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*Research Article*

# Phytochemical and Antioxidant Studies of Albizia Zygia (DC) Jf Macbride and Trema Orientalis (LINN) Blume

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**ABSTRACT**
Oxidative stress and its attendant health challenges are on the increase. To find a plant-based solution to the challenges, phytochemical and antioxidant properties of the leaf extracts of *Albizia zygia* (AZ) and *Trema orientalis* (TO) were investigated. Phytochemical screening of the leaves of AZ and TO was done using standard methods. Hexane, ethyl acetate, and methanol extracts of AZ (AZH, AZE, and AZM) and TO (TOH, TOE, and TOM) were evaluated for their free radical scavenging power in comparison with ascorbic acid (AA), using five different methods. In the DPPH antioxidant assay, AZH, AZE, AZM, TOH, TOE, TOM, and AA had $IC_{50}$ values of 2.06, 0.28, 0.60, 0.93, 0.40, 0.34, and 0.22 µg/mL respectively. In the ABTS assay, AZH, AZE, AZM, TOH, TOE, TOM, and AA had $IC_{50}$ values of 1.86, 0.41, 1.58, 2.29, 1.70, 0.73, and 0.27 µg/mL respectively. In the $H_2O_2$ antioxidant assay, AZH, AZE, AZM, TOH, TOE, TOM and AA had $IC_{50}$ values of 0.130, 0.040, 0.220, 1.04, 0.044, 0.038, and 0.040 mg/mL respectively. The results of the studies established that both AZ and TO leaves contain tannins, flavonoids, reducing sugars, cardiac glycosides, saponins, terpenoids, and alkaloids, while combined anthraquinones was found in AZ only. Both AZ and TO are rich in phytochemicals with antioxidant properties. The antioxidant assays showed that AZE extract had the highest antioxidant activity compared to other extracts. This study submits that *A. zygia* and *T. orientalis* possess potent antioxidant principles that could be developed for the management of oxidative stress.

**Keywords:** Phytochemical screening, oxidative stress, Secondary metabolites, Quantitative phytochemicals.

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## Introduction

Herbs are rich in secondary metabolites which can be explored as potential chemotherapeutics for treatment and prevention of diseases Most free radicals such as, hydroxyl radical (·OH), the superoxide radical ($O_2^-$), lipid peroxide radicals and hydrogen peroxide ($H_2O_2$) are being implicated in some disease conditions. These include: oxidative stress, cancer, gastrointestinal inflammation, asthma, cataracts, cardiovascular disease, diabetes mellitus, liver disorder, periodontal disease, and other inflammatory processes (Adebayo et al., 2011). Antioxidants counteract free radicals attack in the body, thereby helping to ameliorate oxidative stress induced diseases (Olugbuyiro et al., 2017). The quality of medicinal plants is usually contingent on their antioxidant power (Leit et al., 2018). Natural antioxidants are steadily being explored as potent agents to combat free radicals generated by rapidly evolving environmental pollutants and unhealthy lifestyles, which interrupt the body's systems (Effiong et al., 2024). Phytochemicals such as tannins, flavonoids and phenols are rich sources of plant antioxidants. Medicinal plants are being engaged for the management of diseases and possible generation of drug leads. In developing countries, plants serve as the primary source of health care among some communities while in some developed countries, they serve as alternative and complementary medicines (De-Campos et al., 2021). *Albizia zygia* (AZ) and *Trema orientalis* (TO) are medicinal plants

found in many parts of the world, while AZ is used to treat ophthalmia, diarrhea, malaria, toothache, female sterility, fever, and wounds. Its leaf decoctions are used to treat diarrhea and fever (Odeku and Fell, 2005). TO is used for the treatment of diabetes mellitus, respiratory diseases, oliguria, asthma, bronchitis, malaria, gonorrhea, and toothache (Adinortey et al., 2013, Babatunde et al., 2015, Al-Robai et al., 2022, Yanes, 2007). Medicinal plants and natural antioxidants with hypoglycemic activity are hypothesized as a possible solution to oxidative stress, due to a plethora of phytochemicals and non-nutritional compounds (Osikoya et al., 2017). The presence of certain phytochemicals and their free radical scavenging ability in these medicinal plants have made them promising agents to be considered for drug development. This study therefore reports the phytochemical constituents of the plant extracts and free radical scavenging abilities of the hexane, ethyl acetate and methanol extracts of *Albizia zygia* and *Trema orientalis*.

## Materials and Methods

### *Plant material collection and preparation of extracts*.

The leaves of *A. zygia* and *T. orientalis* were collected from Oyo-East Local Government area (LGA) of Oyo, Oyo State of Nigeria, in March 2019. Identification and authentication of the leaves were carried out at Forestry Research Institute of Nigeria (FRIN), Ibadan. Herbarium specimens were deposited at FRIN herbarium and the leaves were assigned voucher numbers FHI.112968 and FHI.112967. The leaves were air dried at room temperature for 5 days and then oven dried at 45°C prior to pulverization. Each powdered leaf sample was extracted successively with three solvents, over increasing polarity scale, starting with n-hexane, then ethyl acetate and finally methanol. The extracts were strained and filtered and then the filtrate evaporated in *vacuo* at $45^0$ C. Thus, hexane, ethyl acetate and methanol extracts of each leaf samples were obtained. The concentrated extracts were kept in desiccator for further analysis.

### *Qualitative Phytochemical Screening*

Identification of the secondary metabolites in the plant leaves of AZ and TO was carried out using standard methods (Olugbuyiro, 2002). Generally, about 1 g of the plant material was dissolved in 100 mL of methanol/water 50:50 to serve as the stock solution of the plant extract.

### *Test for Tannins*

The leaf extract (1mL) was diluted to 5 mL with distilled water in a test tube. One drop of freshly prepared 10% $FeCl_3$ was added, and a bluish-black colouration indicated the presence of tannins.

*2.2.2 Test for Flavonoids*

*Shinoda test*: A small amount of magnesium turnings was added to 3 mL of the extract solution, then a few drops of conc. HCl were run on the side of the test tube. At the upper layer, the appearance of orange, pink or reddish colour indicated the presence of flavonoids.

### *Test for reducing sugar (Glycone test)*

To 2mL of extract solution, 3 drops of 20% NaOH was added to the solution and boiled on a water bath for 5 minutes with an equal volume of Benedict's qualitative reagent. The presence of reducing sugar is indicated by the formation of brick red precipitate.

### *Test for Cardiac glycosides*

A portion of the extract (1 g) was dissolved in 5 mL methanol and diluted with distilled water to 10 mL. A few drops of lead acetate solution were added and shaken together. It was then filtered after standing for a few minutes. The filtrate was extracted with aliquots of chloroform. The chloroform extract was divided into two portions in evaporating dishes and evaporated to dryness on a steam bath.

a. *Keller-Killer test*: The dry residue was dissolved in 2 mL of glacial acetic acid containing one drop of 10 % ferric chloride solution in a clean test tube. 2 mL of conc. $H_2SO_4$ was carefully poured on the side of the test tube to form a layer below the acetic acid. The formation of a purple or reddish-brown "ring" at the interface and green colour in the acetic acid layer indicated the presence of deoxy sugar.

b. *Liebermann's test*: About 0.5 g of the methanol extract was dissolved in 2 mL of acetic anhydride mixed with 2 drops of chloroform. Two drops of conc. $H_2SO_4$ were added carefully. A greenish colour was indicative of steroids.

### *Test for Saponins*

a. *Frothing test*: 1 mL of the crude extract was mixed with 3-4mL of distilled water in a test tube and shaken vigorously for 2 mins. The formation of persistent foam for over 15 minutes confirmed the presence of saponins.

b. *Emulsifying property*: 3 drops of olive oil were added to 2 mL of the extract and shaken vigorously for 30 minutes. Formation of stable emulsion indicates the presence of saponins.

### *Test for Terpenoids*

About 5 mL of aqueous plant extract and 2.0 mL of chloroform were mixed, and 3 mL of concentrated $H_2SO_4$ was carefully added to form a layer. An appearance of reddish brown colour in the inner face indicates the presence of terpenoids.

### *Test for phenols*

To 2 mL of the extract solution, 1 mL NaOH was added, a change of colour from yellow to colorless or lighter colour which returned to the original colour upon the addition of dilute HCl was indicative of the presence of phenols.

### *Test for Alkaloids*

The extract solution (3 mL) was mixed separately with each of Mayer's reagent, Wagner's reagent and Dragendroff's reagent. Turbidity or precipitation with any of the reagents was taken as preliminary evidence for the presence of the alkaloids in the sample.

### *Test for Anthraquinones*

a. *Free anthraquinones*: About 5 ml of the chloroform extract of the plant sample was mixed with 5 mL of 10% $NH_3$ solution. Presence of a pink colouration in the ammoniacal phase indicated the presence of free anthraquinones.

b. *Combined anthraquinones*: About 3 mL of the extract was boiled with 5 mL of 10% HCl for 5 minutes and filtered while hot. The cool filtrate was partitioned against equal volume of

chloroform ($CHCl_3$), avoiding vigorous shaking. The chloroform layer was transferred to a test tube. An equal volume of 10% $NH_3$ was added to the chloroform extract and observed for 1-2 hours. A delicate pink, red or violet colour indicated the presence of combined anthraquinones.

***Quantitative Phytochemical Determination***

Quantification of phytochemicals in the plant samples were carried out using standard methods.

***Determination of total tannins***

Total tannins content was evaluated using a modified method (Van-Burden, 2021, Ajiboye et al., 2013). About 500 mg of sample was weighed into a 100 mL conical flask, and shaken on a mechanical shaker for 1 hr. with 50 mL distilled water. The resulting solution was filtered into 50 mL volumetric flask and made up to the 50 mL mark. Thereafter 5 mL of the filtrate was pipetted into a test tube and mixed with 2 mL of 0.1 M $FeCl_3$ in 0.1 N HCl and 0.0008 M potassium ferrocyanide ($C_6FeK_4N_6$). Absorbance was measured within 10 minutes using UV-spectrophotometer at 120 nm.

***Determination of flavonoids***

Aluminium chloride colorimetric method (Chang et al., 2002) with some modifications was used to determine flavonoid content. 1 mL of the extract was mixed with 1 mL of methanol, 0.5 mL aluminium chloride (1.2%) and 0.5 mL potassium acetate (120 mM). The mixture was allowed to stand for 30 min at room temperature; then the absorbance was measured at 415 nm. Quercetin was used as standard. Flavonoid content is expressed in terms of quercetin equivalent (mg/g of extracted compound).

***Determination of reducing sugar***

Constituent reducing sugar in the leaf samples were estimated by the Dinitro Salicylic Acid (DNS) method (Jain et al., 2020). Freshly prepared DNSA reagent (a mixture of 1 g of DNSA dissolved in 20 mL of 2 N NaOH and 30 g of sodium potassium tartrate ($KNaC_4H_4O_6{\cdot}4H_2O$) dissolved in 50 mL distilled $H_20$, made up to the 100 mL mark). Standard sugar (glucose) was prepared in six varying concentrations ranging from (0-750 mg/mL) to prepare the calibration curve. 3 mL of each concentration was measured into different test tubes as well as the samples. 1 mL of DNS reagent was added to each test tube and thoroughly mixed. The test tubes were plugged with cotton and boiled on water bath for 5 minutes. After which they were allowed to cool to room temperature. Absorbance of the standard and the test samples were read against the blank at 540 nm. The concentrations of the samples were estimated from the standard curve, Glucose was used as standard (100mg/mL).

***Determination of cardiac glycosides***

Cardiac glycoside content in the sample was evaluated using Buljet's reagent procedure (El-olemy et al., 1994). One gram (1 g) of the fine powder of the leaf samples was soaked for 2 hours in 10 mL of 70% alcohol. The resulting solution was filtered and the extract was further purified using lead acetate (Pb $(C_2H_3O_2)_2$) and sodium hydrogen phosphate ($Na_2HPO_4$) solution. Thereafter, freshly prepared Buljet's reagent (made up of 95 mL aq. picric acid ($C_6H_3N_3O_7$) and 5 mL 10% aq. NaOH) was added. Blank solution contained the Buljet's reagent and $dH_2O$ only. The concentration of glycosides present is proportional to the difference between the absorbance of sample solution and the absorbance of the blank solution taken at 418 nm.

***Determination of total saponins***

Each powdered sample (20 g) was weighed into a conical flask and 100 mL of 20% aqueous ethanol was added to the sample using a standard method (Ajiboye et al., 2013). The samples were heated over a hot water bath for 4 hours with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 200 mL 20% ethanol. The combined extracts were reduced to 40 mL over water bath at about 90°C. The concentrate was transferred into a 250 mL separating funnel and 20 mL of diethyl ether ($(C_2H_5)_2O$) was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated and 60 mL of n-butanol ($C_4H_9OH$) was added. The combined n-butanol extracts were washed twice with 10 mL of 5% Aq. NaCl. The remaining solution was heated on a water bath. After evaporation the samples were dried in the oven to a constant weight; the saponins content was calculated.

$$\% \text{ Saponins} = \frac{\text{Weight of Residue}}{\text{Weight of Sample}} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots \text{ Equation (1)}$$

***Determination of terpenoids***

Terpenoid was quantified using a described method (Indumathi et al., 2014). A leaf sample of 100 mg was soaked in 9 mL of ethanol for 24 hrs. The resulting extract was filtered, and further partitioned in separating funnel with 10 mL of ether. The ether extract was collected into a pre-weighed evaporating dish and allowed to dry. After the evaporation of ether, the percentage of total terpenoids was calculated as initial weight of sample minus the weight of extract divided by the weight of sample multiplied by 100.

$$\% \text{ Terpenoid} = \frac{\text{Weight of Sample} - \text{Weight of extract}}{\text{Weight of Sample}} \times 100 \quad \ldots\ldots\ldots\ldots \text{Equation (2)}$$

***Determination of Phenols*:**

Determination of phenols was done with the aid of Soxhlet extractor, following phenols (Keay et al., 1964, Ezeonu and Ejikeme, 2016). Two (2 g) of the leaf sample in 100 mL of ether was defatted for 2 hrs. About 0.5 g of the defatted leaf samples were thereafter boiled for 15 minutes with 50 mL ether for

extraction of phenolic compounds. To 5 mL of this extract, 10 mL of $dH_2O$, 2 mL of 0.1 N $NH_3OH$ and 5 mL of Conc. amyl alcohol ($C_5H_{11}OH$) were added, this mixture was left to react for 30 minutes for colour development. The standard curve for phenol was prepared using 1 mg/mL tannic acid ($C_{76}H_{52}O_{46}$) with varying concentration (0.2-1.0 mg/mL). To each concentration, 10 mL of $dH_2O$, 2 mL of 0.1 N $NH_3OH$ and 5 mL of Conc. amyl alcohol ($C_5H_{11}OH$) were equally added giving a condition similar to that of sample. Absorbance of sample solutions and standards were measured against blank at 505 nm using a UV-Spectrophotometer.

***Determination of total alkaloids*:**

The total alkaloids were determined using standard method (Ajiboye et al., 2013, Harborne, 1973). Five gram (5 g) of each leaf sample was carefully weighed into 250 mL beaker. 200 mL of 10% acetic acid ($CH_3COOH$) prepared in ethanol ($CH_3CH_2OH$) was used for extraction by cold maceration for 4 hours at room temperature. The extract was filtered and concentrated to about 60 mL on a water bath. Conc. ammonium chloride ($NH_4Cl$) was added in drop until precipitation was completed. The solution was allowed to stand for 12 hours to precipitate and settle. The precipitate was collected and washed with dil. Ammonium hydroxide ($NH_4OH$) and then filtered. The residue was alkaloid which was dried and weighed on a pre-weighed filter paper.

$$\% \text{ Alkaloids} = \frac{\text{Weight of Residue}}{\text{Weight of Sample}} \times 100 \quad \text{…….. Equation (3)}$$

***Estimation of Antioxidant Activity***

Antioxidant capacity and free radical scavenging ability of the six leaf extracts of AZ and TO were determined using these five methods;

***DPPH Free Radical Scavenging Assay***

The ability of each plant to donate hydrogen atom was determined by the discoloration of methanol solution of DPPH (2, 2-diphenyl-1-picrylhydrazyl). The assay (Liu et al., 2009) added 1 mL of 0.01 M of DPPH to 1 mL of methanol solution of extract or Ascorbic acid (standard) at different concentrations (0.000125 – 0.004 mg/mL). The mixture was kept in the dark for 30 minutes at room temperature. Thereafter, absorbance was measured against a blank at 517 nm. Percentage inhibition was estimated using this equation:

$$\% \text{ Inhibition} = \left[ \frac{\text{Abs(c)-Abs(t)}}{\text{Abs(c)}} \right] \times 100 \quad \text{……. Equation (4)}$$

Where Abs (c) is the absorbance of control (DPPH)
Abs (t) $_t$ is the absorbance of test sample.

A graph of the % Inhibition versus the concentration of sample was plotted (Figure 1). Using linear regression equation (y = mx + c), the inhibitory concentration ($IC_{50}$) value was obtained by extrapolation from the linear graph, using $IC_{50}$ = (50-c)/m.

***ABTS Radical Scavenging Activity***

The ABTS radical ($ABTS^{*+}$) scavenging activity of the leaf extracts was determined according to (Gorinstein et al., 2009) but with slight modification as described by (Shahinuzzaman et al., 2020). ABTS *+ radicals were pre-generated by adding 4 mL of 2.45 mM of potassium persulphate ($K_2S_2O_8$) and 4 mL of 7 mM 2,2-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid (ABTS) in equal proportion (at a ratio of 1:1), this resulting solution was kept in the dark for 15 hrs. and at room temperature. 1 mL of the radical solution was diluted with ethanol to obtain an absorbance of 0.700 ± 0.02 units at 745 nm, from which 3.9 mL of ABTS $^{*+}$ radicals was added to 0.1 mL of each sample as well as ascorbic acid standard. The absorbance was read at 745 nm after 6 minutes of incubation at room temperature against aq. Ethanol (75%) which was used as blank. While the negative control is the absorbance of ABTS for each sample. ABTS Scavenging percentage inhibition was calculated using (Equation 5) and $IC_{50}$ was determined by plotting the line graph.

$$\text{ABTS Scavenging (\%)} = \left[ \frac{\text{Abs(c)-Abs(t)}}{\text{Abs(c)}} \right] \times 100 \quad \text{……. Equation (5)}$$

Where Abs (c) is the absorbance of control (ABTS)
Abs (t) $_t$ is the absorbance of test sample.

***Hydrogen Peroxide Scavenging Assay***

A solution of 40 mM $H_2O_2$ was prepared in phosphate buffer (50 mM, pH 7.4). Five concentrations (0.01-0.05 mg/mL) of the leaf samples (AZH, AZE, AZM, TOH, TOE and TOM) and standard (Ascorbic Acid) were prepared. And then 1.2 mL of each concentration for all samples were measured and 0.6 mL

of $H_2O_2$ were added, the reaction was incubated at room temperature for 10 minutes, absorbance was thereafter taken at 230 nm. The control was 0.6 mL of $H_2O_2$ and 1.2 mL of $dH_2O$. Percentage inhibition as well as $IC_{50}$ values were obtained by calculation and extrapolation from line graph.

$$\% \text{ Inhibition} = \left[ \frac{\text{Abs(c)-Abs(t)}}{\text{Abs(c)}} \right] \text{ x } 100 \quad \text{…….. Equation (6)}$$

Where Abs (c) is the absorbance of control
Abs (t) $_t$ is the absorbance of test sample.

***Total Antioxidant Capacity***

Plant extract of 0.3 mL was combined with 3 mL of Phosphomolybdenum reagent (comprising of 0.6 M of Sulfuric acid, 28 mM Sodium phosphate and 4 mM Ammonium molybdate) solution in test tubes. Ascorbic acid was used as standard to generate the calibration curve (0.1 -0.5 mg/mL). The reaction mixture was capped and incubated at 95 ℃ for 90 minutes, then cooled to room temperature with reference to (Ravisankar et al., 2014). Absorbance was read against a blank of 0.3 mL methanol at 695 nm using UV/Vis Spectrophotometer. The concentration of each extract was extrapolated from the line equation generated from the ascorbic acid calibration curve and the result expressed as mg AAE/mL.

***Ferric Reducing Antioxidant Power***

Each extract solution (0.5 mL) was mixed with 1.25 mL of phosphate buffer (0.2 M, pH 6.6) and 1.25 mL of 1 % Potassium ferricyanide in a test-tubes as described by (Kajaria et al., 2012). The reaction mixture was heated on a water bath at 50 ℃ for 20 minutes, after which it was cooled to 25 ℃, thereafter 1.25 mL of trichloroacetic acid (10%) was added and vortexed. The incubated mixture was centrifuged for 10 minutes at 3,000 rpm. An aliquot of 1.25 mL was taken and mixed with 1.25 mL of distilled water and 0.25 mL of 0.1% $FeCl_3$. It was further incubated at room temperature for 10 minutes and absorbance was measured at 700 nm. Ascorbic acid (0.035-1 mg/mL) was used as standard, each concentration of AA was subjected to similar conditions as the leaf extracts, the absorbance values obtained from AA was used to generate a calibration curve, from which the concentration of each sample was extrapolated and expressed as ascorbic acid equivalent per g of powder (mg AAE/g powder). A high degree of absorbance indicated the stronger reducing power.

**Results and Discussion**

**Table 1:** Classes of secondary metabolites in the leaves of AZ and TO

| s/n | Secondary Metabolite | AZ | TO |
|---|---|---|---|
| 1. | Tannins | +++ | +++ |
| 2. | Flavonoids | +++ | ++ |
| 3. | Reducing sugar | +++ | ++ |
| 4. | Cardiac glycosides | +++ | ++ |
| 5. | Saponins | + | +++ |
| 6. | Terpenoids | ++ | +++ |
| 7. | Phenols | +++ | +++ |
| 8. | Alkaloids | +++ | +++ |
| 9. | Combined anthraquinones | +++ | - |

+: present, ++: present at moderate level, +++: moderate at high level: - not detected
AZ- *Albizia zygia* and TO-*Trema orientalis*.

Phytochemical study of medicinal plants is essential as it unveils the secondary metabolites and their probable medicinal application. Phytochemicals can be an antioxidant (e.g. phenolic, flavonoids, or terpenoids), anti-biotic (alkaloids and saponins), anti-obesity (flavonoids and saponins) anti-inflammatory (e.g. flavonoids), anti-fungal (e.g. tannins) etc. (Aiyelaagbe et al., 2009.

The phytochemical screening of AZ and TO leaves revealed the presence of tannins, flavonoids, reducing sugars, cardiac glycosides, saponins, terpenoids and alkaloids, while AZ alone was found to possess combined anthraquinones (Table 1). The positive test of AZ corroborates the assertion (Borges et al., 2020) about the West African AZ which normally tests positive to alkaloids, sterol, tannins, and haemolysis, unlike the AZ grown in Florida, which tests negative to alkaloids, sterol, tannins, and haemolysis, but tests positive to flavonoid. Also, Abere et al., (2014) reported the presence of alkaloids, tannins, flavonoids and saponins in the leaf of AZ. Karuppannan et al., (2013) as well reported the presence of triterpenoids, saponins, diterpenoids, lignans and pyridine glycoside in the leaf of AZ. Similarly, Odeyemi et al., (2014) also reported the presence of alkaloids, flavonoids, tannins, saponins and cardiac glycosides in the leaves of AZ. However, there was a report (Uwaya et al., 2017) on the absence of alkaloids, reducing sugar, tannins and anthraquinones in AZ which was in contrast to the findings of this study. These reports (Abere et al., 2014, Karuppannan et al., 2013, Odeyemi et al., 2014) are in support of the findings of this study on *Albizia zygia*.

The phytochemicals found in TO are tannins, flavonoids, reducing sugar, cardiac glycosides, saponins, terpenoids and alkaloids, while combined anthraquinones was not detected as found in AZ. The findings are similar to the previous reports

(Adjileye et al., 2019, Geetha et al., 2019). In a review (Adinortey et al., 2013), it was gathered that the leaf of TO contains triterpenoids, saponins, tannins and flavonoid. Also, some workers (Ahino-Mary and Ganthi 2017) reported that the leaf of TO contains terpenoids, alkaloids, phenolic compounds, saponins, tannins, and reducing sugars. They also reported the presence of sugars, amino acids, and anthraquinones, which was not detected in this study. A report (Hemalatha et al., 2013) succinctly attributed the antioxidant property of *Trema orientalis* to the presence of tannins and flavonoids. Tannins are known for their free radical scavenging property, hence are strong antioxidant. Also, flavonoids have been implicated for the antioxidant property (Varadharajan et al., 2022, Mutha et al., 2021, Ullah et al., 2020, Rasheed et al., 2019).

Results of the qualitative phytochemical tests were equally confirmed by the quantitative analysis of the leaf samples, which showed that AZ has higher quantity of flavonoids, reducing sugar, cardiac glycosides, alkaloids and slightly higher quantity of tannins than TO, while TO contains higher amount of saponins, terpenoids and phenols (Table 2).

**Table 2**: Quantitative phytochemicals in the leaves of AZ and TO

| s/n | Phytochemical | AZ (mg/100g) | TO (mg/100g) |
|---|---|---|---|
| 1. | Tannins | 37.92 ± 0.06 | 37.39 ± 0.44 |
| 2. | Flavonoids | 85.94 ± 0.23 | 68.65 ± 1.02 |
| 3. | Saponins | 45.33 ± 0.68 | 49.45 ± 0.46 |
| 4. | Reducing sugar | 32.58 ± 1.10 | 25.48 ± 0.14 |
| 5. | Cardiac glycosides | 68.53 ± 1.66 | 55.02 ± 1.19 |
| 6. | Terpenoids | 21.78 ± 0.10 | 22.91 ± 0.92 |
| 7. | Phenols | 53.62 ± 0.64 | 57.16 ± 2.07 |
| 8. | Alkaloids | 59.08 ± 0.36 | 54.83 ± 2.83 |

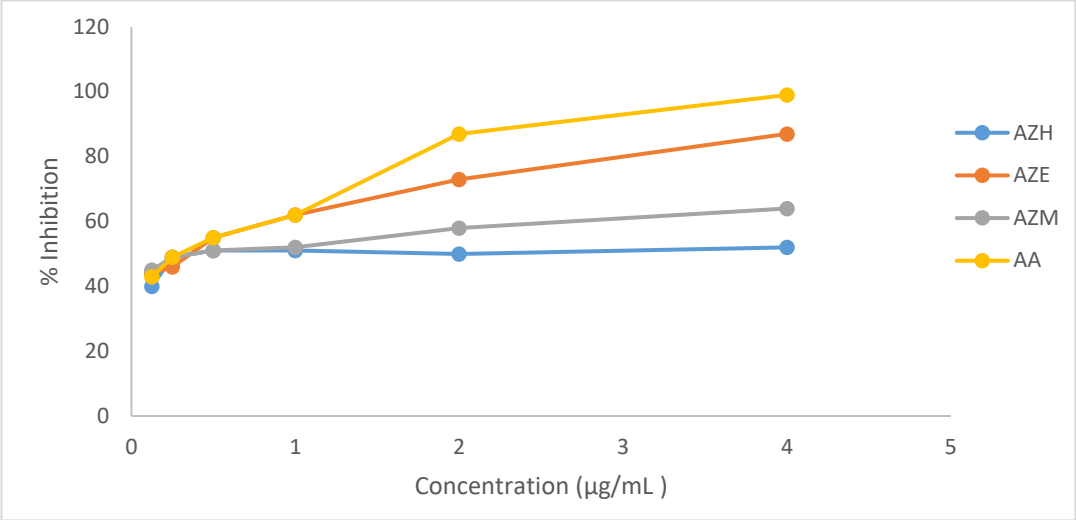
Each value is a mean of three determination ±SEM. AZ- *Albizia zygia* and TO-*Trema orientalis*.

Table 2 shows the quantity of named phytochemicals in AZ and TO. AZ had a slightly higher amounts of flavonoids, tannins, reducing sugar, and cardiac glycosides than TO. Cardiac glycosides which usually contain both glycosides (sugar) and aglycone (non-sugar) are known for their ability to increase the force of heart contraction without concomitant increase in oxygen consumption, they are also being implicated for their anti-cancer property (Skubnik et al., 2021, Morsy, 2017, Patel, 2016).

The qualitative and quantitative tests of both plants showed the presence of saponins. Though TO had a saponin value which was slightly above that of AZ. Saponins have a characteristic soap like nature which makes them foam in water (Fink and Flipp, 2022, Rai, 2021). They are glycosides made up of the hydro-phobic sapogenin and the hydrophobic sugar moiety (Guclu-Ustundag and Mazza, 2027). They possess cholesterol lowering property by preventing re-absorption, they have antioxidant activity and reduce the occurrence of stroke, tumors and cancers (Guclu-Ustundag and Mazza, 2027). There are reports (Adinortey et al., 2013, Uwaya et al., 2017, Ajayi et al., 2018, Fabowale et al., 2020) that confirmed the presence of saponins, flavonoids, tannins and triterpenoids in the methanol leave extract of TO. However, there was a report on the absence of saponins in TO (Panchal et al., 2015). This difference in the chemical composition of plants may be due to disparity in ecological region and soil contents available for plant up take.

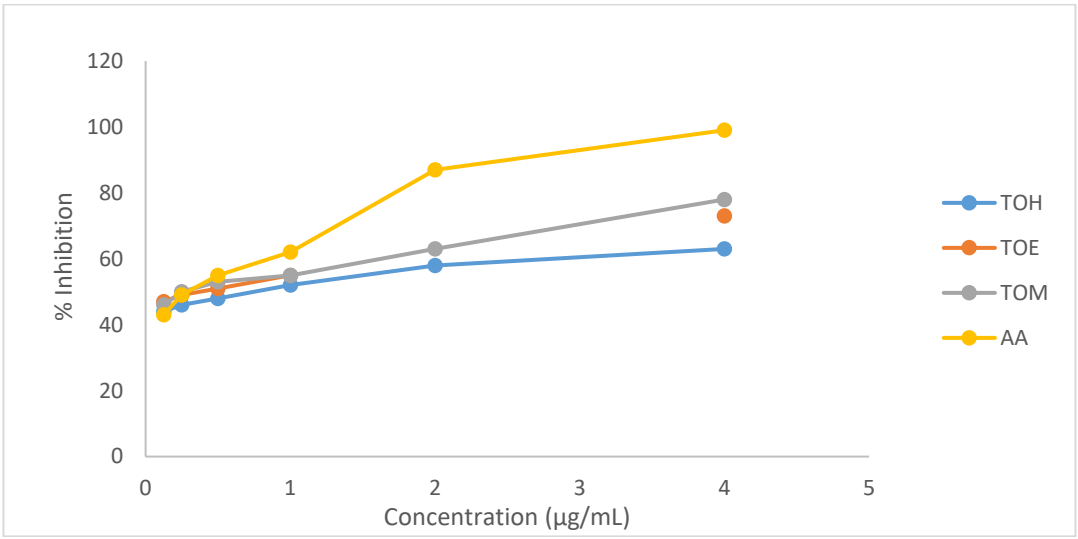
Flavonoids are known for their strong antioxidant property, they show free radical scavenging capacity, as well as antioxidative activity. Flavonoids are reputable for reducing lipid peroxidation hence possesses high antioxidant capacity, they thereby prevent cell damage, increases collagen fibrils, accelerates wound healing and promote the generation of protein (Varadharajan et al., 2012). The antioxidant studies of the two plants show that AZ would have higher antioxidant property than the TO as reflected in the quantity of the constituent flavonoids (Table 2). The antioxidant studies show that the free radical scavenging power of AZ is higher than that of TO.

Figure 1 and Figure 2 show the DPPH scavenging ability of AZH, AZE, AZM, TOH, TOE, and TOM in comparison to that of ascorbic acid, a standard antioxidant compound. The percentage of inhibition was plotted against the concentration (range from 0-4 µg/mL). The ethyl acetate extract of AZ (AZE) had the highest free radical scavenging effect, AZE significantly ($p < 0.05$) exhibited the highest percentage inhibition (87±0.009) at the highest concentration of 4 µg/mL, which translated to the least $IC_{50}$ value of 0.28 µg/mL. This is followed by the methanol extract of TO (TOM), TOM significantly ($p < 0.05$) exhibited a relatively high percentage inhibition (78±0.000) at the highest concentration of 4 µg/mL, which translated to an $IC_{50}$ value of 0.34 µg/mL. AZE had the lowest $IC_{50}$ value (0.28 µg/mL) which indicates high antioxidant property, this was closely followed by TOE with an $IC_{50}$value of (0.34 µg/mL) (Table 3). The hexane extracts of both plants AZH (2.06 µg/mL) and TOH (0.93 µg/mL) have highest $IC_{50}$ values which indicates they have little or no radical scavenging power.



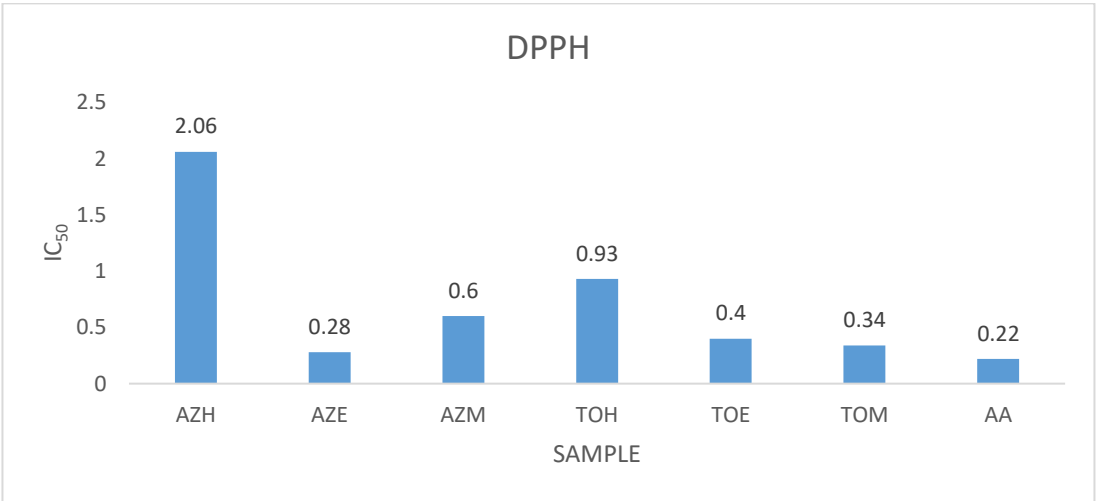

AZH- hexane extract of *A. zygia* AZE- ethyl acetate extract of *A. zygia* AZM- methanol extract of *A. zygia*

**Figure** 1: DPPH Radical Scavenging activity of AZH, AZE, AZM and Ascorbic Acid (standard)




TOH- hexane extract of *T. orientalis* TOE- ethyl acetate extract of *T. orientalis* TOM- methanol extract of *T. orientalis*

**Figure 2**: DPPH Radical Scavenging activity of TOH, TOE, TOM and Ascorbic Acid (standard)

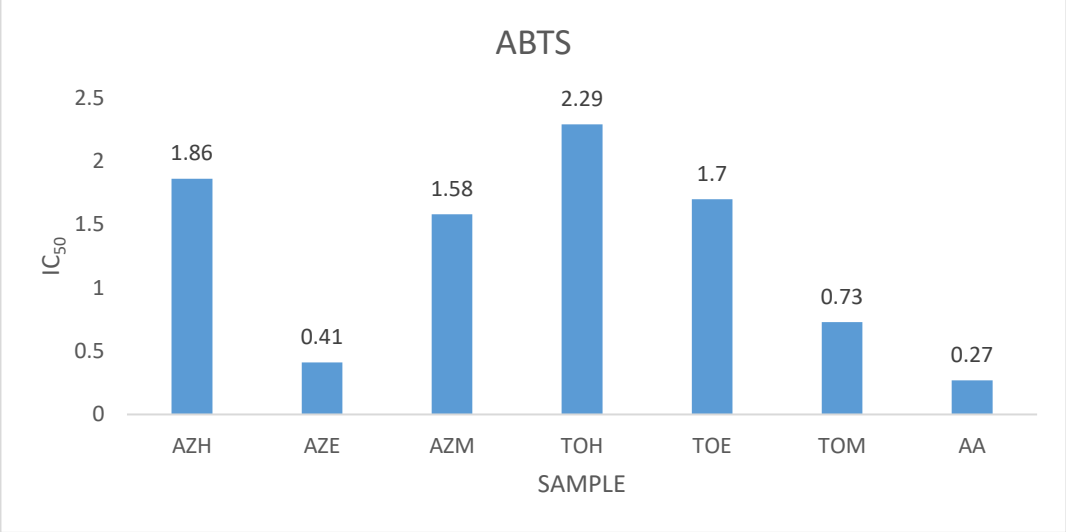



AZH- hexane extract of *A. zygia* AZE- ethyl acetate extract of *A. zygia* AA-Ascorbic Acid AZM- methanol extract of *A. zygia* TOH- hexane extract of *T. orientalis* TOE- ethyl acetate extract of *T. orientalis* TOM- methanol extract of *T. orientalis*

**Figure 3**: IC $_{50}$ values of the antioxidant activities of the extracts and standard drug using DPPH method

The antioxidant study using ABTS assay method gave a result similar to that of DPPH method. AZH, AZE, and AZM had an $IC_{50}$ values of 1.86 µg/mL, 0.41 µg/mL, and 1.58 µg/mL respectively, while the TOH, TOE and TOM had $IC_{50}$ values of

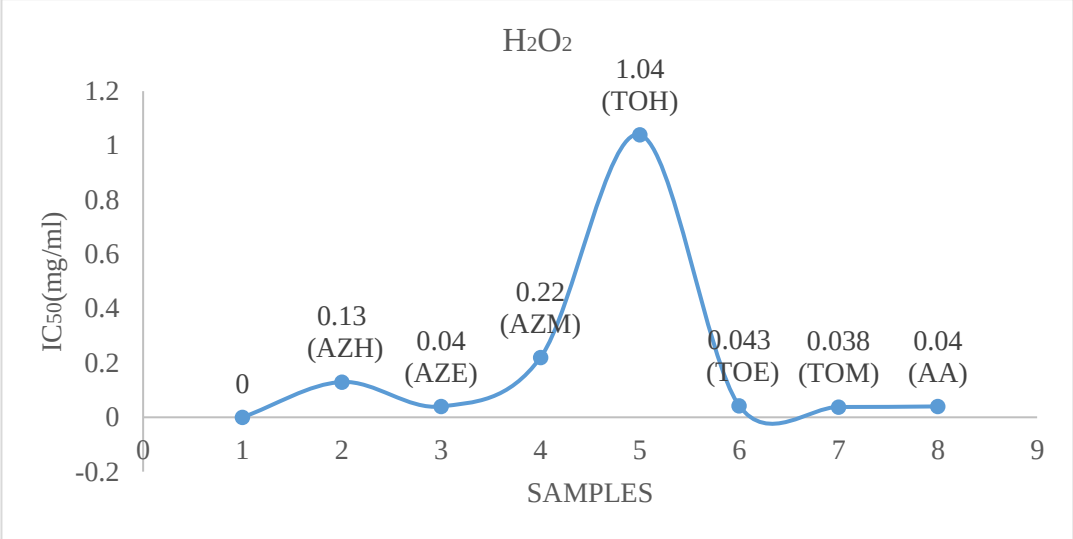
2.29 µg/mL, 1.70 µg/mL, and 0.73 µg/mL respectively, with the ascorbic acid having an $IC_{50}$ value 0.27 µg/mL. Here also, AZE had the lowest $IC_{50}$ values out of the six extract (0.41 µg/mL), followed by TOM (0.73 µg/mL), then AZM (1.58 µg/mL) and then TOE (1.70 µg/mL). The hexane extracts of the two plants maintained the lowest free radical scavenging abilities with the high $IC_{50}$ values of 1.86 µg/mL and 2.29 µg/mL for AZH and TOH respectively.




AZH- hexane extract of *A. zygia* AZE- ethyl acetate extract of *A. zygia* AA-Ascorbic Acid AZM- methanol extract of *A. zygia* TOH- hexane extract of *T. orientalis* TOE- ethyl acetate extract of *T. orientalis* TOM- methanol extract of *T. orientalis*

**Figure 4**: $IC_{50}$ values of the antioxidant activities of the extracts and standard drug using ABTS method.

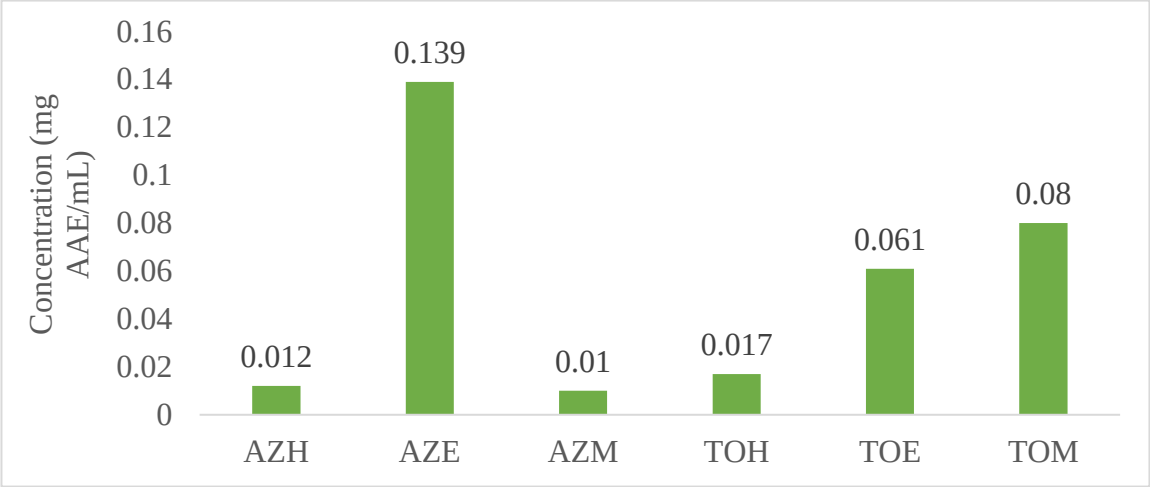
In $H_2O_2$ antioxidant assay, AZE and TOM had very close $IC_{50}$ values of 0.040 mg/mL and 0.038 mg/mL respectively. Interestingly, the standard drug had the same strength of activity with the $IC_{50}$ value of 0.040 mg/ml which fell in the range of the two test drugs-AZE and TOM.




AZH- hexane extract of *A. zygia* AZE- ethyl acetate extract of *A. zygia* AA-Ascorbic AcidAZM- methanol extract of *A. zygia* TOH- hexane extract of *T. orientalis*
TOE- ethyl acetate extract of *T. orientalis* TOM- methanol extract of *T. orientalis*

**Figure 5**: IC $_{50}$ values of the antioxidant activities of the extracts and standard drug using $H_2O_2$ method

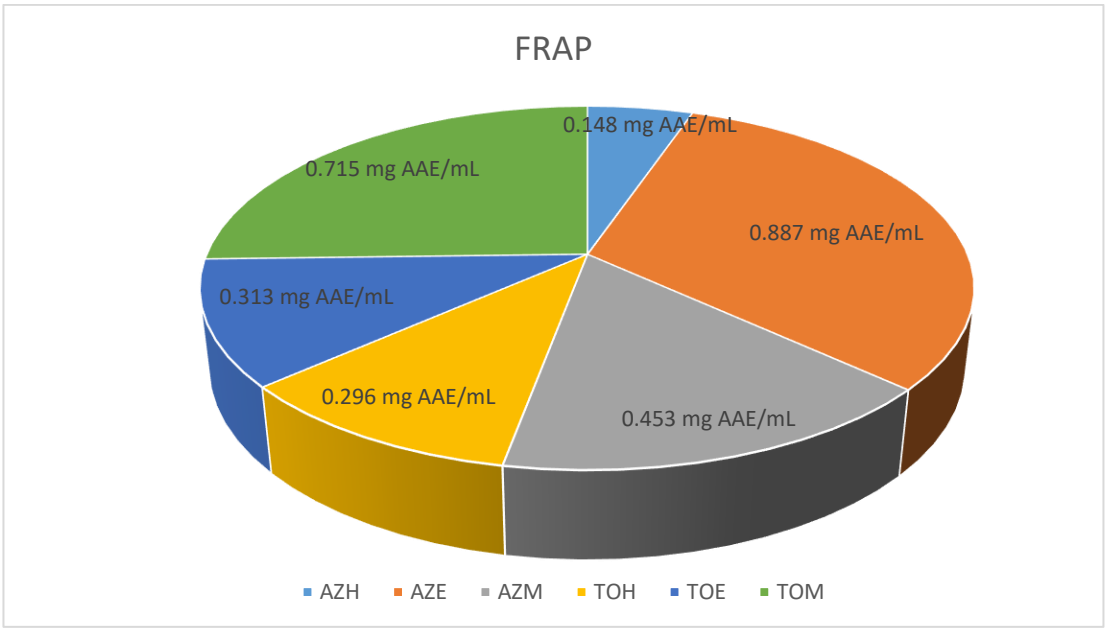
In TAC antioxidant assay, the ascorbic acid equivalent of each sample was determined from a calibration curve generated from varying concentration of AA standard (0.1- 0.5 mg/mL). The concentrations of AZH, AZE, AZM, TOH, TOE and TOM were 0.012, 0.139, 0.010, 0.017, 0.061, and 0.080 mg AAE/mL.




AZH- hexane extract of *A. zygia* AZE- ethyl acetate extract of *A. zygia* AZM- methanol extract of *A. zygia* TOH- hexane extract of *T. orientalis* TOE- ethyl acetate extract of *T. orientalis* TOM- methanol extract of *T. orientalis*

**Figure 6**: Ascorbic Acid equivalence of AZH, AZE, AZM, TOH, TOE, and TOM in TAC method

In FRAP antioxidant assay, the ascorbic acid (AA) equivalent of each sample was also determined from a calibration curve generated from varying concentration of AA standard (0.032-1 mg/mL). The concentrations of AZH, AZE, AZM, TOH, TOE and TOM were 0.148, 0.887, 0.453, 0.296, 0.313, and 0.715 mg AAE/mL.




AZH- hexane extract of *A. zygia* AZE- ethyl acetate extract of *A. zygia* AZM- methanol extract of *A. zygia* TOH- hexane extract of *T. orientalis* TOE- ethyl acetate extract of *T. orientalis* TOM- methanol extract of *T. orientalis*

**Figure 7**: Ascorbic Acid equivalence of AZH, AZE, AZM, TOH, TOE, and TOM in FRAP method.

The outcome of the study indicates that both AZ and TO plants have antioxidant properties and that the presence of flavonoids, phenols and terpenoids plays key role in the antioxidant properties of the selected plants. In both samples, and all antioxidant assays used, the least free radical scavenging capability was found in the hexane extracts, this is because the n-hexane solvent would only extract the fatty compounds with long straight chains, attributed to low antimicrobial and antioxidant activities (Borges et al., 2020, Aldughaylibi et al., 2022). It was observed that the ethyl acetate extract had the highest antioxidant activity compared with the other extracts. Although in the $H_2O_2$ antioxidant assay TOM had a slightly higher $IC_{50}$ value of 0.038 mg/mL as against 0.040 mg/mL of AZE. The findings in this study are similar to the previous research outcomes (Oloyede and Ogunlade, 2013). 54], where the extracts of AZ stem bark had better antioxidant activities than the three standards including ascorbic acid, α -tocopherol and butylated hydroxyl anisole in both hydrogen peroxide and DPPH methods. Generally, TOM ranked second in all the five assays used for the antioxidant study. It was previously reported that TO exhibited an anti-radical activity that was similar to that of ascorbic acid (Salprima et al., 2011).

**Conclusion**

The findings of this study reveal AZ and TO leaves contain tannins, phenols, flavonoids, saponins, cardiac glycosides, reducing sugar, terpenoids and alkaloids. The quantitative analysis of the plant constituents also indicates that AZ has slightly higher quantity of tannins, flavonoids, reducing sugar, cardiac glycosides and alkaloids than TO, While TO contains higher amount of saponins, terpenoids, tannins and phenols than AZ. The non-polar hexane extracts have least antioxidant activity compared to mid polar and polar extracts (the ethyl acetate and methanol extracts respectively). On the whole, though both AZ and TO have antioxidant properties, and AZE has the strongest free radical scavenging potential.

**Consent for publication**

All authors have given consent for the publication.

**Conflict of Interest**

The authors declare that there is no conflict of interest.

**Data availability**

Data will be made available on request**.**

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