

**INTERNATIONAL JOURNAL OF SCIENCE FOR GLOBAL SUSTAINABILITY****Assessment of Phytochemicals, Antioxidant and Antimicrobial Activities of Aqueous Ethanol Extract and Fractions of *Azadirachta indica* Stem Bark****<sup>1</sup>Feyisara Banji Adaramola\*, <sup>2</sup>Samuel Adegoke Adewole, and <sup>3</sup>Oluwaseun Adetayo Adewole**<sup>1</sup>Department of Basic Sciences, Chemistry Unit, Babcock University, Ilishan-Remo, Nigeria<sup>2</sup>Department of Agriculture and Industrial Technology, Babcock University, Ilishan-Remo, Nigeria<sup>3</sup>Department of Biochemistry, School of Basic Medical Sciences, Benjamin S. Carson (Snr.) College of Health and Medical Sciences, Babcock University, Ilishan-Remo, Nigeria\*Corresponding author's Email & Phone No.: [adaramolaf@babcock.edu.ng](mailto:adaramolaf@babcock.edu.ng); +2348039446793

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**ABSTRACT**

In this study, the aqueous ethanol extract of *Azadirachta indica* stem bark was obtained by Soxhlet extraction and partitioned into different solvents. The extract and fractions were then assessed for their phytochemical contents, antioxidant, and antimicrobial activities. The results showed that saponins, flavonoids, phenol, and tannins were present in all the samples. Also, the ethanol extract had the highest flavonoid (281.15 mgQE/g), phenolic (714.82 mgGAE/g), and tannin (156.54 mgTAE/g) contents, as well as the highest DPPH scavenging activity (95.71%) and total reducing power (1.27). However, among the fractions, butanol fraction showed the highest contents of flavonoids (235.20 mgQE/g), phenolics (413.42 mgGAE/g) and the highest DPPH scavenging activity (93.87 %) while the highest tannin content (86.90 mgTAE/g) and total reducing power (1.06) was observed in the ethyl acetate fraction. The lowest concentration of flavonoid (34.60 mgQE/g) and lowest antioxidant activity was obtained for the hexane fraction while lowest contents of phenolics (102.32 mgGAE/g) and tannins (41.21 mgTAE/g) were both observed in the water-soluble fraction. The most active fraction against both *Staphylococcus aureus* and *Enterobacter cloacae* was ethyl acetate. Meanwhile, the ethanol extract, hexane fraction, and water-soluble fractions showed the highest activity against *Candida albicans*, *Salmonella typhi*, and *Escherichia coli*, respectively. This research reveals that the stem bark of *Azadirachta indica* contains notable antioxidant and antimicrobial properties, making it a promising source for developing potent therapeutic drugs and functional foods.

**Keywords:** *Azadirachta indica*; stem bark; fractions; antimicrobial; antioxidant**1.0 INTRODUCTION**

Medicinal plants have been used for centuries to treat a wide range of illnesses, from minor aches or injuries and skin irritations to severe and life-threatening diseases. Recently, their widespread use has made them an integral part of healthcare systems around the world, particularly in rural settings where people have limited access to modern treatments. In fact, in developing countries, approximately eighty percent of primary health care is estimated to be alternative medicine (Rupani & Chavez, 2018), while in industrialized countries, alternative medicine remains a popular complementary form of care. Increasingly, researchers are exploring the potential of medicinal plants, which have been a valuable source and an

inspiration for the development of many modern medicines, and they are uncovering new compounds with improved therapeutic effects. This is due to the low cost, easy accessibility and fewer side effects of medicinal plants when compared to synthetic drugs. The effectiveness of medicinal plants is attributed to their active compounds, including alkaloids, flavonoids, terpenoids, phenolic compounds and glycosides, which possess biological activities such as antioxidant, anti-inflammatory, antimicrobial and immunomodulatory activities (Heyman *et al.*, 2017). Additionally, some of these plant-derived compounds have also demonstrated anti-cancer, anti-diabetic and anti-hypertensive activities. Besides their therapeutic benefits, medicinal plants

also possess nutritional values. They possess nutrients such as proteins, minerals, vitamins, antioxidants, fatty acids, and dietary fiber which are essential for improving one's overall health. One of such nutrient is omega-3 fatty acids present in medicinal plants which may help reduce inflammation, while dietary fiber can help improve digestion, reduce cholesterol, and lower the risk of diabetes. One such highly valued medicinal plant possessing great nutraceutical potentials is *Azadirachta indica*, which has the potential to be a viable option for the prevention and treatment of a wide range of diseases.

*Azadirachta indica*, commonly known as Neem, is an evergreen tree of the *Meliaceae* family which is widely cultivated and distributed throughout Asia, Africa, and other tropical regions of the world (Ibrahim *et al.*, 2022). It usually grows to a height of 30-60 feet, with a straight trunk and long, spreading branches, forming a broad, round crown (Shakib *et al.*, 2020). This tree has a rough, dark brown bark with wide, longitudinal fissures separated by flat ridges. Its compound, imparipinnate leaves come in clusters of 5-15 leaflets, alternating with each other and bearing many-flowered panicles, mostly in the axils of the leaves (Mohammad *et al.*, 2016). Its ovate-shaped seed pods are about one cm long, with sweet-scented, white, oblanceolate petals. It produces yellow, ellipsoid and glabrous drupes that are 12-20 mm long; initially green, they turn yellow upon ripening (Mohammad *et al.*, 2016) and have an aromatic, garlic-like odor. The neem plant has a long history of use in medicine and nutrition, dating back centuries. It has been utilized in traditional Ayurvedic and Unani medicine to treat a wide range of health issues. Nearly every part of the neem plant, including the seeds, oil, stem bark, flowers, gum, fruits, and leaves, have been used to treat diseases such as leprosy, diabetes, intestinal helminthiasis, respiratory disorders, cancer, constipation, and skin infections (Islas *et al.*, 2020; Shakib *et al.*, 2020). Millions of people around the world use neem twigs as chewing sticks to maintain good dental hygiene (Gupta *et al.*, 2017). The neem tree has attracted significant interest from the scientific community due to its demonstrated abilities as an anti-cancer agent, antimicrobial agent, insecticide, immune system modulator, and

antiviral agent (Paul *et al.*, 2011; Raut *et al.*, 2014; Chaudhary *et al.*, 2017; Ghosh *et al.*, 2019; Sarkar *et al.*, 2020). Several compounds with proven biological activities have been extracted from various parts of the neem tree. These include azadirachtin, gedunin, nimbolide, gallic acid, and catechins. The biopesticidal properties of neem are due largely to the presence of azadirachtin (Kilani-Morakchi *et al.*, 2021). Meanwhile, nimbin has been shown to have multiple beneficial effects, including antipyretic, fungicidal, antihistamine, antiseptic, anti-inflammatory, and antioxidant properties (Naik *et al.*, 2014). Neem, initially known as a potent pesticide and fertilizer in agriculture, has in recent years been seen as a safe and versatile antimicrobial with applications in food production, storage, packaging, and consumption (Chaudhary *et al.*, 2017). The neem stem bark has therapeutic properties that can control gastric hypersecretion and gastroduodenal ulcers by exhibiting anti-ulcer and anti-secretory effects. According to Ahmad *et al.* (2015), fabrics dyed with neem bark extract has anti-lepidopteran properties that are more effective than those of neem leaf extract. This is due to the higher levels of azadirachtin, cyanogenic glucosides, and nimbin found in neem bark extract. This study evaluates the aqueous ethanol extract and fractions of *Azadirachta indica* stem bark for their phytochemical compositions, antioxidant and antimicrobial activities.

## 2.0 MATERIALS AND METHODS

### 2.1 Collection, Preparation and Extraction of Sample

The stem bark of *A. indica* was obtained from its tree growing within the premises of Babcock University, Ogun State, Nigeria. It was thoroughly washed with distilled water to remove dirt and cut into very small pieces with a knife. Thereafter, these were oven dried at 50 °C for 48 h. The dried sample was pulverized with a laboratory blender (LEXUS MG-2053 OPTIMA) and used for the extraction step. For the extraction process, 100 g of the sample were extracted into 800 mL aqueous ethanol (80 % v/v) for 24 h using a Soxhlet apparatus. The extraction solvent was then removed *in vacuo* using a rotary evaporator (Eyela N-1001) at 40 °C in order to recover the stem bark extract. The extract was placed on a water bath at

40 °C for about 6 h to ensure complete removal of residual solvent. The resulting dark wine solid extract was stored in a well-labelled glass bottle for further analysis.

## 2.2 Partitioning of Crude Aqueous Ethanol Extract

A portion of the resulting crude aqueous ethanol extract (EE) of *A. indica* stem bark was reconstituted in distilled water in the ratio 1:3 (extract: distilled water) and then successively partitioned by solvent-solvent extraction into n-hexane, ethyl acetate and butanol. Each of the resulting solvent fractions; n-hexane fraction (NHFR), ethyl acetate fraction (EAFR), butanol fraction (BUFR) and the residual fraction called water soluble fraction (WSFR) was collected separately and concentrated under reduced pressure at 40 °C with a vacuum rotary evaporator (Eyela N-1001). The extract and its fractions were immediately analyzed for their phytochemical compositions, antimicrobial and antioxidant activities using various standard protocols. The percentage yields of the ethanol extract and each solvent fraction relative to the crude aqueous ethanol extract were also calculated using equation 1;

$$\text{Yield (\%)} = \frac{\text{Weight of extract}}{\text{Weight of sample}} \times 100 \quad 1$$

## 2.3 Qualitative Phytochemical Analysis

The phytochemical screening of the extract and fractions of the stem bark of *A. indica* was conducted using the methods outlined by Kumar *et al.* (2007) and Sivaraj *et al.* (2011). The aim was to identify the presence of secondary metabolites, such as alkaloids, cardiac glycosides, saponins, steroids, phytosterols, phenols, flavonoids, anthraquinones, and tannins in the samples.

## 2.4 Quantitative Phytochemical Analysis

### 2.4.1 Flavonoid Content

The flavonoid content of the extract and its fractions was determined using the colorimetric method of Ohikhena *et al.* (2018). About 0.5 mL aliquots of the ethanol extract or fraction (1 mgmL<sup>-1</sup>) and quercetin standards (10–100 µgmL<sup>-1</sup>) were placed in separate test tubes and 2 mL of distilled water was added to each. Thereafter, 0.15 mL of sodium nitrite (5 %) was added and the mixture was left to stand for 6 min. Then, 0.15 mL of AlCl<sub>3</sub> (10

% w/v) was added and the mixture was left to stand for an additional 5 min. Finally, 1 mL of sodium hydroxide (1 M) was added and the mixture was made up to 5 mL with distilled water. Absorbance was measured at 420 nm on a UV-Visible Spectrophotometer (JENWAY 6305), and the flavonoid content was expressed as milligram Quercetin equivalent per gram of the extract.

### 2.4.2 Phenolic Content

The Folin-Ciocalteu method, as described by Wintola & Afolayan (2011), was used to measure the total phenolic content of the extract and its fractions. This involved mixing 0.5 mL of a 1 mg/mL plant extract or fraction and gallic acid standards (ranging from 10-100 µgmL<sup>-1</sup>) with 2.5 mL of a 1:10 diluted Folin-Ciocalteu reagent in a test tube. The mixture was then combined with 2 mL of 7.5 % w/v sodium carbonate solution, mixed well, and left to stand for 30 min at 40 °C to allow for color development. The absorbance of the mixture was then read at 765 nm using a UV-Visible Spectrophotometer (JENWAY 6305), and the phenolic content was calculated as gallic acid equivalents (GAE) using a calibration curve of gallic acid standards with an R<sup>2</sup> value of 0.9971. Results were expressed as mg GAE per gram of dry extract.

### 2.4.3 Tannin Content

The tannin content of the extract and its fractions was determined using a modified version of the method described by Akter *et al.* (2016). The sample solution, consisting of 1.25 mgmL<sup>-1</sup> of ethanol and 50 µL of the sample, was mixed with 3 mL of a 4 % vanillin-methanol reagent and 1.5 mL of an 11.6 M HCl solution. After thorough mixing, the mixture was allowed to stand for 15 minutes before measuring its absorbance at 500 nm using a JENWAY 6305 UV-Visible Spectrophotometer, with a blank as the reference. The total tannin content was then calculated as tannic acid equivalent and expressed as mg tannic acid equivalent per gram of dry extract or fraction (mgTAEg<sup>-1</sup>) by comparison with a calibration curve of tannic acid standard solutions (10–100 µgmL<sup>-1</sup>).

## 2.5 Antioxidant Activity Assay

### 2.5.1 Total Reducing Power

The total reducing power (TRP) of the extract and fractions of *A. indica* stem bark was determined by the method of Subramanian *et al.* (2013), which involved adding 1 mL of each sample at concentrations of 20, 40, 60, 80 and 100 µg mL<sup>-1</sup> to phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and aqueous potassium ferricyanide solution (2.5 mL, 1 % v/v), followed by incubation at 50 °C for 30 min. Then, trichloroacetic acid solution (2.5 mL, 10 %) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The supernatant (2.5 mL) was mixed with distilled water (2.5 mL) in a test tube, followed by 0.5 mL of ferric chloride solution (0.1 % w/v) in a test tube. The absorbance of the reaction mixture was measured at 700 nm using a UV-Visible Spectrophotometer (JENWAY 6305), with the reducing power being directly proportional to the absorbance.

### 2.5.2 DPPH Free Radical Scavenging Activity

The antioxidant activity of the *A. indica* stem bark extract and fractions was determined by measuring their ability to scavenge free radicals using the 2, 2-diphenyl-2-picrylhydrazyl (DPPH) radical assay. This method, described by Álvarez-Casas *et al.* (2014), involved adding an aliquot of the extract or fraction at concentrations of 20, 40, 60, 80 and 100 µg mL<sup>-1</sup> to 4 mL of a methanol-based DPPH solution (0.1 mM). The mixture was then incubated in the dark for 30 minutes at room temperature and the absorbance was read at 517 nm using a UV-Visible Spectrophotometer (JENWAY 6305). The results were expressed as the percentage inhibition of DPPH free radical, calculated using equation 2.

$$\% \text{ inhibition of DPPH} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100 \quad 2$$

where Abs control is the absorbance of the DPPH without plant extracts, and Abs sample is the absorbance of the DPPH after adding extracts. The IC<sub>50</sub> value, which represents the concentration of the plant extract that caused 50 % inhibition of radical was obtained by interpolation from linear regression analysis. A lower IC<sub>50</sub> value indicates a higher antioxidant activity.

## 2.6 Antimicrobial Assay

### 2.6.1 Microorganisms and Media Preparation

In this study, the pure clinical isolates of various test organisms including *Staphylococcus*, *Escherichia coli*, *Enterobacter*, *Salmonella* and *Candida albicans* were used. These isolates were obtained from stock cultures in the Department of Microbiology at Babcock University in Ogun State, Nigeria. For the antimicrobial assay, Mueller–Hinton agar (Oxoids Ltd., Basingstoke, Hampshire, UK) and Potato Dextrose Agars (Rapid Labs) were prepared according to the manufacturer's instructions. Twenty eight grams and thirty nine grams of the respective agars were separately dissolved in 1 L of distilled water and homogenized on a hot plate. The media were sterilized in an autoclave at 121 °C for 15 minutes. The agars were then poured into plates and allowed to solidify in a hot air oven at 40 °C.

### 2.6.2 Susceptibility Test

The antimicrobial activity of the extract and its fractions against clinical isolates was evaluated using the agar well diffusion method. The test bacteria were grown in nutrient broth for 24 hours, and the fungus was grown in potato dextrose broth for 5 days. The test organisms were prepared by suspending colonies picked from 24 hour old cultures grown on nutrient agar in saline solution to achieve an optical density of approximately 0.1 at 600 nm. The suspension was then diluted 1:100 by adding 0.1 mL of the bacterial suspension to 9.9 mL of sterile nutrient broth before being used. This assay was performed according to the method outlined by Olajuyigbe & Afolayan (2012). The test organisms were swabbed onto sterile Mueller-Hinton agar plates for bacteria and on potato dextrose agar plates for the fungus. Wells were then bored into the agar medium using a heat sterilized 6 mm cork borer, and filled with 100 µl of each extract concentration and with 100 µL of ciprofloxacin (2.5 µg/mL) and fluconazole (2.5 µg/mL). The plates were left to stand on the laboratory bench for 1 hour to allow proper diffusion before being incubated at 37 °C for 24 hours for the bacterial isolates and 3-5 days for the fungus. Dimethyl sulfoxide was used as the blank, while ciprofloxacin (for bacteria) and fluconazole (for fungi) were used as standard drugs. After the

incubation period, the diameter of each zone of inhibition was recorded and expressed in millimeters. Minimum inhibitory concentration of the extract on the organisms was determined at lower concentrations using the same method. The entire microbial assay was conducted under strict aseptic conditions, and all analyses were carried out in triplicates.

### 2.7 Statistical Analysis

All analyses were carried out in triplicates and data obtained were reported as mean  $\pm$  SD of three replicates. Data were also plotted using Microsoft excel and Origin 9.0 Pro software.

## 3.0 RESULTS AND DISCUSSION

### 3.1 Phytochemical Screening

Phytochemical screening is a method used to identify the presence of various classes of bioactive compounds in plant materials. The results of a phytochemical screening of ethanol extract (EE) and fractions (HF, EAF, BF, and WSF) of *A. indica*

stem bark are presented in Table 1. The results reveal that saponins, flavonoids, phenol and tannins were present in all the samples. Meanwhile, only cardiac glycosides were absent in HF, phytosterols and anthraquinones were absent in BF, while alkaloids, steroids, phytosterols, and anthraquinones were absent in WSF. However, all the phytochemicals assayed were present in both EE and EAF. These results indicate that the stem bark of *A. indica* contains a variety of phytochemicals which are known to have important biological activities. Some of the biological activities that have been reported in literature for these phytochemicals include anti-inflammatory, antiparasitic, antioxidant, immunomodulatory, anticancer, astringent, antibacterial and antifungal activities (Sharma *et al.*, 2010; Czemplik *et al.*, 2011; Amin *et al.*, 2013; Manasa *et al.*, 2014;). Based on these results, it is evident that the stem bark of *A. indica* contains a rich variety of phytochemicals which may be useful for the development of novel drugs and therapeutic agents.

**Table 1: Phytochemical screening of ethanol extract and fractions of *A. indica* stem bark**

S/N	Phytochemical	Test	EE	HF	EAF	BF	WSF
1.	Alkaloids	Wagner's test	+	+	+	+	-
2.	Cardiac Glycosides	Keller-Killani test	+	-	+	+	+
3.	Saponins	Frothing test	+	+	+	+	+
4.	Steroids	Salkowski's test	+	+	+	+	-
5.	Phytosterols	Lieberman Buchard test	+	+	+	-	-
6.	Phenols	Ferric chloride test	+	+	+	+	+
7.	Flavonoids	Alkaline test	+	+	+	+	+
8.	Anthraquinones	Anthraquinone test	+	+	+	-	-
9.	Tannins	Ferric chloride test	+	+	+	+	+

+ = present

- = absent

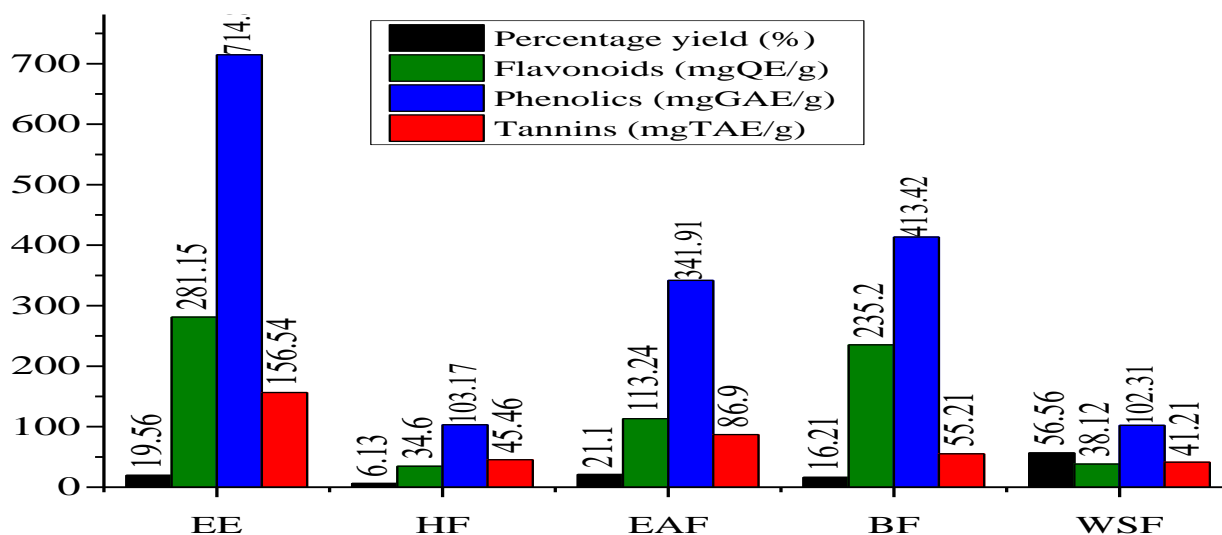
### 3.2 Percentage Yield and Quantitative Phytochemical Analysis

Figure 1 shows the percentage yields, flavonoid, phenolic and tannin contents of *Azadiracta indica* stem bark's aqueous ethanol extract and its fractions. The crude extract constituted 19.56 % of the stem bark, and the fractions HF, EAF, BF and WSF respectively yielded 6.15 %, 21.10 %, 16.21 % and 56.56 % of the ethanol extract. The yield of the aqueous ethanol extract obtained in this study was notably high. This could be attributed to the fact that aqueous ethanol is able to extract a wide range of compounds from the plant materials. Additionally, the type of extraction method used

could also have a significant impact on the amount of bioactive compounds extracted. Studies have suggested that irrespective of the extraction solvent and plant material, hot solvent systems under reflux condition are more efficient for the recovery of bioactive plant components, resulting in higher extract yields (Bushra *et al.*, 2009). Higher extraction temperatures can also increase the solubility of certain bioactive compounds, resulting in higher yields. Hence, the Soxhlet extraction method employed in this study could be responsible for the relatively high yield of the aqueous EE. Generally, the aqueous ethanol extract had the highest flavonoid content (281.15 mgQE/g),

phenolics (714.82 mgGAE/g) and tannins (156.54 mgTAE/g). However, the fraction with the highest flavonoid content (235.20 mgQE/g) was BF, followed by EAF (113.24 mgQE/g), WSF (38.12 mgQE/g) and HF (34.60 mgQE/g). In terms of phenolic content, BF also had the highest content (413.42 mgGAE/g), followed by EAF (341 mgGAE/g), HF (103.17 mgGAE/g) and WSF (102.31 mgGAE/g). As for the tannin content, EAF had the highest (86.90 mgTAE/g), followed by BF (55.21 mgTAE/g), HF (45.46 mgTAE/g) and WSF (41.21 mgTAE/g). The observed variation in the yields and phytochemical compositions of the fractions could be attributed to the varying polarity

of the extraction solvents used, as well as the different extractability of bioactive compounds (Adaramola *et al.*, 2018). The presence of phytochemicals such as flavonoids, phenols and tannins has been correlated with medicinal plants' biological activities including antioxidant, antimicrobial, anti-inflammatory, anti-cancer, antidiabetic, cardio-protective and skin-protective activities (Dziąło *et al.*, 2016; Andreu *et al.*, 2018; Meng *et al.*, 2018). Hence, the significantly high phytochemical contents of the BF and EAF suggests that these fractions of the EE would be more pharmacologically active than the HF and WSF



**Figure 1: Percentage yield and quantitative phytochemical content of ethanol extract and fractions of *A.indica* stem bark**

### 3.3 Antioxidant Activity

The antioxidant activity of EE and its fractions was investigated by assaying their DPPH free radical scavenging activity and total reducing power at different concentrations (20, 40, 60, and 80  $\mu\text{g mL}^{-1}$ ) of each of the samples. The DPPH radical scavenging activity is an assay commonly used to evaluate the antioxidant activity of compounds, as it measures the ability of a compound to scavenge the stable DPPH radical. In this assay, the sample was added to a solution of DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical, which is a stable radical that can be easily measured spectrophotometrically. The degree of discoloration of the DPPH radical solution from

purple to yellow was proportional to the scavenging activity of the sample. On the other hand, the total reducing power assay measures the ability of an extract or fraction to reduce a chromogenic compound, typically ferric ion ( $\text{Fe}^{3+}$ ) to ferrous ion ( $\text{Fe}^{2+}$ ), which reflects its antioxidant capacity. Antioxidants in the extract act as reducing agents, donating electrons to the  $\text{Fe}^{3+}$  ions and reducing them to  $\text{Fe}^{2+}$  ions. This reduction reaction is monitored spectrophotometrically by measuring the increase in absorbance at a particular wavelength usually 700 nm. The higher the concentration of antioxidants in the extract, the more electrons will be donated to the  $\text{Fe}^{3+}$  ions, resulting in a greater increase in absorbance. This

increase in absorbance is proportional to the total reducing power of the extract, and thus serves as a measure of its overall antioxidant activity. That is, the higher the absorbance, the higher the reducing power of the extract and the higher its antioxidant activity.

### 3.3.1 DPPH Free Radical Scavenging Activity and Total Reducing Power

The results of the percentage DPPH scavenging activity of the aqueous ethanol extract and fractions of *Azadirachta indica* stem bark are shown in Figure 2a. The results showed that, of all the tested samples, the crude extract had the highest DPPH scavenging activity, with 95.71 % inhibition of DPPH free radical at a concentration of 80  $\mu\text{g mL}^{-1}$  while the hexane fraction had the lowest activity, with only 24.91 % inhibition at 80  $\mu\text{g mL}^{-1}$ . However, it is noteworthy that the DPPH free radical scavenging activity of the extract was closely followed by that of the BF with a 93.87 % inhibition of DPPH at the 80  $\mu\text{g mL}^{-1}$  concentration; making it the most potent fraction of the extract for the scavenging of DPPH free radicals. This was followed by the EAF with 86.41 % inhibition of DPPH at the same 80  $\mu\text{g mL}^{-1}$  concentration. For the DPPH scavenging activity, the IC<sub>50</sub> values (Fig. 3) of the samples were also determined, with the crude extract having the lowest value of 1.96  $\mu\text{g mL}^{-1}$  and the hexane fraction having the highest value of 176.19  $\mu\text{g mL}^{-1}$ . The IC<sub>50</sub> value represents the concentration at which 50 % inhibition of the DPPH radical occurs, and a lower IC<sub>50</sub> value indicates a higher antioxidant activity and vice-versa. The other fractions, ethyl acetate, butanol and water-soluble showed moderate antioxidant activities with IC<sub>50</sub> values of 39.84, 28.31 and 148.10  $\mu\text{g mL}^{-1}$  respectively. The much higher IC<sub>50</sub> values of the HF and WSF indicate that these fractions may contain fewer antioxidant

compounds, making them less effective at scavenging DPPH radicals. Likewise, the results of the total reducing power (TRP) assay (Fig. 2b) show that EE exhibited the highest TRP with an absorbance of 1.27 while the least TRP was observed in the HF with an absorbance of 0.42. Meanwhile, the TRP of EAF (1.06) was the highest among the fractions and it superseded that of BF (0.99), unlike in the DPPH scavenging activity where the reverse was the case. Additionally, IC<sub>50</sub> values of the TRP assay (Fig. 3) showed that EE had the lowest IC<sub>50</sub> value of 7.87  $\mu\text{g mL}^{-1}$ , followed by EAF with 9.62  $\mu\text{g mL}^{-1}$ , BF with 13.57  $\mu\text{g mL}^{-1}$ , WSF with 44.81  $\mu\text{g mL}^{-1}$  and the least was observed in the HF with 102.86  $\mu\text{g mL}^{-1}$ . It was generally noted that increasing the concentration of all samples led to an increase in both their DPPH scavenging activity and total reducing power. As the concentration increased from 20 to 80  $\mu\text{g mL}^{-1}$ , both the crude extract and hexane fraction for instance, demonstrated a significant increase in the percentage inhibition of DPPH free radicals. Specifically, the crude extract showed a remarkable rise in percentage inhibition from 56.69 % to 95.71 %, while the hexane fraction exhibited an increase from 6.43 % to 24.91 %. This confirms that the antioxidant activity of the extract and fractions is concentration-dependent. The higher inhibition of DPPH and TRP observed in the EE and its EAF and BF fractions could be attributed to the presence of phytochemicals such as flavonoids, tannins, and phenolic acids, which are known to have antioxidant activity (Aljbery and Chen, 2018). These findings support the traditional use of *Azadirachta indica* stem bark as a natural remedy for various diseases and also suggests that *Azadirachta indica* stem bark extract has promising antioxidant activity, which could be employed to develop potential therapeutic drugs or functional foods.

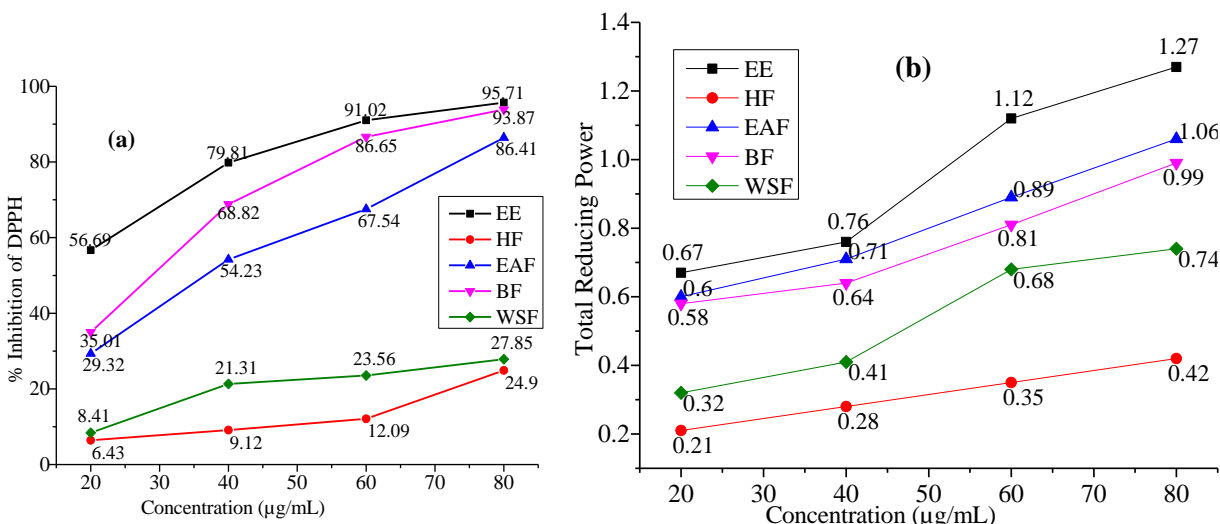


Figure 2: (a) DPPH scavenging activity and (b) total reducing power of *A. indica* stem bark aqueous ethanol extract and fractions

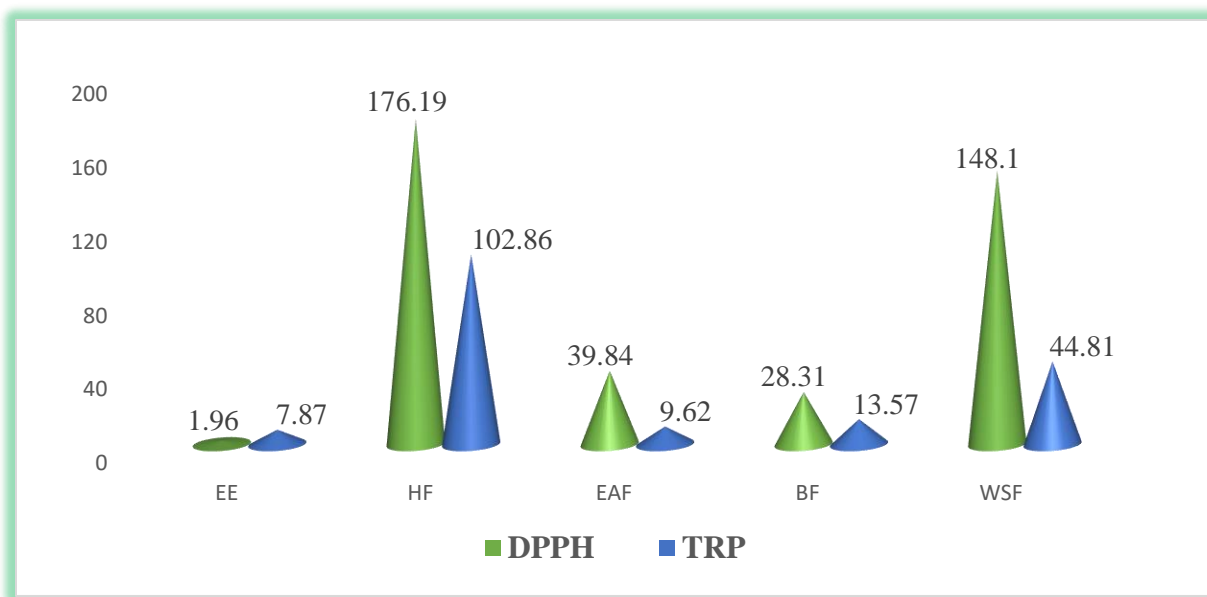


Figure 3: IC<sub>50</sub> values (µg/mL) of aqueous ethanol extract and fractions of *A. indica* stem bark for DPPH scavenging and total reducing power activities.

### 3.4 Antimicrobial Activity

The emergence of multidrug-resistant microorganisms is an increasingly serious and growing health concern, posing a major threat to global health. This is largely due to the overuse, misuse, and abuse of antibiotics, resulting in the development of resistant strains of bacteria, fungi, and viruses. These resistant microorganisms pose significant challenges due to their resistance to traditional antibiotics and the potential for more

severe disease, prolonged illness, increased healthcare costs, and even death. In response to this crisis, researchers are continuously exploring alternative sources of antimicrobial agents, such as medicinal plants, which have been found to contain compounds such as saponins, tannins, flavonoids, phenolics with antimicrobial properties (Khan *et al.*, 2018) that can effectively control or inhibit the growth of multidrug-resistant microorganisms. In an effort to assess their potential as alternative

sources of antimicrobial agents, this study investigated the antimicrobial potential of aqueous ethanol extract of *Azadirachta indica* stem bark and its fractions with the aim of determining the efficacy in controlling the growth of selected microorganisms. The results of the susceptibility pattern of the test organisms to the EE and its fractions are presented in Table 2 while the minimum inhibitory concentration (MIC) of the extract and fractions are stated in Table 3. These results showed variation in the susceptibility of the organisms to the extract and its fractions. From the results in Table 2, it is clear that EAF was the most active against both *Staphylococcus aureus* and *Enterobacter cloacae* with zones of inhibition of 16.5 mm and 16.0 mm respectively at 20 mgmL<sup>-1</sup>. Additionally, EE, HF and WSF were most active against *Candida albicans* (18.5 mm), *Salmonella typhi* (19.0 mm) and *Escherichia coli* (12.5 mm), respectively. It is worthy of note that *Enterobacter cloacae* and *Salmonella typhi* were completely resistant to WSF while *Escherichia coli* showed complete resistance to HF and EAF. The results also showed that the inhibitory effect of the ethanol extract and its fractions against the test organisms was slightly concentration dependent as the diameter zone of inhibition increased with increasing concentration. The MIC results (Table 3) was in agreement with the diameter zones of inhibition results in Table 2. Minimum Inhibitory Concentration (MIC) is the lowest concentration of an antimicrobial agent that prevents the visible growth of a microbe *in vitro*. It is a critical parameter in antimicrobial studies, as it provides

valuable information on the susceptibility of pathogens to different antimicrobial agents, which can be used to guide the selection of appropriate treatments and to monitor changes in susceptibility over time. From Table 3, it is clear that EAF had the highest activity against *Staphylococcus aureus* and *Enterobacter cloacae* with MIC of 2 mgmL<sup>-1</sup> and 3 mgmL<sup>-1</sup>, respectively. Also, the EE and HF showed the highest activity against *Candida albicans* and *Salmonella typhi*, with both having MIC of 2 mgmL<sup>-1</sup> while WSF showed the highest activity against *Escherichia coli* with MIC of 5 mgmL<sup>-1</sup>. Meanwhile, the standard drugs, ciprofloxacin and fluconazole, generally outperformed the EE and its fractions in the inhibition of the test organisms. However, it is worthy of note that *Salmonella typhi* showed resistance to ciprofloxacin but was highly susceptible to the extract and all its fractions. This could be attributed to the diverse and interdependent nature of the bioactive compounds present in the complex mixture of the extract and fractions of *Azadirachta indica* (Adaramola *et al.*, 2018). Also, differences in the type and concentration of phytochemicals found in the extract and various fractions may account for the variations observed in the susceptibility of the tested organisms to the EE and its fractions. Generally, the results of this study suggest that the aqueous ethanol extract and its fractions of *Azadirachta indica* stem bark have the potential to inhibit the growth of the tested microorganisms, indicating that the extract could be employed as an alternative source of antimicrobial agents.

**Table 2: Zone of inhibition of *Azadirachta indica* stem bark extract and fractions against test organisms**

S / N	Microorganism	Extract/ Fraction	Diameter zone of inhibition (mm)				
			5 mgmL <sup>-1</sup>	10 mgmL <sup>-1</sup>	15 mgmL <sup>-1</sup>	20 mgmL <sup>-1</sup>	Ciprofloxacin/ Fluconazole (2.5 µgmL <sup>-1</sup> )
1	<i>Staphylococcus aureus</i>	EE	10.5 ± 0.11	13.0 ± 0.10	14.0 ± 0.11	15.0 ± 0.02	22.0 ± 0.20
		HF	10.5 ± 0.11	12.5 ± 0.12	13.5 ± 0.11	14.5 ± 0.05	
		EAF	11.5 ± 0.22	14.5 ± 0.10	15.0 ± 0.21	16.5 ± 0.03	
		BF	11.5 ± 0.03	12.5 ± 0.13	13.0 ± 0.22	16.0 ± 0.02	
		WSF	10.0 ± 0.02	12.0 ± 0.12	15.0 ± 0.02	14.0 ± 0.01	
2	<i>Enterobacter cloacae</i>	EE	11.0 ± 0.33	13.5 ± 0.13	14 ± 0.05	15.5 ± 0.31	22.0 ± 0.21
		HF	R	R	12.0 ± 0.03	13.0 ± 0.21	
		EAF	14.0 ± 0.01	14.5 ± 0.11	15.0 ± 0.12	16.0 ± 0.24	
		BF	R	11.0 ± 0.15	13.0 ± 0.17	14.0 ± 0.11	

3	<i>Escherichia coli</i>	WSF	R	R	R	R	
		EE	R	10.0 ± 0.12	12.0 ± 0.04	12.0 ± 0.31	24.0 ± 0.35
		HF	R	R	R	R	
		EAF	R	R	R	R	
		BF	R	R	10.5 ± 0.10	12.5 ± 0.10	
4	<i>Salmonella typhi</i>	WSF	R	11.23	12.0 ± 0.22	12.0 ± 0.11	
		EE	10.5 ± 0.20	16.5 ± 0.33	17.5 ± 0.02	18.0 ± 0.03	R
		HF	10.5 ± 0.03	14.5 ± 0.13	17.5 ± 0.02	19.0 ± 0.03	
		EAF	10.5 ± 0.11	15.5 ± 0.03	16.5 ± 0.13	17.5 ± 0.23	
		BF	10.5 ± 0.32	14.5 ± 0.12	15.0 ± 0.12	16.0 ± 0.12	
5	<i>Candida albicans</i>	WSF	R	R	R	R	
		EE	12.0 ± 0.03	15.0 ± 0.31	16.5 ± 0.02	18.5 ± 0.11	23.0 ± 1.03
		HF	R	R	12.0 ± 0.13	12.0 ± 0.12	
		EAF	12.0 ± 0.12	14.0 ± 0.22	15.0 ± 0.12	16.5 ± 0.23	
		BF	11.0 ± 0.13	12.0 ± 0.20	13.5 ± 0.14	14.5 ± 0.21	
		WSF	11.0 ± 0.14	11.0 ± 0.17	12.0 ± 0.16	12.0 ± 0.23	

**R= Resistant**

**Table 3: Minimum Inhibitory Concentration of test organisms in mgmL<sup>-1</sup>**

Extracts	<i>Staphylococcus aureus</i>	<i>Enterobacter cloacae</i>	<i>Escherichia coli</i>	<i>Salmonella typhi</i>	<i>Candida albicans</i>
EE	3	5	6	3	2
HF	3	12	>20	2	12
EAF	2	3	>20	5	3
BF	3	8	13	5	4
WSF	5	>20	5	>20	4

#### 4.0 CONCLUSION

This study revealed that the ethanol extract and fractions of the stem bark of *Azadirachta indica* contain a variety of phytochemicals with records of important biological activities such as anti-inflammatory, antioxidant, anti-cancer, antidiabetic, cardio-protective, and skin-protective properties. The highest antioxidant activity was observed in the crude aqueous ethanol extract but both the butanol and ethyl acetate fractions also exhibited considerable antioxidant activity. Additionally, the extract and its fractions displayed promising antimicrobial properties. The butanol and ethyl acetate fractions, in particular, demonstrated particularly promising antioxidant and antimicrobial activities, making them potential alternative sources for antimicrobial agents and novel drugs, therapeutic agents or functional foods. The findings of this study corroborate traditional or folkloric use of *Azadirachta indica* stem bark to treat various infectious, inflammatory and oxidative stress-related diseases.

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