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# PHYTOCHEMICAL SCREENING AND *IN-VITRO* ANTIOXIDANT PROPERTIES OF THE STEM BARK OF *TRICHILIA TESSEMANNII* (HARMS) (MELIACEAE).

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## ABSTRACT

This study has investigated the phytochemical constituents by screening the pulverized plant material and the antioxidant potential of the stem bark of *Trichilia tessemannii* (Harms) (Meliaceae). The phytochemical investigation revealed the presence of plant secondary metabolites such as; tannins, reducing sugars, anthraquinones, alkaloids, saponins, glycosides, cardiac glycosides, terpemoids, coumarins and phytosterol. Antioxidant activity was determined by the use of DPPH free radical scavenging assay, total phenol content assay and total antioxidant capacity assay on the total crude methanol extract of the stem bark. The total crude methanol extract was found to possess DPPH free radical scavenging activity of $EC_{50}$ 0.02078 mg/ml, a total antioxidant capacity of 444.7 ± 28.58 mg/g equivalent of ascorbic acid and a total phenol content of 970.2 ± 78.69 mg/g of tannic acid equivalent. The implication of this study suggests that the stem bark of *Trichilia tessemannii* has major phytochemical principles and also has a good natural antioxidant source that may be indicated in the treatment and management of diseases associated with oxidative stress.

**KEYWORDS:** phytochemicals, *Trichilia tessemannii*, Total antioxidant capacity, Total phenol content.

## 1. INTRODUCTION

*Trichilia tessemannii* is among the eighteen (18) African species of trichilia. With over ninety (90) species found worldwide, trichilia is known to have interesting phytochemical principles that produce very complex chemical structures. The distribution of *Trichilia tessmannii* (Harms) is nearly the same as that of *Trichilia monadelpha* (Thonn) JJ de wilde.[1] *Trichilia tessmannii* (Harms) is a medium-sized forest tree with a height of about 30 m. It has a straight cylindrical base of about 70 cm in diameter bearing a dense crown. The leaves are pari-pinnate with opposite leaflets with soft orange hairs beneath. The fruit is a three (3) chambered, sub-globbose, stalked capsule of a size of about 3 cm across. The fruits are pinkish to purple or purplish red in color. There are two (2) seeds in each chamber of the fruit. This tree is easily recognized by the grayish and slightly scaly back which peels off in plates. The older trees slowly exude a little sweet-scented creamy or yellowish latex.[2,3] The medicinal value of plants resides in the bioactive phytochemical constituents produced by plants.[4] Phytochemicals are naturally occurring compounds produced by plant and are used by plants to perform important biological functions to sustain plant life.[5] These pytochemicals may be grouped into primary (e.g. sugars, amino acids, proteins and chlorophyll) and secondary (e.g., alkaloids, tannins, terpenoids and phenolic compounds) phytochemical.[6] Current research has indicated that medicinal plants possessing constituents such as phenols, coumarins, vitamins (vit. C and vit. E), flavonoids and terpenoids are major sources of potential antioxidant agents.[7]

Oxygen is a very important molecule used by majority of living organisms for their existence. Oxygen however is a very reactive molecule that could damage healthy living cells by producing reactive oxygen species (ROS) or free radicals.[8] Irrespective of the significant physiological role played by these reactive species in the living organism, the tendency to cause havoc such as death to healthy cells under uncontrollable circumstances, is a matter of deep concern to science.[9] These free radicals have been known to be associated with some chronic and degenerative diseases such as cancer, aging, alzheimer's disease, parkinson's disease, coronary heart disease, diabetes and inflammation.[10] Therefore the search for agents with the capacity to enhance the resolution of these issues is very paramount. Antioxidants have been found to avert these problems. These molecules or agents prevent the reactive oxygen species from being formed on one hand and on the other, are able to eliminate these ROS before harm is caused to vital components of the living organism.[11,12]

During these recent years, plant-derived antioxidants have received enormous attention and so there is continuous search into medicinal plants to identify new and potent antioxidant agents.[13] In spite of the numerous folkloric claims and uses of *Trichilia tessemannii*, there have not been any reported scientific biological activities on this plant. This research therefore seeks to identify the phytochemical principles in the pulverized stem bark and then to determine the antioxidant potentials of the total crude methanol extract of *T. tessemannii*.

## 2. MATERIALS AND METHODS

### 2.1 Materials

The following drugs and chemicals of analytical grade were employed in the research; Diphenyl-picryl-hydrazyl (DPPH) (Sigma Aldrich USA), Tannic acid (Fluka, U.K), Disodium phosphate, Ammonium molybdate, Folin-ciocalteu reagent and Ascorbic acid (all from BDH, Chemical Laboratory, England, U.K).

### 2.2 Plant material collection and authentication

The stem bark of *T. tessemannii* collected from Asakraka-Kwaku (Eastern region, Ghana) in January, 2013 was authenticated at the Department of Herbal Medicine, Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology (KNUST), assigned the Voucher specimen No. KNUST/T.T/2013/S005 and deposited at the Department's herbarium.

### 2.3 Preparation of the stem bark extract

The stem bark was washed with water after dead cell scraping, chopped into smaller pieces for air-drying at room temperature for 7 days and coarsely powdered. 200 g of pulverized plant material was serially extracted by soxhlet extraction with 500 mL each of petroleum ether, ethyl acetate and methanol for 48 hours. The obtained extracts were filtered and evaporated to dryness using a rotary evaporator (R-114, Buchi, Switzerland) at reduced temperature and pressure. The concentrates were further dried to obtain a yield of 10.53% total crude methanolic extract that was used in the preliminary antioxidant screening.

### 2.4 Qualitative phytochemical screening

The pulverized plant materials were subjected to preliminary phytochemical screening for the identification of secondary plant metabolites using standard procedures described by Evans[14] and Sofowora.[2] Below are brief descriptions of the methods used for the detection

of the various secondary plant metabolites such as saponins, tannins, alkaloids, and glycosides.

### 2.4.1 Tannins

About 0.5 g of pulverized plant material was extracted with 20 ml of boiling water for 5 minutes, cooled and filtered.1 ml of the cooled filtrate was diluted to 10 ml with distilled water and 5 drops of 1 % lead acetate solution added and observed for the presence of a white precipitate.

### 2.4.2 Reducing sugars

About 0.2 g of plant material was extracted by warming with 5 ml dilute HCl on a water bath for 2 minutes. The mixture was filtrated and the filtrate made distinctly alkaline with several drops of 20 % NaOH. 1 ml each of Fehling's solution A and B were added to the alkalized filtrate and heated on a water bath for 2 minutes while observing for the formation of a brick-red precipitate.

### 2.4.3 Saponins

About 0.2 g of pulverized plant was shaken vigorously with about 10 ml of water in a stoppered test tube and observed for the presence of a persistent froth. .

### 2.4.4 Anthraquinones/ anthracene glycosides

About 0.2 g of the pulverized plant material was boiled with 2 mls of dilute $H_2SO_4$ and 5 % aqueous $FeCl_3$ for 5 minutes. The mixture was filtered hot, cooled and shaken with an equal volume of chloroform. The chloroformic layer was separated and shaken with half its volume of dilute ammonia solution and observed for the formation of a rose-pink coloration.

### 2.4.5 Cyanogenetic Glycosides

0.2 g of the pulverized plant material was place in a conical flask and moistened with a few drops of water. A strip of sodium picrate paper was suspended by means of a cork in the neck of the flask and warmed gently on a water bath. The change in color of the test paper was observed to indicate the presence of cyanogenetic glycosides.

### 2.4.6 Cardiac glycosides

About 0.5 g of the pulverized plant material was extracted with 70 % alcohol and the mixture filtered. 5 ml of the alcoholic filtrate was mixed with 1 ml of glacial acetic acid with traces of ferric chloride. Concentrated Sulphuric acid was carefully poured down the sides of the tube.

A reddish brown ring is formed at the interface due to the presence of aglycone (steroidal) that indicates the presence of a cardiac glycoside.

### 2.4.7 Alkaloids

0.2 g of pulverized plant material was boiled with 2 % sulphuric acid and filtered. To 1 ml of filtrate, 5 drops of dragendorf's reagent was added. The presence of the characteristic orange brown precipitate indicates the presence of alkaloids. This is confirmed by the absence of a buff white precipitated upon addition of Mayer's reagent to 1 ml of the acidic extract.

### 2.4.8 Flavonoids

About 0.2 g of pulverized plant material was boiled with 5 ml of water for 2 minutes and filtered. To 1 ml of the filtrate, 20 % NaOH was added. There was a formation of an intense yellow coloration which was further exposed to fumes of concentrated HCl. The yellow coloration disappears or turns colorless, indicating the presence of flavonoids.

Lead acetate test: A few drops of lead acetate solution were added to 1 ml of the filtrate. Formation of a yellow color precipitate indicates the presence of flavonoids.

### 2.4.9 Coumarins

A small amount of the pulverized plant material was extracted with chloroform and filtered to obtain a chloroformic extract. 5 ml of the chloroformic extract was evaporated to dryness and the residue dissolved in hot distilled water and cooled. About 0.5 ml of 10 % ammonia solution was added to the extract and observed under UV light (long wave length; 366 nm). The occurrence of an intense bluish green fluorescence under UV light indicates the presence of coumarins and their derivatives.

### 2.4.10 Steroids

Powdered plant material (0.2 g) was shaken with chloroform and filtered. A few drops of acetic anhydride were added to1 ml of the filtrate after which concentrated sulphuric acid was carefully poured down the side of tube. The formation of a bluish- green coloration at the interface indicates the presence of a steroidal ring.

### 2.4.11 Terpenoids

A chloroformic extract was obtained by shaking 0.2 g of the pulverized plant material with chloroform and filtered. To 1 ml of the filtrate, a few drops of concentrated sulphuric acid

was added, shaken and allowed to stand. Effervescence followed by the appearance of a clear reddish brown color at the interface indicates the presence of terpenoids.

### 2.5 *In vitro* antioxidant assay

#### 2.5.1 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

This method involves the reduction of purple methanolic DPPH solution with a maximum absorption at 517 nm to a yellow color. There is the formation of 1,1-diphenyl-2-picrylhydrazyl hydrate (DPPH.H) a stable radical when the yellow color is formed.[15, 16]

Various concentrations of the extract (total crude methanol) ranging between 0.00098 mg/ml to 0.125 mg/g were prepared and used in the experiment. The concentration of the DPPH solution used was 20 mg/L in methanol. The reaction mixture was made up of 3.0 ml of DPPH and 1.0 ml each of the different concentrations of the extract and incubated for 30 minutes in the dark. The absorbance of each concentration was determined at 517 nm using a UV-spectrophotometer (Cecil CE 7200 spectrophotometer, Cecil instrument limited, Milton Technical Centre, England).

Ascorbic acid with concentrations ranging from 0.00098 mg/ml to 0.0625 mg/ml and methanol (vehicle) were employed as positive and negative controls respectively. The test procedures were repeated in triplicates. A graph of concentration against % DPPH scavenging is plotted to estimate the $EC_{50}$.

The percentage DPPH scavenging ability was calculated according to the equation blow;

$$\% \text{ DPPH radical scavenging activity} = \left[\frac{A_0 - A_1}{A_0}\right] x\ 100 \quad \text{.....Equaton 1}$$

Where; $A_O$ is the absorbance of the negative control and

$A_1$ is the absorbance of extract or positive control

#### 2.5.2 Total antioxidant capacity

This method of assay involves the reduction of Mo (vi) to Mo (v) by the test sample (extract) and the formation of a green phosphomolybdenum (v) complex at acidic pH.[17]

Concentrations in the range 0.00098 mg/ml to 0.125 mg/ml of the extract were prepared. A standard reagent was obtained by mixing 0.6 M sulphuric acid, 28 mM disodium phosphate and 4 mM ammonium molybdate. 3 mls of the standard reagent was added to 1 ml each of the various concentrations of the extract to obtain test samples which were incubated at 95 $^0$c

for 24 hrs. The test samples were allowed to cool at room temperature, centrifuged for 10 mins and the absorbances of the supernatant fluids (complex), measured at 695 nm using a spectrophotometer.

The procedure was repeated using Ascorbic acid as the reference sample with concentration range 0.00098 mg/ml to 0.03125 mg/ml. A calibration curve of absorbance against concentration was plotted for the Ascorbic acid and the total antioxidant capacity of the extract was expressed in terms of ascorbic acid equivalent (mg/g of the extracted compound). The procedures were carried out in triplicates.

### 2.5.3 Total Phenol Content

This method depends on the reduction of folin-ciocalteu reagent (made up of phosphotungstic and phosphomolybdic acids) by phenols present in the extract to blue oxides with a maximum absorption at 760nm.

Extract concentrations ranging from 0.00098 mg/ml to 0.125 mg/ml were prepared. For the test mixture, 0.5 ml of extract was added to 0.1 ml of folin-ciocalteu reagent (0.5 N) and incubated at room temperature for 15 minutes. 2.5 ml of saturated sodium carbonate was added to the mixture after which the incubation was continued for a further 30 minutes at room temperature. The absorbances for the test mixtures were taken at 760 nm with a UV-spectrophotometer. Reference mixtures were prepared with Tannic acid (0.00098 mg/g - 0.125 mg/g) as the positive control. A calibration curve of absorbance against concentration was plotted for the Tannic acid and the total phenol content expressed in terms of tannic acid equivalent (mg/g of the extracted compound).[18] The procedures were repeated in triplicates.

### 2.6 Data Analysis

The concentrations responsible for 50 % of the maximum effect ($EC_{50}$) for the extract were determined using interactive computer least squares method, with the following non-linear regression (three-parameter logistic) equation.

$$Y = \frac{a+(b-a)}{1+10^{(\text{Log}EC_{50}-x)}} \quad \text{.....Equation 2}$$

Where X is the logarithm of concentration and Y is the response. Y starts at ‘a’ (the bottom) and goes to ‘b’ (the top) with a sigmoid shape. Graph pad prism windows version 6 (Graph pad software, San Diego, CA, USA) was used to estimate the $EC_{50}$ of both the extract and the

standard drug. With the aid of the Graph pad prism, the mg/g equivalent of the extract in terms of ascorbic acid and tannic acid were also estimated.

## 3. RESULTS

### 3.1 Phytochemical screening

Table 1 summarizes the results from the preliminary phytochemical screening performed on the pulverized stem bark of *Trichilia tessmannii*. It revealed the presence of major secondary plant metabolites which included; alkaloids, coumarins, tannins, saponins, sterols, reducing sugars, cardiac glycosides, anthraquinones and terpenoids. However it revealed the absence of cyanogenetic glycosides and flavonoids.

**Table 1: Phytochemical constituents of the powdered stem bark of *Trichilia tessmannii***

| Test | Results |
|---|---|
| Tannins | + |
| Reducing sugar | + |
| Saponin glycosides | + |
| Anthraquinones | + |
| Cyanogenetic glycosides | - |
| Cardiac glycosides | + |
| General Alkaloids | + |
| Flavanoids | - |
| Terpenoids | + |
| Coumarins | + |
| Steroids | + |

***Key: + present and – absent***

### 3.2 Antioxidant assay

#### 3.2.1 DPPH scavenging activity

The results of the DPPH scavenging activity is as shown in the Table 2 below. The DPPH scavenging assay determines the ability of the agent (extract) to scavenge free radicals. From the results obtained it may be observed that the DPPH free radical scavenging activity was concentration dependent. The $EC_{50}$ value recorded was 0.0207 mg/ml for the extract and 0.004806 mg/g for the ascorbic acid used as the reference drug. Fig. 1 shows the percentage DPPH scavenging activity against log concentration of extracts of *Trichilia tessmannii* (TT) and ascorbic acid.

**Table 2: $EC_{50}$ values of the stem bark extract of *Trichilia tessmannii* and Ascorbic acid**

| Extract / drug | $EC_{50}$ |
|---|---|
| *T. tessemannii* | 0. 02078 |
| Ascorbic acid | 0.004806 |

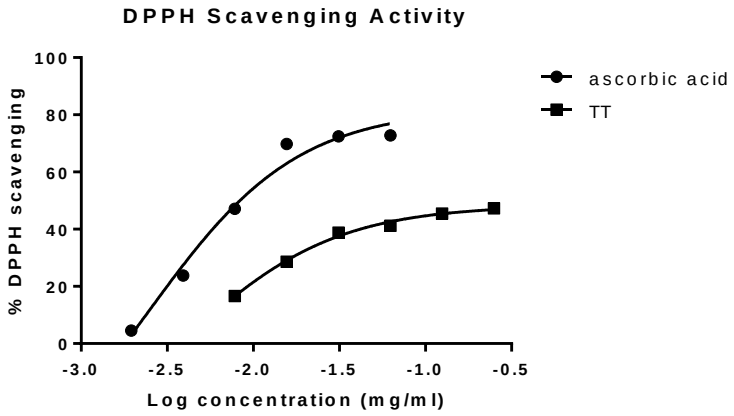


**Figure 1: Percentage DPPH scavenging activity against log concentration of extracts and ascorbic acid.**

### 3.2.2 Total Antioxidant capacity (TAC)

Fig. 2 shows the calibration curve of ascorbic acid as the reference drug ($r^2$ = 0.9933). The antioxidant activity was expressed as mg of ascorbic acid equivalent per g of the extract (Fig. 3). The results for the Total antioxidant capacity showed a concentration dependant increase. There was an increase in the Total antioxidant capacity as the concentration of the extract increased. The total antioxidant capacity of the extract was estimated to be 444.7 ± 28.58 mg/g expressed as ascorbic acid equivalent (Table 3).

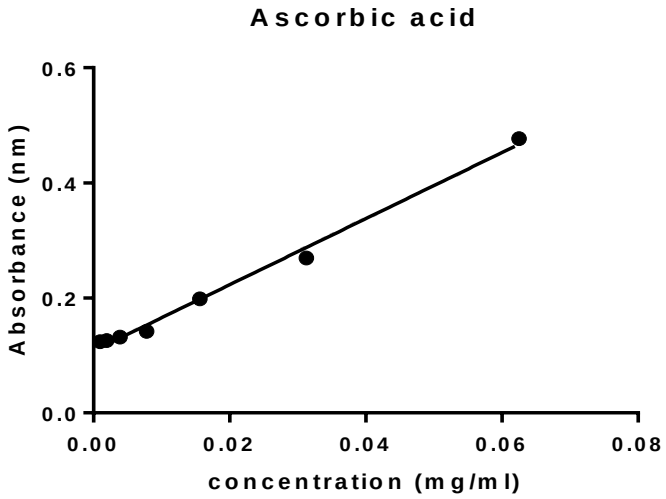


**Figure 2: Calibration curve for ascorbic acid**

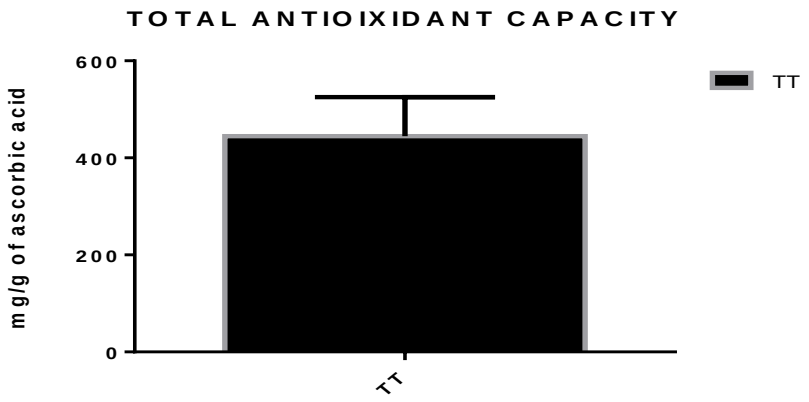


**Figure 3: Total antioxidant capacity of *T. tessemannii***

### 3.2.3 Total Phenol content (TPC)

The total phenol content of the extract was determined by using the folin-ciocalteu reagent with tannic acid used as the reference drug. Fig. 4 shows the calibration curve for tannic acid ($r^2$ = 0.9873). The total phenol content of the extract was estimated to be 970.2 ± 78.69 mg/g expressed as tannic acid equivalent (Table 3).

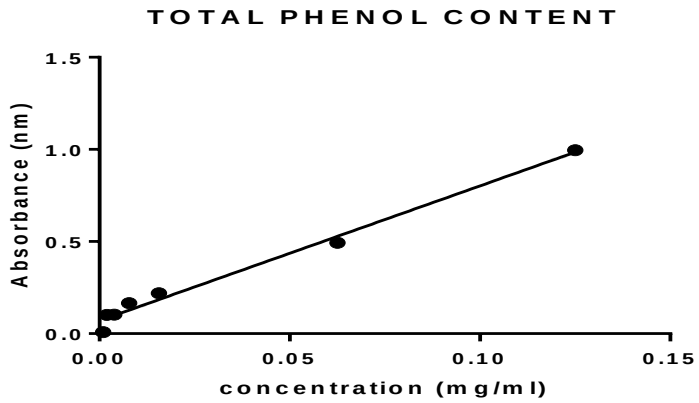


**Figure 4: Calibration curve for tannic acid**

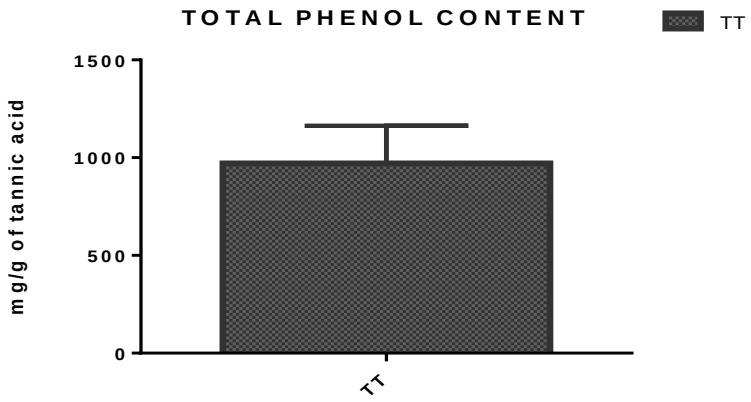


**Figure 5: Total phenol content of the extract**

**Table 3: Total antioxidant capacity and total phenol content expressed in mg/g equivalent**

| Extract / test | mg /g equivalent |
|---|---|
| *T. tessmannii* (TAC) | 444.7 ± 28.58 of ascorbic acid |
| *T. tessmannii* (TPC) | 970.2 ± 78.69 of tannic acid |

## 4. DISCUSSIONS

Thousands of secondary plant metabolites have been isolated and reported in literature with many of them possessing powerful physiological properties that affects humans and have therefore been used as medicines.[5, 19]

This study reports for the first time the phytochemical constituents and the antioxidant properties of the stem bark of *T. Tessmannii*. The phytochemical analysis of the pulverized stem bark of the plant showed the presence of major secondary plant metabolites such as alkaloids, coumarins, tannins, saponins, sterols, reducing sugars, cardiac glycosides, anthraquinones and terpenoids. Cyanogenetic glycosides and flavonoids were however absent. Saponins and some alkaloids have been known to possess selective antibacterial effects and have proven useful in the treatment of many diseases.[20, 21] Alkaloids, a group of compounds containing nitrogen have been proven to have useful medicinal properties that improve the health of humans. Some of these useful isolated compounds include morphine and cocaine which act on the nervous system;[22] vincristine and vinblastine used in the treatment of some cancers[23] and quinine which is used in the treatment of malaria.[24] Some terpenoids (diterpens; taxol) are also used in the treatment of some solid tumors.

Also some phenolic compounds such as salicylic acid have demonstrated anti-fungal properties. Aspirin, an acetylated derivative of salicylic acid has been used to reduce inflammation, pain and fever.[25] Some flavonoids have also been proven to have important anti-inflammatory, anti-allergic and anti-cancer activities and have been shown to have potent antioxidant properties as well.[26]

The presence of these phytochemicals in *T. Tessmannii* may be responsible for the acclaimed traditional medicinal uses of this plant.

Antioxidants on the other hand are agents that neutralize or terminate the chain reaction initiated by free radicals either by providing extra electrons needed to make the pair or by breaking down the free radical molecule to a harmless moiety.[27, 28] Antioxidants stop the

chain reaction of free radical formation and improve our health by boosting the immune system.[29]

From the results of the DPPH scavenging assay, it was observed that there was an increase in the percentage DPPH scavenging activity with increase in the concentration of the extract and the reference drug (ascorbic acid). There was however a decrease in DPPH absorbance as the concentration of the extract increased. The $EC_{50}$ value recorded was 0.0207 mg/ml for the extract and 0.004806 mg/g for the ascorbic acid used as the reference drug. It may be suggested that the extract contains some constituents that have the ability to quench free radicals via proton or electron donation to make the DPPH radical stable; thus reducing it to the form DPPH.H which has a characteristic yellow color.[16] The donation of the electrons to the free radical helps in the termination of the chain reaction. The values obtained from the estimation of the Total antioxidant capacity and Total phenol content assays were 444.7 ± 28.58 mg/g of ascorbic acid and 970.2 ± 78.69 mg/g of tannic acid respectively.

Research has shown that polyphenolic and phenolic compounds are abundant in medicinal plants and have been demonstrated to possess potent antioxidant properties.[15, 30] This serves as a rationale for their content estimation. Current research has also proven that certain secondary metabolites of medicinal plants such as polyphenols, coumarins and terpenoids possess potent antioxidant properties and may be used in the management of diseases that are linked with oxidative stress.[31]

Furthermore polyphenols, and vitamins such as E and C have been shown to aid in the delay of ageing by neutralizing the actions of free radicals.[32] These suggest that the antioxidant potentials of this plant may be as a result of the presence of certain phytochemicals such as coumarins, terpenoids polyphenol and other phenolic compounds. Further studies would however be required to fractionate, isolate and characterize the active principles responsible for the antioxidant activity observed.

## 5. CONCLUSIONS

This study has shown that the stem bark of *T. Tessmannii* contains major secondary plant metabolites such as alkaloids, coumarins, tannins, saponins, sterols, reducing sugars, cardiac glycosides, anthraquinones and terpenoids. The results however revealed the absence of cyanogenetic glycosides and flavonoids. The total crude extract also demonstrated potent antioxidant properties.

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**COMPETING INTERESTS**

The authors declare that they have no competing interests.

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