkaloids. Also, Wagner's reagent was used to confirm the presence of alkaloids with a brownish yellow precipitate.

Tannins

Extract (0.5g) was mixed with 10 ml of distilled water, boiled and filtered. A dropper was used to add few drops of 0.1% ferric chloride into the solution. A blue black colouration indicated the presence of tannin.

Saponins

Extract (1 g) was added to 10 ml of distilled water and the solution was vigorously shaken. An observation of persistent froth confirmed saponins. To get a stable emulsion, 3 drops of olive oil was added to the mixture to further confirm the presence of saponins.

Terpenoids (Salkowski Test)
Chloroform (4 ml) was added to 1 g of extract followed by 3 ml of conc. H_2SO_4 to form a layer. The interface was observed for a reddish brown colour, indicating the presence of terpenoids.

Anthraquinones
Extract (0.5 g) was mixed with 10 ml of conc. H_2SO_4, boiled and filtered while warm. Then, 5 ml of chloroform was added and the mixture shaken, gently. The chloroform layer was transferred into another test tube after which 1 ml of dilute NH_3 was added. The solution was observed for a pinkish red colour change.

Cardiac Glycosides (Keller-Killiani Test)
Extract (0.5 g) was diluted in 5 ml of distilled water while in another test tube, a mixture of 2 ml glacial acetic acid and one drop of ferric chloride solution was made. The latter mixture was added to the former and the mixture stirred. This mixture was underlayed with 1 ml of conc. H_2SO_4 and the formation of a brown ring, at the interface indicated the presence of cardenolides.

Flavonoids
Three methods were used to test for flavonoids. First, diluted Ammonia (5 ml) was added to a portion of an aqueous filtrate of the extract. Then conc. H_2SO_4 (1 ml) was added. A yellow colouration that disappeared on standing indicated the presence of flavonoids. Second, a few drops of 1% Aluminium solution were added to a portion of the filtrate. A yellow colouration indicated the presence of flavonoids. Third, a portion of the extract was heated with 10 ml of ethyl acetate, over a steam bath for 3 mins. The mixture was filtered and 4 ml of the filtrate was mixed with 1 ml of diluted Ammonia solution. A yellow colouration indicated the presence of flavonoids.

Steroids
A few drops of Acetic anhydride were added to 1 ml of the extract, the mixture was boiled then cooled. Then, a few drops of conc. H_2SO_4 were added from the sides of the test tube. The formation of a brown ring at the junction of the two layers and a green ring at the upper layer, indicated the presence of steroids.

Phenols
Extract (0.5 ml) was dissolved in 5 ml distilled water and 3 drops of ferric chloride were slowly added. The green or blue colouration indicated the presence of phenols.

Quantitative Phytochemical Screening
Extracts from the leaves and bark were quantitatively assessed for phytochemicals using the spectrophotometric method.

Total Flavonoid Content
Total flavonoid content was determined using the Aluminium chloride colorimetric assay. Extract (1 ml) was mixed with 4 ml of distilled water in a 10 ml volumetric flask. Then, 0.30 ml of 5% sodium nitrite and 0.3 ml of 10% Aluminium chloride were added after 5 minutes. After another 5 minutes, 2 ml of 1 M Sodium hydroxide was treated and diluted to 10 ml with distilled water. A set of reference standard solutions were prepared by dissolving 3.2 mg in 95% ethanol and then dissolving in Quercetin (20, 40, 60, 80 and 100) μg/ml. The absorbance test and standard

solutions were performed as against the reagent blank at 510 nm using the UV/Visible spectrophotometer and expressed in mg of QE/g of extract.

Total Phenolic Content (TPC)

Following the method described by Marinova *et al*. (2005), the total phenolic content (TPC) was determined using Folin-Ciocalteu assay. An aliquot (1 ml) of extract and 9 ml of distilled water were mixed in a 25 ml volumetric flask, after which 1 ml of Folin-Ciocalteu reagent was added to the mixture before vigorous shaking. After 5 minutes, 10 ml of 7% sodium carbonate solution was added to the mixture and made up to 25 ml. A set of reference standard solutions of Gallic acid were prepared in the same manner (80, 120, 160, and 200 μg/ml). Using distilled water, a blank reagent was prepared, 1 ml of Folin-Ciocalteu phenol reagent was added and the mixture, shaken. After incubation for 75 minutes, at room temperature, the absorbance against the blank reagent was determined at 550 nm with UV-Visible spectrophotometer and expressed in mg Gallic acid equivalents (GAE).

Determination of Tannin Content

Folin - Ciocalteu assay method was used to determined tannin content (Singh *et al*., 2012). Extract (1 ml) was mixed with 7.5 ml of distilled water and 0.5 ml of Folin - Ciocalteu phenol reagent. After 5 mins, 1 ml of 35 % Na_2CO_3 solution was diluted in 10 ml of distilled water and added to the mixture. The mixture was shaken and incubated at room temperature for 30 mins. A set of reference standard solutions of Gallic acid (32, 63, 125, 250, 500 and 1000 μg/ml) were prepared in the same manner described. Absorbance for test and standard solutions were measured against the blank at 725 nm with a UV/Visible spectrophotometer and expressed in mg of GAE /g of extract.

Determination of Total Alkaloid Content

Powdered samples (5 g) were mixed with 200 ml of 20% Acetic acid in a 250 ml beaker. The solution was covered and left to stand for 4 hours. The solution was then filtered and place in a water bath for 30 mins. Concentrated ammonium hydroxide was then dropped into the solution till precipitation was complete. The solution was allowed to settle, and the precipitate was separated by filtration, the filtrate was weighed and expressed as shown in eqn. 1:

$$Total\ Alkaloids\ (\%) = \frac{weight\ by\ residue}{weight\ of\ sample\ taken} \times 100 \ldots (1)$$

Determination of Total Saponin Content

Saponin quantification was achieved based on the method of Koomson *et al*. (2018). Samples (5 g) were measured and placed in a 250 cm^3 conical flask and 100 cm^3 of 20% aqueous ethanol was added. The mixture was placed in a hot water bath at 55°C with continuous stirring for 4 hours. Separation was done through filtration and the residue re-extracted a second time before the mixture was evaporated to 40 cm^3 over a water bath at 90°C. To the concentrate, 20 cm^3 of diethyl ether was added in a separator funnel and the mixture was vigorously shaken. The aqueous layer was recovered while the ether layer was discarded. This purification process was repeated twice. Then, n-butanol (60 cm^3) was added and extracted twice with 10 cm^3 of 5% sodium chloride. After discarding the sodium

chloride layer the remaining solution was heated in a water bath for 30 mins, after which the solution was transferred into a crucible and oven dried to a constant weight. The saponin content was determined as a percentage (eqn. 2):

$$Saponin\ (\%) = \frac{weight\ of\ saponin}{weight\ of\ sample} \times 100 \quad \ldots (2)$$

Determination of Total Terpenoid Content

Total terpenoid content was determined by soaking 100 g of powdered samples in 9 ml of ethanol for 24 hours (Indumathi *et al.*, 2014). The extract was filtered and extracted with 10 ml of petroleum ether using a separating funnel. The ether extract was separated in pre-weighed glass vials and dried (final weight). Ether was evaporated and the yield (%) of total terpenoids content was measured using eqn. 3:

$$Terpenoid\ (\%) = \frac{final\ weight\ of\ sample - initial\ weight\ of\ sample}{weight\ of\ sample} \times 100 \ldots\ldots (3)$$

RESULTS AND DISCUSSION

Qualitative Phytochemical Screening

Nine (qualitative) and seven (quantitative) secondary metabolites were each identified in the leaves and bark of *T. catappa*, respectively (Table 1). Flavonoids have beneficial anti-inflammatory effects that protect cells from oxidative damage which could lead to diseases. Terpenoids have a broad range of biological activities and possess dietary antioxidants that could prevent the progress of cardiovascular disease, diabetes and cognitive diseases such as Alzheimer's and dementia (Atanasov *et al*., 2015). Flavonoids were abundantly present in both leaves and bark of the tree species while traces of terpenoids were found in both plant parts of the species (Rajesh *et al.*, 2016). Alkaloids were in the leaves and bark but the bark had no trace of anthraquinones. Gayathri *et al*. (2019) also confirmed the presence of alkaloids in the leaves of *T. catappa,* while Rajesh *et al.* (2016) confirmed it in both leaves and bark. Alkaloids and anthraquinones have anti-inflammatory, analgesic and stimulant properties, although small dosages are recommended, to avoid food toxins (Kurek, 2019).

Tannins and phenols were abundant in the leaves while the bark had moderate presence of phenols. Tannins are known to accelerate blood clotting, reduce blood pressure, decrease lipid level and adjust immune-responses. Phenols are extremely toxic to humans, when taken orally. Hence, there are reports of anorexia, weight loss, diarrhea, vertigo, black urine colouring, blood and liver damage when humans are repeatedly exposed to phenols (EPA, 2000).

Steroids influence endocrine functions such as sexual differentiation and reproductive life. They also maintain salt and sugar metabolism in the human body. Saponins exhibit antimicrobial properties that prevent oxidative stress. They help to lower cholesterol in animals and humans (El-Aziz *et al*., 2019). Saponins and steroids were abundant in the leaves, while the bark did not have steroids. This is at variance with Gayathri *et al*. (2019), who reported the absence of saponins in the leaves of the same species. Botelho *et al.* (2019) also identified cardiac glycosides in the leaves and bark of

T. catappa. This metabolite has an effect on the neural tissues and electrical activities of the heart. The presence of phytochemicals in plant parts is dependent on the location/source of plant material, solvent used for extraction and extraction method (Katiki *et al.,* 2017).

Quantitative Phytochemical Screening

Alkaloid content had the highest yield (leaves: 36.65% and bark: 35.15%) (Figure 1). Saponins were 2.9% (leaves) and 2.25% (bark), while terpenoids were least (0.25% and 0.60% respectively) (Figure 2). Alkaloids, flavonoids and saponins have been previously reported to be in high amounts in both plant parts (Mbengul *et al*., 2013; Ajiboye *et al.,* 2016). This suggests that the species has potentials for use in management of oxidative damage induced by malaria parasite, although caution should also be taken during ingestion due to their stimulant activities (Ajiboye *et al*., 2016; Kurek, 2019).

Total Tannin Content

The standard calibration curve of the Gallic acid (y = 0.0022x – 0.0565) showed linearity in the range of 32 - 1000 µg/ml with a coefficient of determination (R^2) of 0.99 and a maximum absorbance of 2.08 (Figure 3). The tannin content of leaves (4.23 mg/g) was significantly higher than that of the bark (2.83 mg/g) with percentage values approximately 2.46% and 2.29% of every 1000mg sample (Figure 2). The results obtained were lower than that of Ajiboye *et al*. (2016), but higher than that of Azrul *et al.* (2014). Tannins found in small quantities are often referred to as anti-nutrients in diets and possess some level of astringency (Chikezie *et al*., 2008).

Total Phenolic Content

The standard calibration curve deduced a Gallic acid linearity of (y = 0.0124x –1.0827) within a range of 80 – 200 µg/ml with a coefficient of determination (R^2) of 0.96 and a maximum absorbance of 1.525 (Figure 4). Phenol content of leaves (2.07 mg/g) was slightly higher than that of bark (1.76 mg/g) with both values being approximately 1.5% of 1000 mg sample. The results were higher than those reported by Azrul *et al.* (2014) and Tizhe *et al.* (2016). Considering the toxicity of phenols and the fact that plants metabolize it easily, exposure through ingestion should be minimal and with caution. Nevertheless, the phenolic content was minimal and lower than the maximum recommended daily intake (EPA, 2000).

Total Flavonoid Content

The flavonoid content of leaves (19.69 mg/g) was significantly higher than that of bark (15.72 mg/g) with percentage values approximately 4.5% and 3.8% of every 1000 mg of samples, respectively. The standard calibration curve of Quercetin (y = 0.0012x + 0.0894) showed linearity in the range of 20 – 100 µg/ml with a R^2 of 0.98 and a maximum absorbance of 0.210 (Figure 5). The results were higher than that reported by Ajiboye *et al*. (2016), which was 8.04 mg/g for leaf extract. Flavonoids help to eradicate radicals, fight allergies and tumours. They aid metabolism and repair after ingestion. However, the concentration of flavonoids in

Table 1: Qualitative phytochemical screening of *Terminalia catappa* leaves and bark

Phytochemical	Leaves	Bark
Saponins	++	+
Tannins	++	++
Flavonoids	++	++
Cardiac glycosides	+	++
Anthraquinones	+	-
Terpenoids	+	+
Steroids	++	-
Phenol	+	+
Alkaloids	++	++

+ : present; - : absent; ++: abundantly present

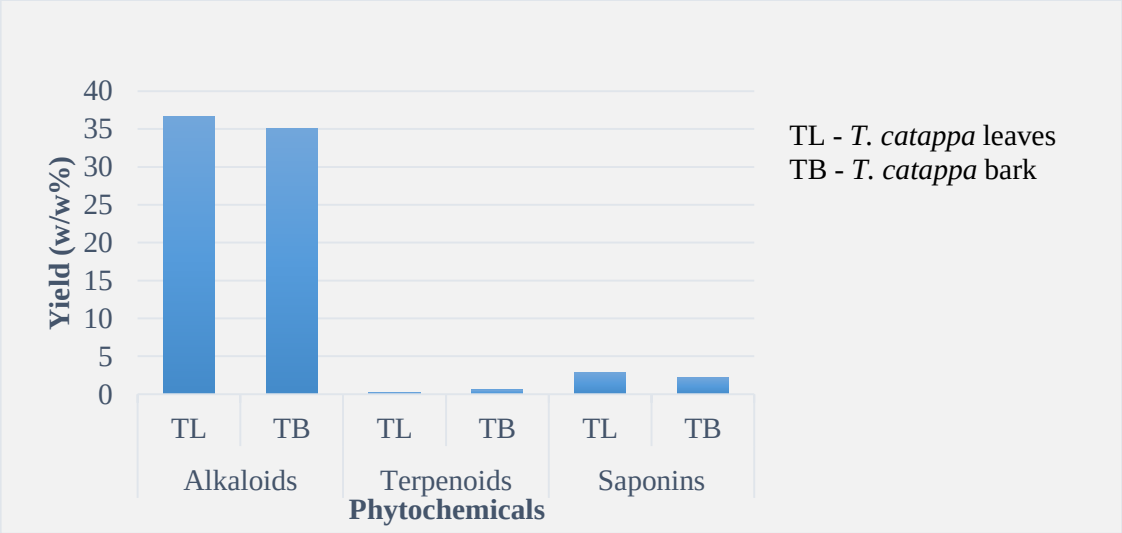

Figure 1. Yield of alkaloids, terpenoids and saponin in *Terminalia catappa* extracts

plant extracts depend on the polarity of solvents used during extract preparation (Rajesh *et al.*, 2016). Other species such as *T. avicennioides* have lower contents of saponins, flavonoids, alkaloids, glycosides and tannins (Chiroma *et al.*, 2018). Tizhe *et al*. (2016) reported that phytochemical quantification of *T. mantaly*, revealed that absorbance equivalence were comparable while extraction procedures were distinctively different. Also, *T. arjuna* bark was reported to contain larger amounts of flavonoids capable of boosting its antioxidant and antimicrobial activity (Shreda *et al.*, 2013).

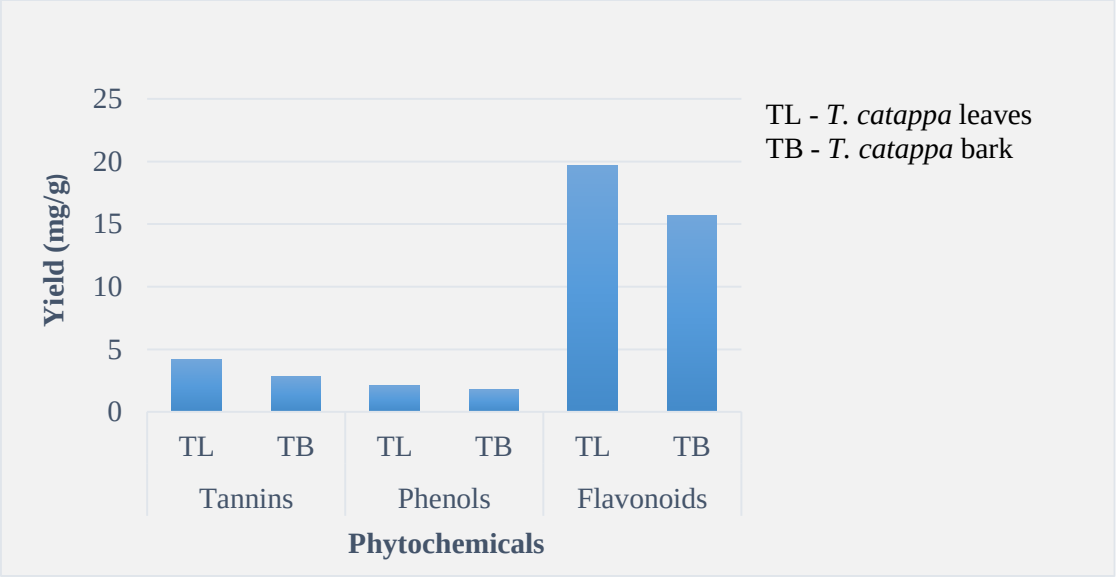

Figure 2. Yield of tannins, phenols and flavonoids in *Terminalia catappa* extracts

CONCLUSION

This study identified beneficial metabolites in the leaves and bark of the species. The higher quantity of alkaloids, flavonoids and saponins and lower quantity of phenols suggest the potential ability of the plant to protect against oxidative stress triggered by free radicals. Plants with beneficial phytochemicals can serve as natural antioxidants to complement human requirements making it imperative for the identification and quantification of these natural compounds. It is recommended that further studies be carried out to understand the biochemical nature of these secondary metabolites.

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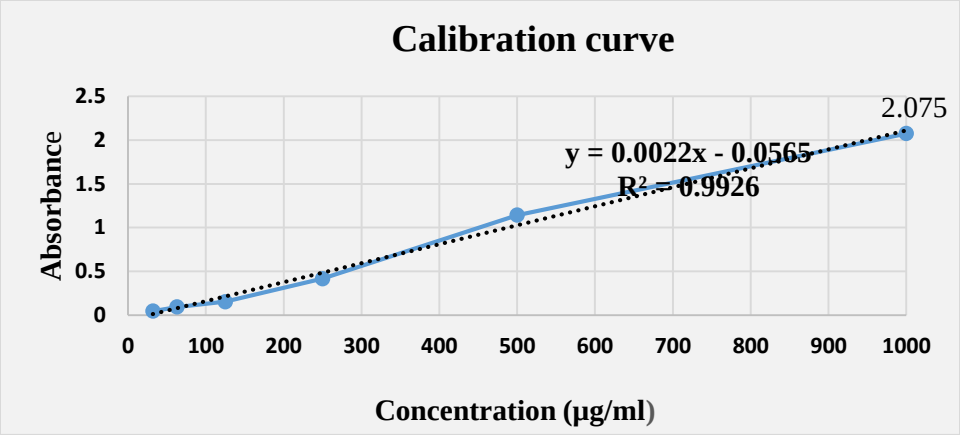

Figure 3. Calibration curve for total tannin content of *Terminalia cattapa* extracts

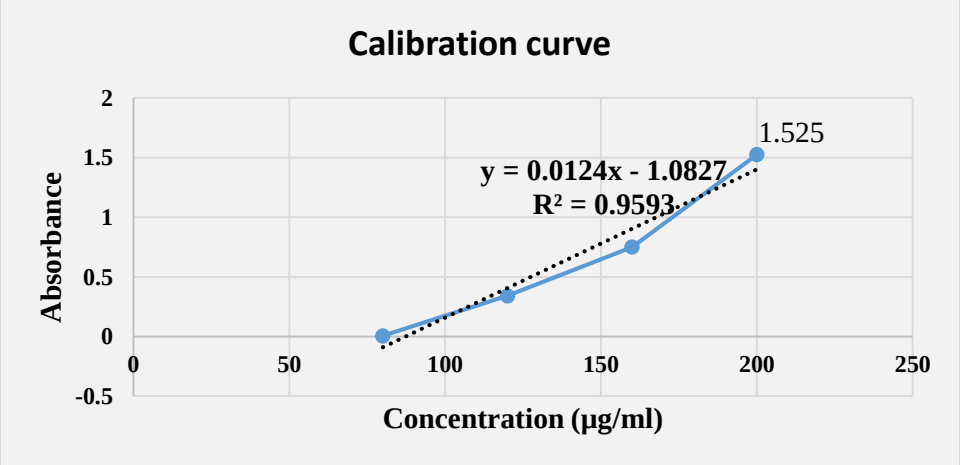

Figure 4. Calibration curve for total phenolic content of *Terminalia cattapa* extracts

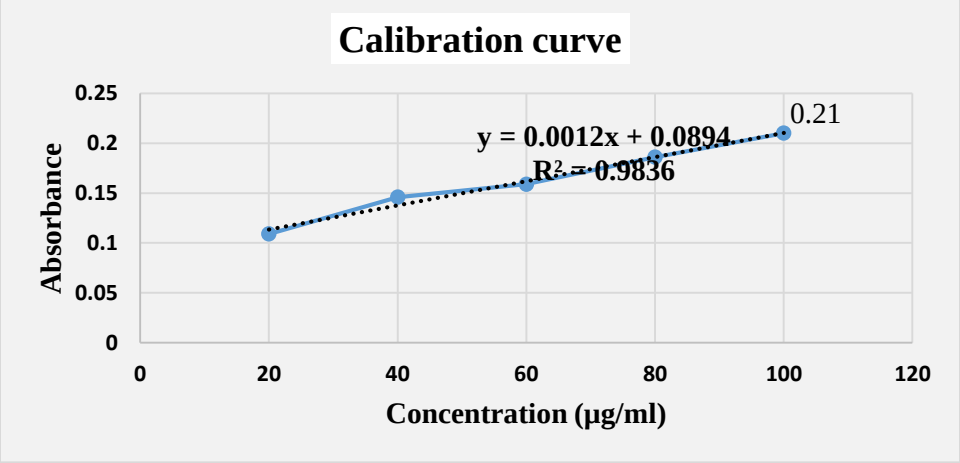

Figure 5. Calibration curve for total flavonoid content of *Terminalia cattapa* extracts