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**OD Olukanni**

Department of Biochemistry,  
Redeemer's University, P.M.B.  
203 Ede, Osun State, Nigeria

**E Lugard**

Department of Biochemistry,  
Redeemer's University, P.M.B.  
203 Ede, Osun State, Nigeria

**E Emmanuel**

Department of Biochemistry,  
Redeemer's University, P.M.B.  
203 Ede, Osun State, Nigeria

**AT Olukanni**

(a) Department of Biochemistry,  
Redeemer's University, P.M.B.  
203 Ede, Osun State, Nigeria  
(b) Department of Cell Biology  
and Genetics, University of  
Lagos, P.M.B. 56, Akoka, Lagos  
State, Nigeria

**F Ayoade**

Department of Biological  
Sciences, Redeemer's University,  
P.M.B. 203 Ede, Osun State,  
Nigeria

**EU Durugbo**

Department of Biological  
Sciences, Redeemer's University,  
P.M.B. 203 Ede, Osun State,  
Nigeria

**Corresponding Author:**

**OD Olukanni**

Department of Biochemistry,  
Redeemer's University, P.M.B.  
203 Ede, Osun State, Nigeria

## Antioxidant and *in vitro* anti-inflammatory activities of *Albizia zygia* (DC) J.F. Mebr and the evaluation of its phytochemical constituents

**OD Olukanni, E Lugard, E Emmanuel, AT Olukanni, F Ayoade and EU Durugbo**

**Abstract**

Medicinal plants are promising sources of natural antioxidant and anti-inflammatory agents and are readily available for ameliorating the increasing burden of diseases related to oxidative damage and inflammation. In the present study, the antioxidant and anti-inflammatory potential of methanol extract of *Albizia zygia*, which is used traditionally as a therapeutic agent, was investigated. The antioxidant activity was examined using DPPH scavenging, and hydrogen peroxide scavenging. The anti-inflammatory activity was studied *in vitro* as the inhibition of proteinase and protein denaturation. The functional groups in the plant extract were identified using Fourier-transform infrared spectroscopy (FTIR), and the bioactive compounds were determined using Gas Chromatography and Mass Spectroscopy (GCMS). The plant extracts demonstrated a positive concentration-dependent antioxidant with IC<sub>50</sub> of 152 µg for DPPH, and anti-inflammatory effects with IC<sub>50</sub> of about 48 µg for DPPH for protein denaturation. The results of FTIR confirmed the presence of relevant functional groups, and the GCMS revealed the presence of various compounds like docosanoic acid, methyl stearate, and squalene, which are known to exhibit antioxidant and anti-inflammatory activities. In conclusion, these results showed that the extracts of *Albizia zygia* could protect against oxidative stress due to the presence of docosanoic acid, methyl stearate, and squalene present in the leaves.

**Keywords:** Anti-oxidant activity, anti-inflammatory potential, GCMS, DPPH

### 1. Introduction

The antioxidant and anti-inflammatory effects of plants are well documented; the diversity of plants and availability varies from region to region. Besides the medicinal values, plants provide an assortment of assets that add to the essential needs of nourishment, apparel, and shelter. However, the medicinal plants are of economic and health significance because they play vital roles in alleviating human suffering. The therapeutic properties of numerous plants have been reported from various societies<sup>[1, 2]</sup>, and their usage is either in the form of traditional preparations or as pure active principles<sup>[3]</sup>. Isolating active principles are, however, not cost-effective, and it takes plants, products out of reach of the populace<sup>[4]</sup>. It is thus essential to understand the phytochemical components of plants as they are in unprocessed plants.

About two-thirds of the world's plant species have medicinal value, and most of these plants possess favorable antioxidant potential<sup>[5]</sup>. Plants antioxidants counteract the Reactive Oxygen Species (ROS), quenching their damaging effects. Some of the antioxidants are naturally occurring or formed in response to abiotic and biotic stress conditions. In response to this condition, plants generate several low molecular weight antioxidants such as vitamin C, vitamin E, phenolic acids<sup>[6]</sup>, and high molecular weight antioxidant secondary metabolites such as tannins that can scavenge free radicals, chelate metal ions and also act as reducing agents. In plants, antioxidant activity is not limited to a particular family or part of a plant<sup>[6, 7]</sup>. Besides the antioxidant activities, plants exhibit other bioactivities such as the anti-inflammatory effects.

Inflammation occurs when living tissues respond to injury initiated by infection, chemical, or physical stimuli. These often result in cellular damage, which activates transcription factors that control the expression of many inflammatory mediators such as eicosanoids, biological oxidants, and cytokines<sup>[8]</sup>. Inflammation also results in the accumulation of plasma fluid and

blood cells in sites of injury and cause edema [8, 9]. Despite inflammation being a defense mechanism by the body, one of its prominent mechanisms involves the release of protease, which breaks down targeted proteins, thus causing associated pains [10]. Protease inhibitors are, therefore, good candidates for use as anti-inflammatory agents. The anti-inflammatory effects of synthetic compounds and natural extracts are often first tested *in vitro* using protease or protein denaturation inhibition. According to Libby [11], common effector mechanisms of inflammation contribute not only to tissue injury, but also to oxidative stress, extracellular matrix remodeling, angiogenesis, and fibrosis. Currently, the management of inflammation commonly involves the use of non-steroidal anti-inflammatory drugs, glucocorticoids, and immunosuppressant drugs [12].

*Albizia zygia*, which is commonly called Senegal Rosewood, is of the family Leguminosae. It is widely found in tropical Africa, and it is a medium-sized, deciduous, gum-producing, shade-tree. Its timber is durable and immune to termites' attack, and it thus of economic importance. In Africa, the leaves and the bark are used as traditional remedies to treat fever, malaria, diarrhea, and edema, conjunctivitis [13]. Odugbemi [14] had associated the bark with the treatment of arthritis and sprain. They are also used as an anti-cough, aphrodisiac, as a worm expeller and to counter female sterility. The roots are toxic and purgative, administration as an enema in overdose can cause death by intestinal hemorrhage. The methanol stem bark extract of *Albizia zygia* was found to have antioxidant and analgesic properties [15]. The flowers of *Albizia spp.* are commonly used to treat anxiety, depression, and insomnia in traditional Chinese medicine [16]. Appiah-Opong *et al.* [17] had reported the cytotoxic effects of extracts and root fractions of *A. zygia* from Ghana against human T-lymphoblast like leukemia (Jurkat), prostate (LNCap) and breast (MCF-7) cancer cell lines. They observed that both aqueous and hydroethanolic root extracts were more cytotoxic to Jurkat cells than the other cell lines. However, some of the fractions were non-cytotoxic. On the contrary, the petroleum ether fraction was cytotoxic towards MCF-7 cells. The hydroethanolic extract of the root exhibited apoptosis via induction of DNA fragmentation in Jurkat cells. Cell morphological changes appeared consistent with the extract-mediated cytotoxicity and DNA fragmentation. Flow cytometric and mitochondrial membrane potential assays also showed significant apoptotic induction confirming apoptosis by the *A. zygia* root extract. There is, however, a dearth of information on the anti-inflammatory activities of the extracts of this plant. In this study, the antioxidant activities and anti-inflammatory activities of the methanol extract of *Albizia zygia* were evaluated.

## 2. Materials and methods

### 2.1 Plant collection and identification

Fresh leaves of *Albizia zygia* were collected within the premises of Redeemer's University Ede, Osun State, Nigeria. The plant specimen was initially identified in the Department of Biological Sciences, Redeemer's University. It was later authenticated at the University of Lagos Herbarium, where a voucher specimen was deposited, and the voucher number LUH 8213 allocated.

### 2.2 Extraction and preparation of plant extracts

The plant materials were washed with distilled water and air-dried at room temperature for five days. The leaves obtained were after that pulverized using a mechanical blender.

Approximately 400 g were macerated in 800 mL methanol for 72 hours in a Grant shaker set at 120 rpm. The solution was filtered with cheesecloth, concentrated using a rotary evaporator (40 °C), and the concentrate was dried to powder in a hood. The powdered extracts were stored in a desiccator at room temperature until further use [18].

### 2.3 Phytochemical screening

The crude methanol leaf extract of *A. zygia* was tested for the presence of phytochemicals such as tannins, saponins, steroids, terpenoids, and flavonoids using standard methods. Some of the phytochemicals were also quantified.

#### 2.3.1 Qualitative phytochemical screening

Powdered plant sample (0.3 g) was dissolved in 20 mL of distilled water, boiled gently, cooled, and filtered. The filtrate was used for the phytochemical tests. The presence of tannins was observed as the formation of blue-black or brownish-green solution on the addition of 3 drops of 0.1% ferric chloride solution to filtrate of the extracts solution. Phlobatanins was confirmed with the presence of red precipitate on the addition of 5 mL of 1% aqueous hydrochloric acid was added to 10mL of the plant extract and boiled for 10 min. Saponins were detected qualitatively as the formation of emulsion on the addition of 3 drops of olive oil to frothing extract's solution achieved by vigorous shaken. Salkowski test was used for steroid detection; a red color observed in the lower chloroform layer indicates the presence of steroids on the addition of an equal volume of concentrated sulphuric acid (2 mL) of the filtrate. The presence of a reddish-brown coloration at the interface of chloroform (1 mL) and sulphuric acid (1.5 mL) added to 2.5 mL of the filtrate shows a positive test of terpenoids. Finally, the appearance of yellow coloration which disappeared on standing, when 5 mL of 1.0 M dilute ammonia solution was added to 10 mL of the aqueous filtrate, shows the presence of flavonoids

#### 2.3.2 Quantitative phytochemical screening

Quantitative screening of phenol and flavonoids were done using the spectrophotometric of Jia *et al.* [19], and Marinova *et al.* [20], respectively. Phenols react with phosphomolybdic acid in Folin-Ciocalteu reagent to produce a blue-colored complex in an alkaline medium, which was measured spectrophotometrically. Briefly, equal volume (1 mL) of Folin-Ciocalteu reagent was added to the sample and allowed to stay for 3 min. Then 1 mL of saturated Na<sub>2</sub>CO<sub>3</sub> solution was added, and the mixture was diluted to 10 mL with distilled water. The reaction mixture was kept in the dark for 90 min, and the absorbance was taken at 725 nm. Gallic, the standard, and distilled water, the control, were given the same treatment. In the method of Marinova *et al.* [20], flavonoids react with aluminum chloride to produce a colored product, which was measured spectrophotometrically. The extract (1 mL) was added to 4 mL of distilled water and 0.3mL of 5% NaNO<sub>2</sub>. After 5 min, 0.3mL of 10% AlCl<sub>3</sub> was added, followed by 2 mL of 1 M NaOH, and the mixture was adjusted to 10 mL. The solution was mixed properly, and the color intensity of the mixture read at 510 nm. Gallic acid and distilled water were also used as the standard and control, respectively.

### 2.4 Antioxidant screening

The antioxidant potentials of the plant extracts were determined as hydrogen peroxide scavenging, and DPPH

## Scavenging.

**2.4.1 Hydrogen peroxide scavenging activity assay**

The ability of plant extracts to scavenge hydrogen peroxide was determined according to the method of Ruch *et al.*,<sup>[21]</sup>. A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4), and concentration was determined spectrophotometrically at 230 nm. The Plant extract (25-50 µg/mL) in distilled water was added to a hydrogen peroxide solution (0.6 mL, 40 mM), and the absorbance of hydrogen peroxide at 230 nm was determined after 19 min against a blank solution in phosphate buffer without hydrogen peroxide. The percentage of scavenging of hydrogen peroxide of plant extracts and standard compounds was calculated using the following equation:

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100\%$$

Where; Absorbance of control = All reagent without the extract.

**2.4.2 DPPH (2, 2-Diphenyl-1-Picrylhydrazyl Radical) scavenging activity**

The scavenging ability of the leaf extract towards the stable free radical DPPH was measured by the method of Mensor *et al.*<sup>[22]</sup>. To 1 mL plant extract or ascorbate standard (1 mg/mL) in a test tube, 2 mL of methanolic DPPH (1 mM) was added and incubated at 37°C for 30 min. Controls (blank) without standard and samples were also prepared. Change in absorbance was measured at 515 nm, and percentage inhibition was calculated as:

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100\%$$

**2.4.3 Determination of reducing power of extract**

The reducing capability of *A. zygia* methanolic extract was measured as its reduction of Fe<sup>3+</sup> to Fe<sup>2+</sup>, as described by Hinneburg *et al.*<sup>[23]</sup>. Different concentrations of extracts in 1 ml of water were mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium hexacyanoferrate (1%). The mixture was heated at 50°C for 30 min, cooled, 2.5 ml of trichloroacetic acid (10%) was added to the mixture, centrifuged at 3000 rpm for 10 min. Three aliquots (125 µL) of the supernatant were transferred into a 96-well microplate, after which 125 µL of distilled water and then 25 µL of FeCl<sub>3</sub> (0.1% aqueous) were added. The reductive power was evaluated at 700 nm against a standard curve of ascorbic acid. Triplicate independent tests were performed, and the reducing activity of the extract was expressed in milligram Equivalent Ascorbic Acid per gram of extract (mg AAE/g).

**2.5 Anti Inflammatory****2.5.1 Proteinase inhibitory activity**

Proteinase inhibitory activity was determined spectrophotometrically according to the method modified by Oyedepo and Famurewa<sup>[24]</sup>. The reaction mixture (2 mL) was containing 0.06 mg trypsin, 1 mL 20mMTris-HCl buffer (pH 7.4) and 1 mL of different concentrations (100 – 500 µg/mL) of extract. The mixture was incubated at 37°C for 5 min, and then 1 mL of 0.8% (w/v) casein was added, before further incubation for 20 min and the 2 mL 70% perchloric acid was added to arrest the reaction. The cloudy suspension formed was centrifuged, and the absorbance of the supernatant was read at 210 nm against the buffer blank. The percentage

inhibition of proteinase inhibitory activity was calculated as:

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100\%$$

the control contains all reagents without the extract.

**2.5.2 Protein Denaturation Assay**

Protein denaturation was performed as described by Elias *et al.*<sup>[25]</sup> with slight modifications. Test solution consisting of 1ml of different concentrations of test sample ranging from 100-500 µg/ml or standard acetylsalicylic acid 200 µg/ml was mixed with 1 mL of egg albumin solution (1mM) and incubated at 27 ±1°C for 15 min. Denaturation was induced by keeping the reaction mixture at 70°C in a water bath for 10 min. After cooling, the turbidity was measured spectrophotometrically at 660 nm. Percentage inhibition of denaturation was calculated from control where no drug was added. Each experiment was done in triplicate, and the values were recorded as mean or readings.

**2.6 FTIR and GCMS analysis**

The determination of functional groups and bioactive components of extracts of *A. zygia* was determined using FTIR and GCMS analyses.

**2.6.1 Fourier transform infrared spectrophotometer (FT-IR)**

Dried powder of methanol extracts of the stem and leaves of *A. zygia* were used for FT-IR analysis. 10 mg of the dried extract powder was blended in 100 mg of KBr pellet, in order to prepare the translucent sample in discs. The powdered sample of each plant specimen was loaded in the FT-IR spectrometer model 8400S (Shimadzu, Japan), with a scan range from 500 to 4000 cm<sup>-1</sup>.

**2.6.2 Gas chromatography-mass spectrometry (GCMS)**

The GCMS analysis of bioactive compounds from the different extracts of the leaves and stem of *A. zygia* was done using Agilent Technologies GC systems with GC-7890A/MS-5975C model (Agilent Technologies, Santa Clara, CA, USA) equipped with HP-5MS column (30 m in length × 250 µm in diameter × 0.25 µm in thickness). Spectroscopic detection by GCMS involved an electron ionization system that utilized high energy electrons (70 eV). Pure helium gas (99.995%) was used as the carrier gas with a flow rate of 1 mL/min. The initial temperature was set at 50 –150°C with an increasing rate of 3°C/min and a holding time of about 10 min. Finally, the temperature was increased to 300°C at 10°C/min. One microliter of the prepared extracts (1%) diluted with respective solvents was injected in a splitless mode. The relative quantity of the chemical compounds present in the extracts of *A. zygia* was expressed as a percentage based on the peak area produced in the chromatogram.

**3. Results****3.1 Phytochemical analysis**

The result of the preliminary qualitative screening for phytochemical constituents in the methanolic extracts of *A. zygia* leaves reveals the presence of tannins, phenols, saponins, flavonoids, and the absence of phlobatannins and terpenoids. The total phenolic and flavonoid contents of *A. zygia* showed a yield of 34.03 and 4366 µg GAE /g, respectively.

### 3.2 Anti-oxidant analysis

The antioxidant activities of the methanol extracts of *A. zygia* were monitored in the form of hydrogen peroxide scavenging, and DPPH scavenging activities. The percentage of hydrogen peroxide scavenging activity of the methanol extract of *A. zygia* showed a dose-dependent activity ranging from

26.64±3.42 to 43.34±2.64 (Figure 1A), and that of the ascorbic acid stand was from 26.64±3.42 to 43.34±2.64. Similarly, the DPPH scavenging activities showed dose-dependent activities with an estimated IC<sub>50</sub> of 152 µg for *A. zygia* and 34 µg for the standard (Figure 1B).

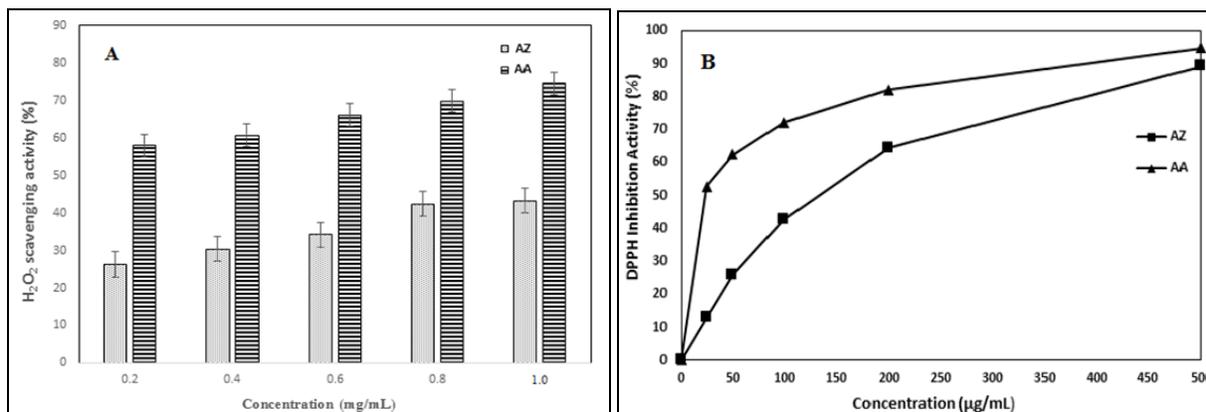


Fig 1(A, B): Anti-oxidant analysis

### 3.3 In vitro anti-inflammatory analysis

The *in vitro* anti-inflammatory activities of *A. zygia* were determined as proteinase inhibitory activities and protein denaturing inhibitory activities (Table 1) Proteinase inhibitory activity of different concentration of extracts showed the

inhibition levels between of 8.61–19.29%, while the % inhibition was in the range of 63.16 – 71.83% at 100 -500 µg/ml concentration of the extracts. The standard (100 µg/mL) showed 59.47 and 61.94% for the proteinase inhibition and protein denaturation studies, respectively.

Table 1: *In-vitro* anti-inflammatory activities of *A. zygia* using proteinase inhibition and protein denaturation assays

Concentration ug/mL	Proteinase Inhibition %	Protein Denaturation %
100	8.61 ± 0.19	63.16 ± 0.40
200	11.52 ± 0.14	64.80 ± 0.30
300	13.30 ± 0.67	66.16 ± 0.40
400	15.77 ± 0.25	68.30 ± 0.40
500	19.29 ± 0.09	71.83 ± 0.20
Aspirin (100)	59.47 ± 0.00	64.96 ± 0.02

### 3.4 Characterization of the methanol leaf extracts of *A. zygia*

Figure 2 shows the result of the FT-IR analysis of the purified methanol leave extract of *A. zygia*. The FTIR spectrum of the extract presents a broad peak at 3385 cm<sup>-1</sup> assigned to the stretching vibration of -OH from phenolic compounds [26]. The presence of infrared bands at 2852 cm<sup>-1</sup>, and 2735 cm<sup>-1</sup> are due to C-H stretching of aldehydes, while the peaks at 2075 and 1701 cm<sup>-1</sup> are attributed to the presence of hydrogen-

bonded C=O bonds. The 1610 cm<sup>-1</sup> and 1518 cm<sup>-1</sup> peaks have also been linked to conjugated C=C of aromatic compounds; this was confirmed by the 1446 cm<sup>-1</sup> peak typical of aromatic stretching vibrations [27]. The infrared bands at 1375 and 1284 cm<sup>-1</sup> are those of C-N stretching vibrations of aromatic compounds. The phosphate esters, P-OR stretching was recorded at 1037 cm<sup>-1</sup>, while other esters are between 700 and 900 cm<sup>-1</sup> [28].

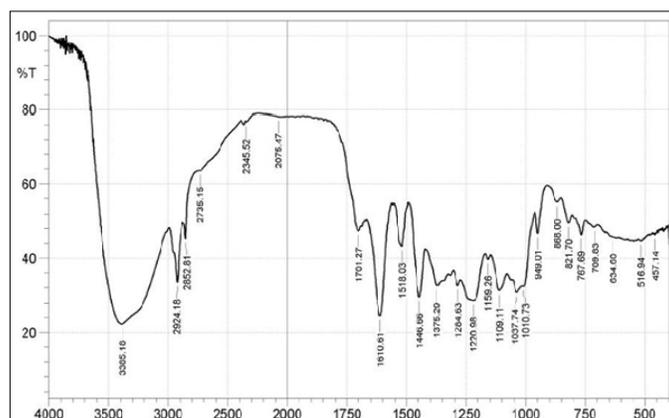


Fig 2: FT-IR analysis of the methanol extract of *Albizia zygia*.

The GC–MS study revealed the presence of nine compounds in methanol extract of the *Albizia zygia* leaves (Table 2). Prominent among the phytochemicals are 9–octadecanoic acid (25.82%), docosanoic acid (20.89%), methyl stearate (15.68%), and cis – 13 – octadecanoic acid (10.31%).

**Table 2:** constituents of the methanol extracts of *Albizia zygia* as shown by GCMS

S/N	Retention time	Area (%)	components
1	18.193	1.91	1, 10 decandiol
2	19.675	6.19	Hexadecanoic acid
3	22.513	25.82	9 – octadecanoic acid
4	22.627	10.31	Cis – 13 – octadecanoic acid
5	22.914	15.68	Methyl Stearate
6	25.998	7.40	18 – methyl nonadecanoate
7	28.344	20.89	Docosanoic acid
8	30.249	2.97	Tetracosanoic acid
9	31.096	8.83	Squalene

#### 4. Discussion

Firstly, the results of the evaluation of phytochemical constituents of *Albizia zygia* methanol extract showing the presence of tannins and saponins and the quantitative phenolic acid and flavonoids estimation demonstrating a substantial amount of phenolic compounds and particularly flavonoids is consistent with previous reports in *Albizia* species [13, 29]. The presence of phenolic compounds in *A. zygia* is further substantiated by the recent isolation of two oleanane-type saponins, which were named zygiaosides [30]. The presence of such phytochemicals such as the phenolics, and more specifically, flavonoids in plants have been associated with their bioactivities [31].

Generally, plants are rich in antioxidants, which are molecules that ameliorate the toxic effects of free radicals in tissues. Free radicals or reactive oxygen species (ROS) such as singlets oxygen, hydrogen peroxides, and oxygen radicals are by-products of normal metabolic processes in both animal and plant tissues. ROS have been linked with aging, stemming from the harmful effects such as structural or functional damage to the cell, enzymes, and genetic material, and the development of inflammation and cancers. Hence, the antioxidant activity of the methanol extract of *A. zygia* was investigated. The inhibition of Hydrogen peroxide and DPPH scavenging activities is with the range observed in other plants. In this study, the hydrogen peroxide scavenging activity of the methanol extract of *A. zygia* was determined. Dose-dependent activities observed in this study is similar to that detected by Limin *et al.* [32], who worked on *Solanum nigrum* L. The disparity between the values of the plant extract to that of the ascorbic acid could be understood since the latter is in pure form while the extract is in its crude form. A similar trend was observed in the DPPH radical scavenging. DPPH radical scavenging assay is typically used to estimate the anti-radical activity of known antioxidants or extract with possibly antioxidant activities. The compounds (hydrogen donors) that interact with DPPH radicals reduce the radicals to corresponding hydrazine. The redox-active antioxidants like flavonoids present in plants contribute to their DPPH radicals scavenging. Moreover, compounds having significant reducing potential can be considered as potential candidates for designing an anti-inflammatory drug that may inhibit the enzyme cyclooxygenase: a key enzyme involved in recruiting inflammation process [33]. In this present study, the results of the DPPH radical scavenging activity of the methanol extract of *A. zygia* showed high values that are comparable to other studies but well below that of the ascorbic acid standard.

Ramya *et al.* [34], have reported that the methanol extracts of purified *S. nigrum* possess high antioxidant activity. The comparative strength of DPPH radical scavenging of the extract with the amount of total phenolic shows some levels of correlation. The similarity between the trend of hydrogen peroxide scavenging and DPPH scavenging is an indication of the possible presence of antioxidant components, which could be further investigated.

Phenolics, terpenoids have been demonstrated to exhibit anti-inflammatory activities by exerting anti-oxidative properties in reducing O<sup>2-</sup> and Malondialdehyde (MDA) production, plasma extraversion and cell migration mainly of leukocytes and potentiate the activity of superoxide dismutase in radical scavenging. In the present investigation, the *in vitro* anti-inflammatory effect of extract of *S. nigrum* and *A. zygia* leaves was evaluated using protein denaturation and protease inhibitor assay. The present findings exhibited dose-dependent anti-inflammatory activity of all analyzed samples in both assays comparable to Aspirin and Acetyl-salicylic used as standard. The anti-inflammatory and antioxidant activities of the extracts from *A. zygia* may contribute to its usefulness in remedying inflammatory ailments such as, the ones for which this plant extracts are used to treat. Although there is a dearth of information on the anti-inflammatory activities of the plant, Amoateng *et al.* [35], had investigated the antipsychotic and antidepressant potentials of hydroethanolic leaf extracts of *A. zygia* in murine models of psychosis and depression on mice. The results provided initial evidence that *A. zygia* extracts do possess antipsychotic-like effects but not antidepressant-like activity.

In a bid to investigate the active principle present in the extract, FTIR and GCMS analyses were conducted. The FTIR is a valued technique for the identification of functional groups in compounds. It provides a unique spectrum with molecular fingerprints [36]. The functional groups of the active components present in the present study were identified using the FTIR Spectrum based on the peaks values and wavenumber in the region of IR radiation. The results of FT-IR analysis confirmed the presence of alcohols, phenols, alkanes, aromatic ring, alkyl halides, ether linkage, and alkynes. This result is similar to the findings of Pakkirisamy *et al.* [37], who performed a similar experiment using methanol extract of *Curcuma caesia*. The GCMS chromatogram of the major compounds detected revealed that docosanoic acid, pentadecanoic acid, 9-Octadecanoic acid, Squalene, Trichothec-9-en-ol, Methyl Stearate, Arsenous acid, are present as the major components in the methanol extract of the plants with the indicated antioxidant, anticancer, antitumor, antibronchitic, anti-inflammatory activities. Previous studies on docosanoic acid revealed that it has high antioxidant activity as well as it could be used as a skin-conditioning agent, emulsifying agent, and surfactant [38]. Sharma *et al.* [39] reported the synergistic effect of fatty acids in the antioxidant, antibacterial and other activities of *P. sylvestris* leaf, and they also argued that pentadecanoic acid has as strong anti-inflammatory, antibacterial and anti-cancerous activity. Fatty acids also serve as precursors of certain hormone-like substances that regulate a wide range of functions such as blood pressure, blood lipid loss, and immune response. Also, GCMS studies have revealed antiarthritic, anticancerous, hypocholesterolemic, nematocidal, pesticide, lubricant, and antiandrogenic activities that were also reported by Kumar *et al.* [40], and Aparna *et al.* [41]. Furthermore, squalene, which is among the major components of the extract, has been reported to have cardioprotective,

antimicrobial, anticancer, and antioxidant activities, the latter as a topical emulsion and oral administration<sup>[42]</sup>.

## 5. Conclusion

From the present investigation, it can be concluded that the phytochemical components and their pharmacological activities which have been identified from methanol extracts of *and A. zygia* has potent antioxidant and anti-inflammatory effects exhibited through different mechanisms. The present findings will strengthen the traditional claims of leaves of *A. zygia* as a remedy for pain and inflammation. Phytochemical analysis revealed the presence of phenols and flavonoids, which were further confirmed by qualitative and quantitative phytochemical studies, and that might have contributed to the said effects. There is, however, a need for further studies to ascertain the extract's bioactive principles, toxicity profile, and agronomic products.

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