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A Comparative Assessment on Antioxidant and Phytochemical of *Trichilia monadelpha* (Thonn) J.J. De Wilde (*Meliaceae*) Plant Extracts

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

This study was designed to evaluate the phytochemical screening and antioxidant activities of *Trichilia monadelpha* plant extracts. The leaves were extracted using two different solvents namely- (n-hexane and methanol). The dry or wet yield of n-hexane extract was 1.3%, while methanolic extract exhibited a percentage yield of 3.7 %. Phytochemical research revealed the presence of secondary plant metabolites such as; alkaloids, flavonoids, cardiac glycosides, terpenoids and saponins. *In-vitro* antioxidant activity was determined using three assays (DPPH free radical scavenging assay, reducing ability and hydroxy radical scavenging activity) with four concentrations (0.25, 5.0, 1.0 and 2.0 mg/L), vitamin C was also used as a standard antioxidant.

The percentage of inhibition was measured, and the results from all assay models showed a concentration-dependent percentage of inhibition by the methanol extract. However, the percentage values for the 2,2, -diphenyl-1-picrylhydrazyl (DPPH), reducing ability and hydroxy radical scavenging activity assays of T. *monadelpha* leaf was lower than that of standard vitamin C. The Pearson's correlation coefficient was evaluated and the results showed that of the two extracts, the methanolic extract had the most antioxidant activity with the methanol extract exhibiting a better significant correlation that had a similar trend to that of the antioxidant compound (vitamin C). The results of this study have shown that the plant *Trichilia monodelpha* contains bioactive compounds which may have contributed to its antioxidant properties.

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1. INTRODUCTION

Plants show enormous versatility in the synthesis of complex materials that do not have immediate noticeable growth or metabolic functions. These complex materials are known as secondary metabolites. Secondary metabolites of plants have recently been referred to as phytochemicals. Phytochemicals are natural and biologically active plant compounds that have potential disease-inhibiting capabilities. Phytochemicals are considered effective in the fight or prevention of diseases due to their antioxidant effect [1,2].

Antioxidants and their chemical properties are currently the subjects of intensive research. This is stimulated by the fact that oxidative stress in vivo may cause various problems, associated with numerous degenerative aging diseases, such as cancer and arteriosclerosis [3]. Oxidative stress caused by reactive oxygen species (ROS) is a specific feature in the pathogenesis of various diseases, including cancer, cardiovascular diseases, diabetes, tumors, rheumatoid arthritis and epilepsy[4]. In recent years, considerable attention has been paid to the antioxidant properties of plants that can be used for human consumption. Plant phenolic compounds are attracting considerable interest in the field of food, chemistry and medicine because of their promising antioxidant potential [5].

Trichilia monadelpha (Thonn) JJ De Wilde (Family: Meliaceae), locally known as Otanduro (Twi) or Tenuba (Nzema) meaning the hatred medicine; is a tree that grows 12-20 m tall and settles well in the lowland high forest and evergreen semi-deciduous secondary jungles, often near river banks [6]. Recent research has shown that some extracts of different parts of *T. Monadelpha* plant exhibit antioxidant, anti-inflammatory, antitumor and antioxidant properties [7,8]. In this present study, considering the relevance of free radical theory of diseases as one of the objective parameters, the amenability of *T. Monadelpha* plant was examined to assess its phytochemical and antioxidant potential. The findings from this work may add to the value of its therapeutic properties.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

All chemicals and reagents (acetic anhydride, ferric chloride, potassium iodide, lead acetate, iodine crystal, ammonium molybdate, sodium thiophosphate, sulphuric acid, ascorbic acid, 2,2-diphenyl-1-picrylhydrazyl, aqueous hydrogen chloride, phenanthroline and glacial acetic acid) used for screnning test and antioxidant activity were of analytical grade obtained from various companies (Goddie Chemicals Int'l Ltd and Chemlod International Energy Ltd).

2.2 Plant Material

The *Trichilia Monadelpha* plant was purchased from traders in Shasha market Akure, Ondo State in Nigeria. It was identified and authenticated by Dr. B. E. Omomoh of the Department of Forestry and Wood Technology Federal University of Technology, Akure. And also, the herbarium specimen (No. 0301.) was submitted to the Department of Forestry and Wood Technology Federal University of Technology, Akure. The plant material was thoroughly washed with distilled water (without squeezing) to remove debris and dust particles. It was dried at room temperature under the shade for a few days to prevent ultra-violet rays from inactivating the chemical components [9,10]. The dried leaves were uniformly pulverized (ground into powder form) using a manual blender (Porkert Manual Grinder No. 32) and transferred to an air-tight poly-ethylene bag for further use.

2.3 Extraction Method

The simple maceration method was adopted for the plant *Trichilia monadelpha* (Figure 1). The concentrated process was carried out by a rotary evaporator (Stuart RE300) using n-hexane and methanol in increasing order of polarity. Extraction was carried out using 300 g of the powdered leaves immersed with 2.5 L of n-hexane in a stoppered flask at room temperature for a period of at least 3 days with intermittent stirring [11]. The efficiency of this method is to soften and break the cell wall of the plant in other to release soluble phytochemicals. After three days, the mixture is pressed or strained by filtration using Whatman filter paper No.1 into a conical flask to obtain the n-hexane extract. The

remaining residue was re-extracted using the same solvent according to the procedure described above to obtain the second extract of n-hexane, this process was performed 6 times to extract an adequate amount of plant material. The same procedure was performed on the plant residue using 1.5 L of methanol in sequence. The two extracts gotten were then concentrated separately using a rotary evaporator under reduced pressure at 40°C to obtain a dense and viscous mass that was then air-dried. The percentage yield for each extract was obtained using the following equation:

$$\text{Percentage yield} = \frac{\text{Extract weight}}{\text{Dried plant weight}} \times 100 \quad (1)$$

2.4 Phytochemical Screening Tests

The phytochemical studies of two different extracts obtained from T. *Monadelpha* leaves were conducted in the Department of Chemical Science, Edwin Clark University, Delta State. Each of the concentrated extracts was subjected to a qualitative test via standard procedures reported by various researchers [12, 13]. To detect the following bioactive compounds: alkaloids, flavonoids, saponins, tannins, terpenoids and cardiac glycosides.

2.5 Antioxidant Assays (*in-vitro*)

2.5.1 DPPH radical scavenging assay

The DPPH radical scavenging activity was carried out as described by Sunil and Ignacimuthu [14] with few modifications. 1 ml of methanolic DPPH (0.15%) was mixed with 3 ml of extract or vitamin C (reference antioxidant) and incubated in a dark room for 30 minutes. Thereafter, absorbance was measured at 515 nm using UV-Vis spectrophotometer (Techcomp UV-2500 UV-Vis-Spectrophotometer). The DPPH radical activity was expressed as a percentage and calculated using equation 1. Distilled water was used as blank.

$$\textbf{\% Scavenging Activity} = \frac{A_c - A_s}{A_c} \times \textbf{100} \quad (2)$$

Where $A_{control}$ is the absorbance of the control, A_{sample} is the absorbance of the sample

2.5.2 Hydroxy radical scavenging assay

Hydroxy radical scavenging activity was investigated using the method described by Bera et al. [15] with few modifications. 1 ml of phosphate buffer (0.2 M, pH 7.2), 1 ml of test solution either extract or vitamin C), 0.02 ml of ferric chloride (0.02 M) and 0.05 ml of phenanthroline (0.04 M) were introduced into a test tube. The reaction was indicated by adding 0.05 ml of 7 mM hydrogen peroxide. After 5 minutes of incubation at room temperature (25°C), absorbance was measured at 560nm using UV-Vis spectrophotometer (Techcomp UV-2500 UV-Vis-Spectrophotometer). Hydroxyl radical scavenging activity was expressed as relative scavenging activity and calculated using equation 2. Methanol was used as the blank.

$$\text{\% Scavenging Activity} = \frac{A_c - A_s}{A_c} \times 100 \quad (3)$$

Where $A_{control}$ is the absorbance of the control, A_{sample} is the absorbance of the sample

2.5.3 Reducing ability

The ability of the extract to reduce was investigated according to Oyaizu [16] method as modified by Okoko and Diepreye [17]. Extract or vitamin C (0.5 ml) was mixed with 0.5 ml of phosphate buffer (0.2 M, pH 6.6) and 0.5 ml of potassium ferricyanide (1%) and incubated at 50°C. After incubation for 20 minutes, 0.5 ml of trichloroacetic acid (10%) was added and centrifuged for 10 minutes at 3000 rpm. A portion of the upper layer (0.5 ml) was mixed with 0.5 ml distilled water and 0.1 ml ferric acid chloride (0.1%). After 10 minutes of incubation at room temperature, absorbance was measured at 700 nm using UV-Vis spectrophotometer (Techcomp UV-2500 UV-Vis-Spectrophotometer). An increase in absorbance showed a greater reducing ability.

2.6 Statistical Analysis

All values were expressed as mean ± standard deviation (SD) of triplicates. Statistical comparisons of the % values were calculated using linear regression analysis. The results were using Ms excel and Origin 6.0 professional software. P-value less than 0.05 is considered statistically significant.

3. RESULTS AND DISCUSSION

3.1 Extraction Yield

Table 1 shows the percentage of yield extracts of *T. mondenelpha* from two different solvents,

which are methanol and n-hexane. Among extraction solvents, the methanol extract (3.2 %) had a higher percentage of extraction yield than the n-hexane extract (2.4 %). This phenomenon occurs due to the polar protic nature and higher dielectric constant of methanol over n-hexane, which facilitates the solubilization of polar Synaptic membrane (SMs) along with some non-polar SMs since most bioactive compounds of plant matrices are highly polarisable [18]. Similar trends in the result of percentage extractive yield have also been previously reported in the solvent extraction of *C. alata* leaf (methanol-11.80% > n-hexane- 9.91%) by Ndukwe et al. [19] and *P.purpureum shoots* (methanol-3.10% > n-hexane- 2.20%) by Jack et al. [20].

3.2 Phytochemical Screening of Plant Extracts

Biochemical screening of the studied plant was investigated for the following metabolites: alkaloid, saponin, cardiac glycosides, tannins, flavonoids, and terpenoids. Qualitative screening with methanol extract indicated the presence of alkaloids, saponins, flavonoids, cardiac glycosides and Terpenoids. Whereas, it was absent in tannins. The n-hexane extract indicated the presence of alkaloids, terpenoids and cardiac glycoside but was absent in saponin, tannin and flavonoid (Table 2). Observed phytochemicals of *T. monadelpha* corroborate with the results of Ben et al. [8] which reported the presence of alkaloids, saponins, flavonoids, cardiac glycosides, saponins and terpenoids in the stem bark of *T. monadelpha.* The phytochemical activities exhibited by the methanolic extract from the leaf of *T. monadelpha* conform to the previous reports of Ndukwe et al. [19] and Jack et al. [20], all of whom linked phytochemical properties with reference to *P.purpureum* shoots and *A.hybridus* leaves to the presence of bioactive secondary metabolites such as alkaloids, flavonoids, cardiac glycosides, saponins and Terpenoids. These phytochemical compounds have been attributed to be the bioactive principle responsible for a variety of biological activities including antimicrobial, antioxidant and anticancer activities [13].

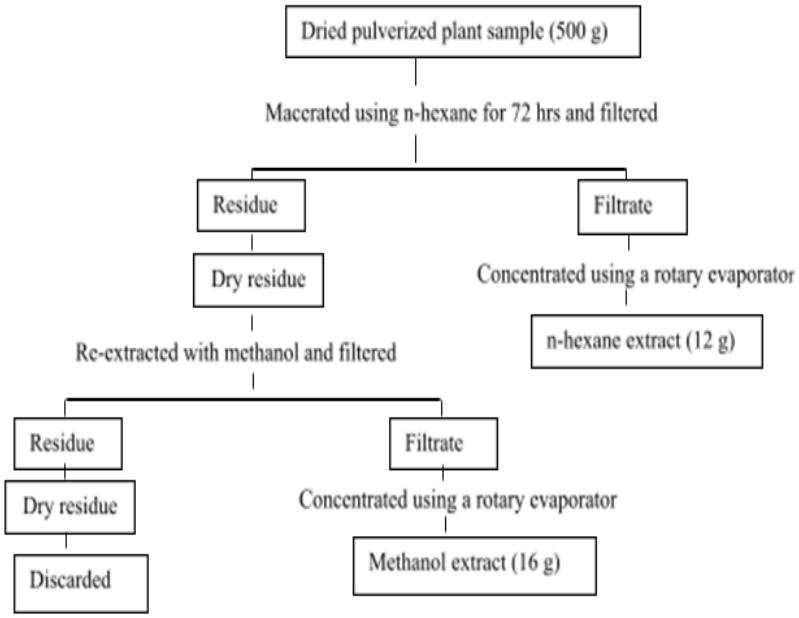

Fig. 1. Flow chat of extraction process

Table 1. Extraction yield of *T. monadelpha* leaf

Extract	Weight of extract (g)	Percentage yield (%)
n-Hexane	12	2.4
Methanol	16	3.2

Table 2. Phytochemical groups present in *T. monadelpha* leaf

Phytochemical group	n-Hexane extract	Methanol extract
Alkaloids	+	+
Saponins	-	+
Tannins	-	-
Flavonoids	-	+
Terpenoids	+	+
Cardiac Glycosides	+	+

KEY: + Present, - Absent

Table 3. Percentage Inhibition of DPPH free radical at 515 nm by *T. monadelpha* leaf extracts

Concentration (mg/L)	Percentage inhibition (%)		
	Methanol Extract	n-Hexane Extract	Vitamin C
0.25	36.00±0.030	27.00±0.090	33.00±0.007
0.50	40.60±0.007	21.00±0.011	33.00±0.020
1.00	44.00±0.020	29.00±0.040	43.00±0.005
2.00	46.00±0.020	40.00±0.090	50.20±0.007

Number of trials (n=3)

Table 4. Percentage Inhibition of Hydroxy radical at 560 nm by *T. monadelpha* leaf extracts

Concentration (mg/L)	Percentage inhibition (%)		
	Methanol Extract	n-Hexane Extract	Vitamin C
0.25	36.00±0.010	37.00±0.160	42.00±0.004
0.50	39.00±0.006	27.00±0.010	43.00±0.006
1.00	40.00±0.006	33.00±0.010	44.00±0.006
2.00	43.00±0.005	40.00±0.010	46.00±0.010

Number of trials (n=3)

Table 5. Reducing power of the extracts at 700 nm of *T. monadelpha* leaf (%)

Concentration(mg/L)	Percentage inhibition (%)		
	Methanol Extract	n-Hexane Extract	Vitamin C
0.25	45.00±0.040	40.00±0.020	48.00±0.001
0.50	43.00±0.020	47.00±0.000	50.00±0.003
1.00	48.00±0.030	50.60±0.006	52.00±0.005
2.00	50.00±0.003	53.00±0.030	54.00±0.010

Number of trials (n=3)

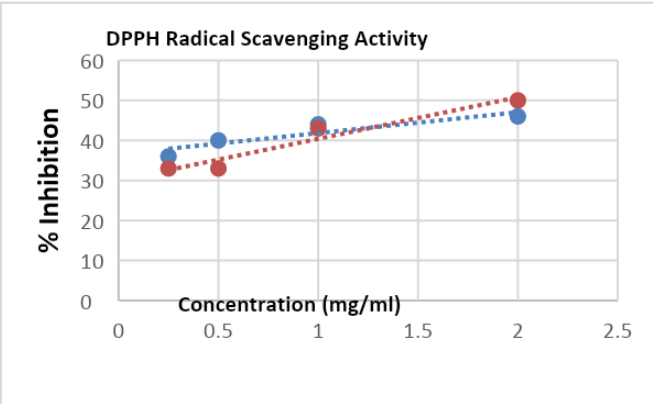

Fig. 2. DPPH free radical scavenging activity of the methanol extract *T. monadelpha* leaf

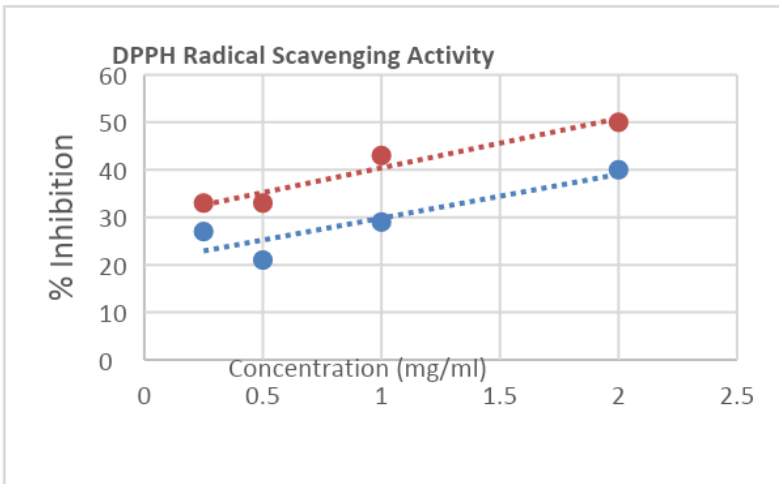

Fig. 3. DPPH free radical scavenging activity of the n-hexane extract o *T. monadelpha* leaf

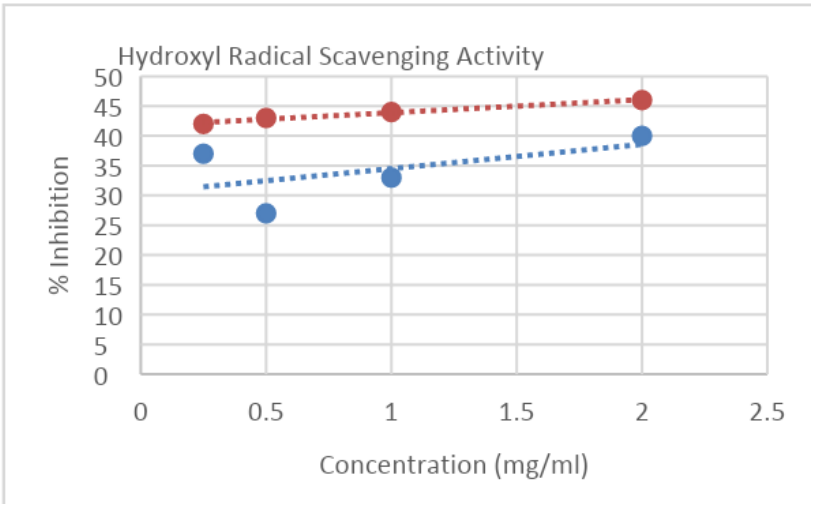

Fig. 4. Hydroxy radical scavenging activity of the n-hexane extract of *T. monadelpha* leaf

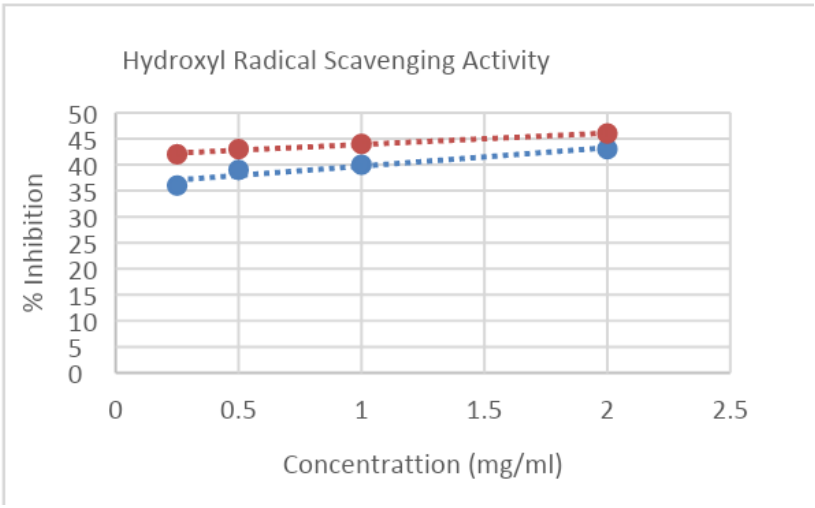

Fig. 5. Hydroxy radical scavenging activity of the methanol extract of *T. monadelpha* leaf

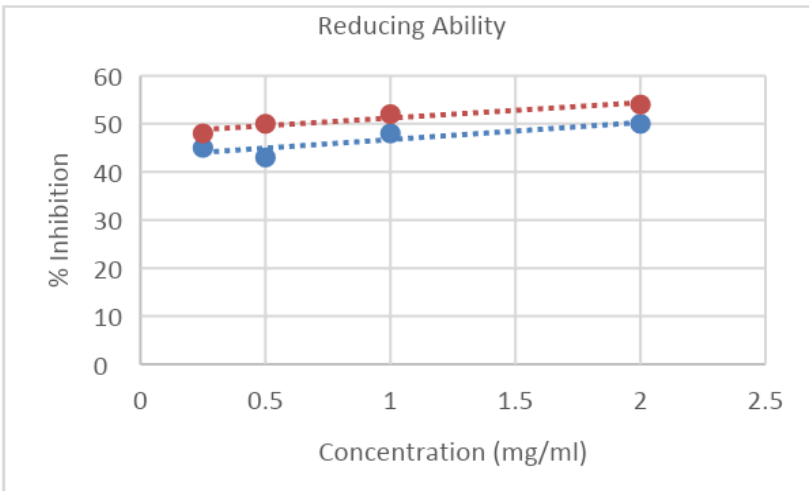

Fig. 6. Reducing ability of the methanol extract of *T. monadelpha* leaf

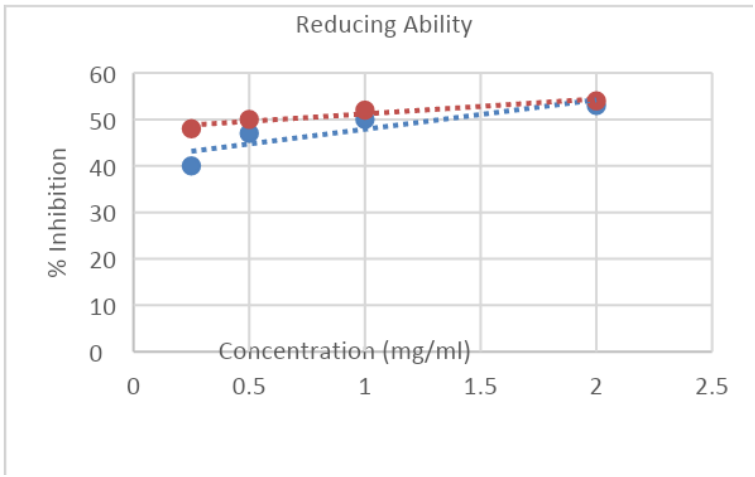

Fig. 7. Reducing ability of the n-hexane extract of *T. monadelpha* leaf

3.3 Antioxidant Activity

There are many methods used to evaluate the free radical scavenging activity of compounds [21]. The antioxidant activity of plant extracts varies with the assay's method because of the complex nature of phytochemicals present in them, and the solvent used for extraction [22]. Therefore, it is important to use several analytical methods and different substrates to evaluate the effectiveness of antioxidants. In this study, we considered using DPPH free radical scavenging activity, hydroxyl radical scavenging activity and reducing ability.

3.3.1 DPPH free radical scavenging activity

The DPPH radical scavenging assay evaluates the ability of extracts to donate hydrogen or to scavenge free radicals. The percentage of inhibition was measured (Table 3) to determine the antioxidant activity of the extracts that are capable of inhibiting free radicals. From the results obtained it was observed that the four varying concentrations (0.25, 5.0, 1.0 and 2.0 mg/L) of different solvent extracts of *T. Monadelpha* leaf showed different percentages of inhibition. Interestingly, the scavenging activity of methanol extract was increased in a concentration-dependent manner. The 2.00 mg/L showed the best antioxidant activity, where among them, the methanolic extract was the highest (46%), followed by n-hexane (40%) respectively but were lower than the standard antioxidant (50%). The DPPH radical scavenging activity of *T. Monadelpha* extracts (methanol and n-hexane) were calculated using regression line equation (y = 5.2174x + 36.604 with R^2 = 0.829

and y = 9.2174x + 20.609 with R^2 = 0.8088 (Figures 2 and 3). The scavenging power of the two extracts was compared with the standard compound (y = 10.4x + 30 with R^2 = 0.94). It was clear from the Pearson's correlation coefficient that there was a significant positive linear relationship with the concentrations of the extracts used, indicating that there are scavenging activities in the extracts of the leaves of *T. Monadelpha*. This result is consistent with a previous report by Sagbo et al. [23] which showed that the scavenging effects on DPPH radical increased with increasing concentration of extract and standard. This activity can be attributed to their ability to provide hydrogen to DPPH free radicals in other to stabilize them [24].

3.3.2 Reducing ability

The reducing power assay conducted in this study sought to establish the in-vitro antioxidant properties of the plant extracts. From the results, we observed a concentration-dependent increase in the percentage of inhibition for the plant extracts and the standard compound (vitamin C) (Table 5), indicating that the extracts can reduce oxidative stress. Significant positive correlations were observed between the extracts and standard (Figures 6 and 7). A similar trend in antioxidant activity was also observed between the n-hexane and methanolic extract with a correlation coefficient of R^2 = 0.7743 and R^2 = 0.7799 respectively (Figures 6 and 7), this explains that the reducing ability is in positive correlation with the concentration of the extracts used, presumably due to its phytochemical content [20]. In comparison, the vitamin C standard had a better correlation coefficient with R^2 = 0.92. The activities of these plant extracts are indications of phytochemicals that could be responsible for the antioxidant activity. In addition, antioxidants could be polar e.g., phenolics, flavonoids, etc., or non-polar e.g., vitamin E in nature and they can act as a radical scavenger by electron-donating mechanism. However, studies have also suggested that plant secondary metabolites such as phenol and flavanol are strongly involved in the antioxidant activities of the methanolic extract [25,26]. Therefore, this finding supports the result of Jack et al. [20] who reported that the reducing power of plants correlates with their phenolic content.

3.3.3 Hydroxy radical scavenging activity

The mutagenic capacity of free radicals is due to the direct interactions of hydroxy radicals with DNA, resulting in DNA breakdown and therefore plays an important role in cancer formation. The hydroxyl radical is one of the most reactive oxygen species in living systems. Its mutagenic capacity of free radicals is due to the direct interactions of hydroxyl radicals with DNA, resulting in DNA breakdown and thus plays an important role in the formation of cancer [27]. Therefore, the removal of OH radicals is very important for protecting biological systems. The hydroxyl radical scavenging activity demonstrated similar activity with the result of 2,2-diphenyl-1-picryhydrazyl (DPPH) radical scavenging activity with an increasing percentage inhibition of the methanolic extract based on increasing concentration. In general, n-hexane extract at 2.00 mg/L (40 %) showed lower antioxidant potential than methanol extract (43 %), as well as standards vitamin C (46%). Here, we assume that the antioxidant activity of the extract (methanol) to quench hydroxyl radicals might directly relate to the inhibition of lipid peroxidation and acts as scavengers of active oxygen species by breaking free radical chains. Comparable scavenging activities of the plant extracts were observed with the standard compound (($y = 4.0696x + 30.435$ with R^2 = 0.3141, $y = 2.1913x + 41.696$ with R^2 = 0.9861 and $y = 3.5478x + 36.174$ with R^2 = 0.9047 (Figures 4 and 5). From the activities shown by the extracts, particularly methanol had a correlation coefficient R^2 = 0.9047 demonstrating a similar linear trend with standard vitamin C. In contrast, n-hexane showed a non-significant correlation (R^2 = 0.3141) indicating that there is little or no scavenging activity in the n-hexane extract. The activity of these plant extracts is an indication of phytochemicals that could be responsible for the antioxidant activity.

4. CONCLUSION

This study evaluated the phytochemical and antioxidant activities of *T. monaldepha* leaf extracts. The results of phytochemical screening revealed that the methanolic extract proved to be more effective than the n-hexane extract. In conclusion, we could say that this research gives us the first picture of the potential antioxidant activity of *T. monaldepha* leaf extracts. These *in-vitro* assays indicate that the extracts of *T. monaldepha* are a significant source of natural antioxidants, which could help to prevent the progression of various diseases caused by free radicals, such as certain cancers. However, the components responsible for the anti-oxidative activity are currently unclear. Therefore, further

studies are needed to isolate and identify active compounds required for the antioxidant properties.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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