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# Pharmacognostic, antioxidant and antimicrobial evaluation of fractions of the stem bark of *Albizia zygia* Benth (Fabaceae)

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## ABSTRACT

The stem barks of *Albizia zygia* Benth (Fabaceae) are used in ethnomedicine for the management and treatment of various infectious diseases. This study was aimed at investigating the pharmacognostic, antioxidant and antimicrobial activities of the plant to aid standardization and continuous usage. Evaluation of fresh and powdered sections of the stem bark was carried out to determine pharmacognostic profile. Chemical tests were employed in phytochemical investigations. The antioxidant activities were carried out using DPPH radical scavenging method. The polyphenolic contents were also evaluated. Inhibitory activity of the crude methanol extract and fractions against clinical strains of *Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella aerogenes, Candida albicans* and *Candida parasilopsis* were compared with ciprofloxacin and nystatin for bacteria and fungi respectively. Pharmacognostic data which can aid sample identification are provided. Phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, and saponins. The fractions showed significant antioxidant activity when compared to the standard with n-butanol fraction having the highest activity. The crude methanol extract and the fractions inhibited the growth of *E. coli, B. subtilis, S. aureus, P. aeruginosa, C. albicans* and *C. parasilopsis* to varying degrees, except the aqueous fraction that was devoid of activity. Extract and fractions of *A. zygia* have demonstrated antimicrobial activity against clinical strains of selected microorganisms and showed significant antioxidant activity. These results showed that *A. zygia* has a role in the treatment of infectious diseases

**KEYWORDS**: *Albizia zygia*, Pharmacognostic, antioxidant, antimicrobial

## INTRODUCTION

Herbal medicines include herbs, herbal materials, herbal preparations and finished herbal products that contain parts of plants or other plant materials as active ingredients [1]. The World Health Organization estimated that 80% of people worldwide rely on herbal medicines for some part of their primary health care. The remaining 20% of individuals living in industrialized countries uses, in at least 25% of cases, pharmaceuticals which have been directly derived from plant products. The use of medicinal plants in complementary and alternative medicine has seen a

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great increase in recent years especially in Asia and Europe. One of the main reasons for the increasing use of traditional medicine is a growing trend for patients to take a more proactive approach to their own health and to seek out different forms of self-care. In the process, many consumers have turned to natural traditional medicinal products and practices, under the assumption that “natural means safe” [2]. Medicinal plants are sources of a number of novel chemical compounds and the metabolites that have effects on human health are known as phytochemicals [3]. Medicinal plants are commonly used in treating and preventing specific ailments and diseases and are generally considered to play a beneficial role in healthcare. Within the last few decades, many plants have been screened for their biological and pharmacological properties by researchers. These efforts are continually being taken to examine the merits of traditional medicine in the light of modern science with a view aimed at adopting effectively beneficial medical practice and discouraging harmful ones [4].

Albizia is a genus of about 150 species of mostly fast-growing subtropical and tropical trees and shrubs in the subfamily Mimosoideae of the family of Fabaceae. The genus is pantropical, occurring in Asia, Africa, America and Australia, but mostly in the Old World tropics. Albizias are important forage, timber, and medicinal plants, and many are cultivated as ornamentals for their attractive flowers [5]. Common names of *Albizia zygia* include West Africa walnut (English), Ayinreta (Efik), Nyieavu (Ibo), and Ayin-rela (Yoruba) [6].

In traditional medicine, bark sap is instilled in the eyes to treat ophthalmia. A bark decoction is administered to treat bronchial diseases, fever (including malaria) and female sterility, and as a purgative, stomachic, antidote, vermifuge and aphrodisiac. Pounded or rasped bark is applied externally to treat yaws, sores, wounds and toothache [7]. *Albizia zygia* has been found to contain bioactive xanthones, benzophenones and flavonoids in the stem bark [8]. In addition to lupeol, chondrillasterol and p-hydroxybenzoic acid, three flavones were isolated for the first time from the bark of *Albizia zygia*. The latter compounds were identified as 3, 4, 7-trihydroxyflavone, 3-Omethoxyfisetin and 3, 7-dihydroxyflavonone. Their structures were determined by Nuclear magnetic resonance and Mass spectrometry techniques and confirmed by comparison with the literature [9]. The methanol extract of the stem bark exhibited antiprotozoal activity ($IC_{50}$ 1.0 μg/ml) against Plasmodium falciparum strain K1, the protozoa responsible for malaria, and *Trypanosoma brucei* and rhodesiense ($IC_{50}$ 0.2 μg/ml), which causes African trypanosomiasis [9]. We had earlier in our laboratory investigated the analgesic and toxicological activities of the stem bark of *Albizia zygia* [10]. The present study was designed not only to establish the Pharmacognostic and antioxidant profile of *A. zygia*, but also to investigate the antimicrobial activities of the fractions as a prelude to isolating the active compounds responsible for the claimed biological activities.

## MATERIALS AND METHODS

### Preparation of plant extract

The stem barks of *A. zygia* Benth were collected in Ediaken-Uselu area of Benin City, Edo State, Nigeria. The plants were authenticated by the curator at the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, Benin City where voucher specimens were deposited. The stem barks were air–dried for three weeks and powdered using an electric mill.

### Macroscopic examination

The following macroscopic characters of the stem bark were noted: size and shape, colour, surfaces, fracture when broken, odour and taste [11, 12].

Microscopic examination

The transverse sections of the stem bark were cleared by warming with chloral hydrate solution, mounted and observed under a compound microscope [13]. The cells were stained with phloroglucinol and hydrochloric acid.

Chemomicroscopic examination

Examination of the powder for starch grains, lignin, mucilage, calcium oxalate crystals, cutin and suberin were carried out using standard techniques [11].

### Phytochemical studies

Screening for secondary plant metabolites was carried out according to previously described methods [11, 14-16]. These included chemical tests for tannins, alkaloids, cardiac, saponin, anthracene and cyanogenetic glycosides.

## Extraction and partitioning

The dried stem bark of *A. zygia* (1.2 kg) was extracted with Methanol (5 X 2L). Evaporating the solvent yielded an extract (82 g) which was subsequently resuspended in water and successively partitioned into Chloroform (3 X 2L) and n-BuOH (3 X 2L). The fractions were investigated for antioxidant and antimicrobial activity.

## Determination of antioxidant activity

The scavenging effect of the crude (80 % methanol extract) and the various fractions on DPPH radical was determined [17]. A 1.0 mL solution of 0.1 mM of DPPH was mixed with 3.0 mL of the crude extract, fractions and ascorbic acid (0.05-0.2 mg/ml concentration). The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm. Ascorbic acid was used as reference standard. The ability to scavenge DPPH radical was calculated by the following equation:

DPPH radical scavenging activity (%) = $[(A0 – A1)/(A0)] \times 100$

Where; A0 was the absorbance of DPPH radical + methanol,

A1 was the absorbance of DPPH radical + sample extract/standard [18].

The 50% inhibitory concentration value ($IC_{50}$) which was indicated as the effective concentration of the sample that was required to scavenge 50% of the DPPH free radical [17] was also determined.

Determination of polyphenolic content

Total phenol contents in the extracts were determined by the method described by Kim [19]. The extract solution (0.5 ml) with concentration of 1000 ug/ml was added to 4.5 ml of deionized water and 0.5 ml of Folin-Ciocalteu's reagent (previously diluted with water 1: 10 v/v) which was added to the solution. After mixing the tubes, they were maintained at room temperature for 5 min followed by the addition of 5 ml of 7% sodium carbonate and 2 ml deionized water. The samples were incubated for 90 min at room temperature and the absorbance was measured with a spectrophotometer at 750 nm. The total phenolic content was expressed as milligrams of gallic acid equivalents (GAE) per gram of extract (mg GAE/g extract). The standard curve was prepared with gallic acid in six different concentrations (12.5, 25, 50, 75, 100 and 150 mg/L).

## Determination of Total Flavonoid

Total flavonoid contents were estimated using the method described by Ebrahimzadeh [20]. 0.5 ml of the crude extract and fractions (1 mg/ml) were mixed with 1.5ml of methanol and 0.1ml of 10% aluminum chloride was added, followed by 0.1ml of 1M potassium acetate and 2.8 ml of distilled water. The mixture was incubated at room temperature for 30 minutes. The absorbance was measured by a spectrophotometer at 415 nm. The results were expressed as milligrams quercetin equivalents (QE) per gram of extract (mg QE/g extract). The standard curve was prepared with quercetin in six different concentrations (12.5, 25, 50, 5, 100, and 150 mg/L).

## Antimicrobial assay

Clinical strains of five bacteria comprising three gram negative bacteria (Escherichia coli, Pseudomonas aeruginosa and Klebsiella aerogenes) and two gram positive bacteria (Staphylococcus aureus and Bacillus subtillis) were used for the antibacterial assay. Two yeasts (Candida albican and Candida parasilopsis) were used for the antifungal assay. The organisms were obtained from the Department of Pharmaceutical Microbiology Laboratory, Faculty of Pharmacy, University of Benin, Benin City, Nigeria. The purity of the culture prior to use was confirmed by conventional cultural, morphological and biochemical methods. The microbial cultures were maintained in Nutrient Agar and Sabouraud Dextrose Agar for bacteria and fungi respectively at 4 °C.

## Preparation of inoculum

An overnight culture was used for the preparation of microbial suspension with a turbidity equivalent to that of 0.5 McFarland's standard.

## Agar well diffusion method

The media were prepared and sterilized at 121°C for 15 min. A total of 30 ml Nutrient agar was seeded with bacterial culture and allowed to solidify and on each plate wells of 10 mm in diameter were made. The open wells were filled with different concentrations of the extract ranging from 20 mg/ml to 100mg/ml, and incubated at 37°C for 24 h. For antifungal assay, Sabouraud agar was used in place of Nutrient agar and the medium incubated at 28 °C for 2 days. All tests were carried out in triplicates. The inhibition

zone diameter were measured and compared with ciprofloxacin and nystatin for antibacterial and antifungal assays respectively [21].

### Statistical analysis

All the data were expressed as mean + SEM (Standard error of student's t-test. P-value of <0.05 was considered statistically significant.

## RESULTS

Macro-micro morphology of *A. zygia* stem bark *Albizia zygia* occurs in single quills. Pieces of bark vary in length. The outer surface is rough, greyish in colour and marked with transversely elongated lenticels. The bark has silver grey patches of lichens, mosses which occur in the outer surface. The inner surface is yellowish in colour. The fracture is short. It has a characteristic odour with a slightly acid taste.

The transverse section shows a layer of cork, cortex and phloem transversed by medullary rays. The cells of the cork are tangentially elongated and arranged in radial rows. The cortex consists of numerous thin-walled cells and they contain starch grains. There are sclerids (stone cells) present in the cortex. The stone cells have thick walls with lumen. The primary phloem which comes after the parenchyma consists of fibers. The secondary phloem which forms the bulk of the bark is transversed by medullary rays. The phloem fibers are lignified.

Chemo-microscopic examination of the stem bark revealed the presence of lignin, starch, mucilage, tannins and cellulose.

### Phytochemical screening

Phytochemical screening of the stem bark of *A. zygia* for secondary plant metabolites revealed the presence of alkaloids, tannins, flavonoids and saponins glycosides (Table 1).

### Antioxidant activity

There was concentration dependent increase in the scavenging activity of the crude extract and fractions ranging from 5 μg/ml to 200 μg/ml. The n-Butanol fraction demonstrated the highest activity.

**Table 1: Phytochemical constituents of *A. zygia* leaves**

| Classes of secondary metabolites | Inferences |
|---|---|
| Alkaloids | + |
| Tannins | + |
| Flavonoids | + |
| Anthracene derivatives | - |
| Saponin glycosides | + |
| Cardiac glycosides | _ |
| Cyanogenetic glycosides | _ |

**Key:** = absent; + = present

## Total phenolic content and flavonoid assay

Table 3 shows the total phenolic and flavonoid content of the crude extract and fractions of *Albizia zygia* stem bark. Total phenolic content are expressed as milligrams of gallic acid equivalents (GAE) per gram of extract (mg GAE/g extract) by reference to the calibration plot. (Fig. 2)

$$y = 0.002x + 0.005 \text{ and } R2 = 0.998$$

Total flavonoid content is expressed as milligram Quercetin equivalents (QE) per gram of extract (mg QE/g extract) as calculated from the appropriate calibration plot. (Fig. 3)

$y = 0.007x – 0.0021$ and $R2 = 0.995$

**Table 2: $IC_{50}$ values of crude extract and fractions**

| Samples (Extract) | $IC_{50}$ (µg/ml) |
|---|---|
| Crude methanol extract | 1,481 |
| Aqueous | 1,773 |
| Chloroform | 210.61 |
| n-Butanol | 190.18 |
| Ascorbic acids | 0.595 |

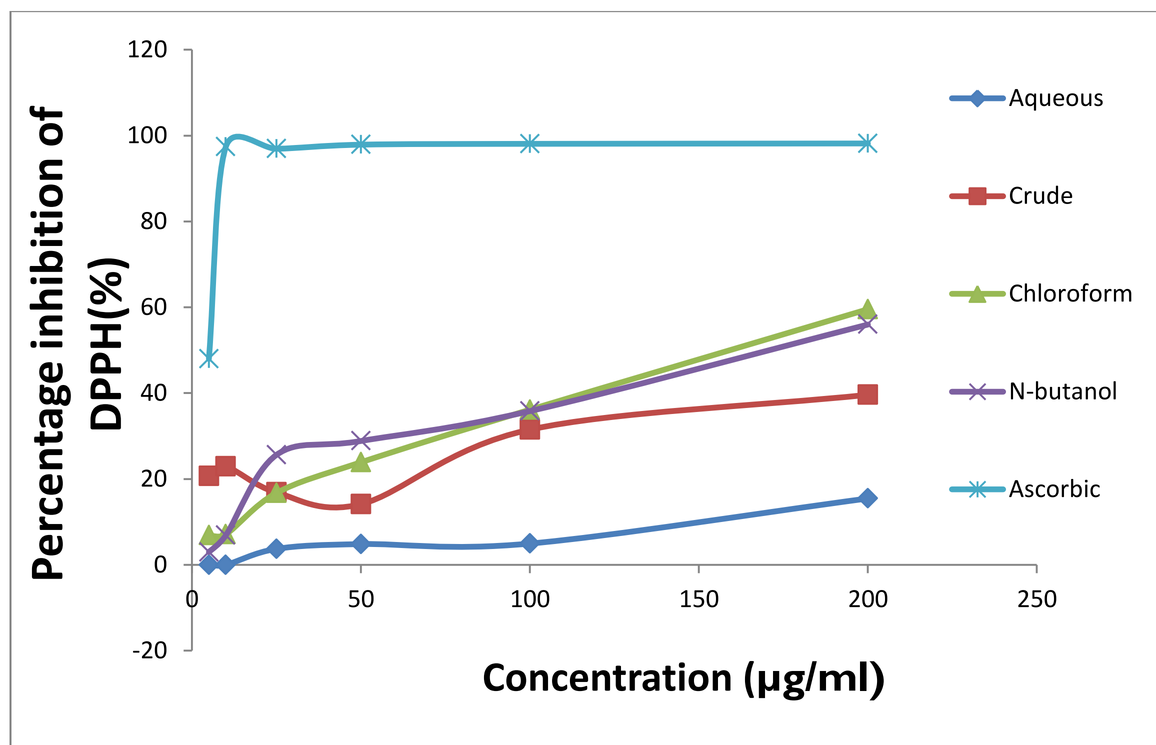


**Figure 1: 2, 2-diphenyl-1-picryl hydraxyl radical (DPPH) scavenging activity of the crude extract and fractions of *A. zygia* compared with the standard (Ascorbic acid)**

**Table 3: Total Phenolic and Flavonoid content of *Albizia zygia***

| SAMPLES | TOTAL PHENOL ± SEM | TOTAL FLAVONOID ± SEM |
|---|---|---|
| Crude | 92.70±6.83 | 7.47±0.14 |
| Chloroform | 59.94±3.16 | 59.37±3.53 |
| N-butanol | 112.70±9.56 | 8.48±1.035 |
| Aqueous | 28.20±4.67 | 8.71±0.2084 |

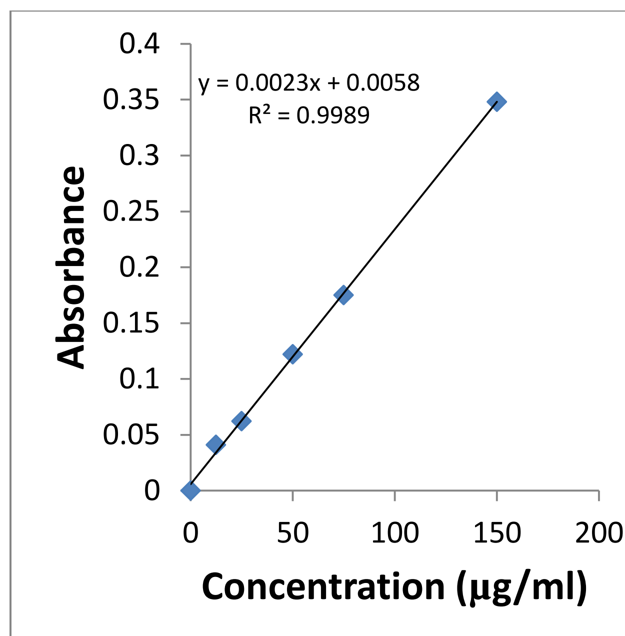


**Figure 2: Calibration Plot for Gallic acid**

## DISCUSSION

The types and distributions of Pharmacognostic characters in plants aid in their classification and identification. Before any crude drug can be included in an herbal pharmacopoeia, Pharmacognostic parameters and standards must be established. The macro- and micro-morphological features described could therefore, serve as a basis of proper identification, collection and investigation of the stem bark of *Albizia zygia*.

Antioxidants are one of the most essential ingredients of today's therapy since they reduce *in vivo* oxidative damages. Plants are good sources of natural antioxidants [22]. DPPH radical scavenging method is widely used to investigate the total antioxidant activities in plants due to the fact that it is simple, rapid and inexpensive [23]. The method is based on scavenging of DPPH free radicals by the antioxidant. While *A. zygia* showed concentration dependent increase in scavenging activity, except for the crude extract at the initial stage, the extracts and fractions had lower antioxidant activities compared to the standard, ascorbic acid. The 50 % inhibitory concentration value ($IC_{50}$) is the effective concentration of the sample that is required to scavenge 50 % of the DPPH free radicals. Among the various fractions, chloroform and n-butanol fractions had high phenolic content and maximum antioxidant property compared to the aqueous fraction. This could serve as a guide in structural activity related experiments.

Phenolic compounds are crucial for plant growth and reproduction, and are produced as a response to environmental factors such as light and pollution and to defend injured plants [24]. In recent years, the importance of antioxidant activities of phenolic compounds and their potential usage in processed foods as natural antioxidant compounds has reached a new level [25]. The results of the total phenolic and flavonoid contents indicated that the n-butanol fraction had the highest total phenol (112.70 ± 9.56 mgGAE/g extract) while the chloroform fraction contained significantly more flavonoids (59.37 ± 3.53 mgGE/g extract) than other fractions. The Folin-Ciocalteu assay has for many years been used to measure total phenolics in natural products. The basic mechanism is an oxidation/reduction reaction and as such can be considered a measure of antioxidant capacity provided the specific conditions are followed to minimize variability and eliminate erratic results. The different levels of antioxidant activities in plants may be due to not only differences in their phenolic contents, but also in their phenolic acid components [26]. In this study, the high content of total phenolic compounds in *A. zygia* may be responsible for its free radical scavenging activity.

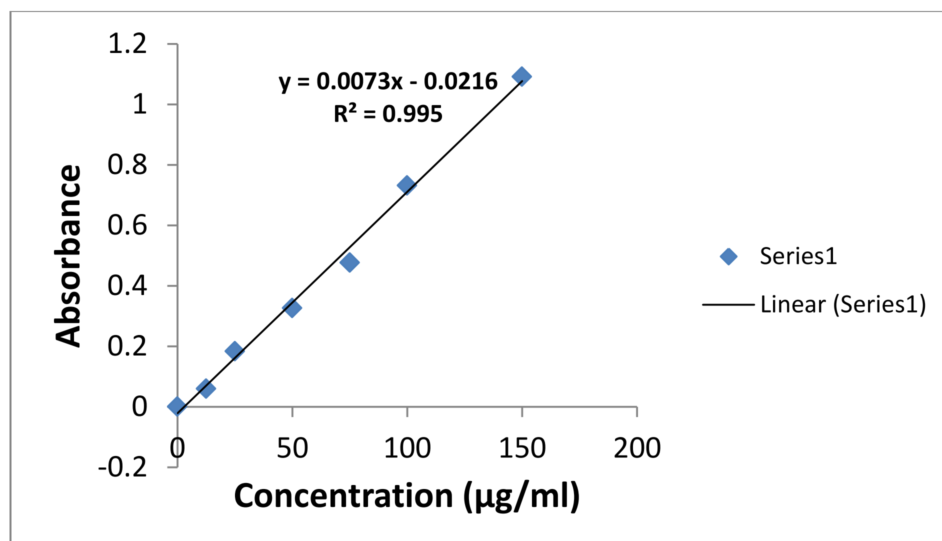


**Figure 3: Calibration Plot for Quercetin**

**Table 4: Antimicrobial activity of 80% methanol extract of the stem bark of *A. zygia***

| Organisms | Diameter of zones of inhibition (mm) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 80% methanol extract (mg/ml) | | | | | Cp (µg/ml) | Nys (µg/ml) |
| | 20 | 40 | 60 | 80 | 100 | 10 | 10 |
| *E. coli* | G | 11±0.03 | 13±0.01 | 14±0.01 | 15±0.02 | 34±0.03 | ND |
| *P. aeruginosa* | G | 11±0.01 | 12±0.03 | 13±0.01 | 14±0.01 | 32±.16 | ND |
| *Kleb.aerogenes* | G | G | 11±0.01 | 12±0.01 | 13±0.01 | 20±0.75 | ND |
| *B. subtilis* | G | G | 13±0.02 | 14±0.02 | 15±0.01 | 30±0.33 | ND |
| *S. aureus* | G | 11±0.01 | 12±0.02 | 13±0.02 | 14±0.02 | 28±0.23 | ND |
| *C. albican* | G | 12±0.02 | 13±0.02 | 14±0.01 | 16±0.02 | ND | 22±0.12 |
| *C. parasilopsis* | G | 11±0.01 | 13±0.02 | 14±0.02 | 15±0.02 | ND | 21±0.23 |

Values are expressed as mean ± SEM, (G) indicates no inhibition zone, (ND) indicates Not Determined, (Cp) indicates ciprofloxacin $P<0.05$, (Nys) indicates Nystatin $P<0.05$

**Table: 5: Antimicrobial activity of n-Butanol fraction of the stem bark of *A. zygia***

| **Organisms** | **Diameter of zones of inhibition (mm)** | | | | | | |
|---|---|---|---|---|---|---|---|
| | **n-Butanol fraction (mg/ml)** | | | | | **Cp (µg/ml)** | **Nys (µg/ml)** |
| | 20 | 40 | 60 | 80 | 100 | 10 | 10 |
| *E.coli* | 16±0.01 | 17±0.01 | 19±0.02 | 20±0.01 | 21±0.03 | 31±0.68 | ND |
| *P.aeruginosa* | 23±0.03 | 24±0.02 | 25±0.01 | 27±0.01 | 28±0.01 | 30±0.67 | ND |
| *Kleb. aerogenes* | 12±0.02 | 15±0.03 | 16±0.01 | 17±0.02 | 18±0.01 | 22±0.33 | ND |
| *B.subtilis* | G | 11±0.02 | 12±0.01 | 14±0.01 | 15±0.01 | 34±0.54 | ND |
| *S.aureus* | 20±0.03 | 23±0.02 | 24±0.03 | 25±0.01 | 26±0.01 | 29±0.43 | ND |
| *C. albican* | G | G | 11±0.02 | 12±0.01 | 13±0.01 | ND | 23±0.22 |
| *C. parasilopsis* | G | G | 11±0.02 | 13±0.03 | 12±0.01 | ND | 24±0.63 |

Values are expressed as mean ± SEM, (G) indicates no inhibition zone, (ND) indicates Not Determined, (Cp) indicates ciprofloxacin $P<0.05$, (Nys) indicates Nystatin $P<0.05$

**Table: 6: Antimicrobial activity of Chloroform fraction of the stem bark of *A. zygia***

| Organism | Diameter of zones of inhibition (mm) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Chloroform fraction (mg/ml) | | | | | Cp (µg/ml | Nys (µg/ml) |
| | 20 | 40 | 60 | 80 | 100 | 10 | 10 |
| *E.coli* | 15±0.01 | 16±0.01 | 17±0.01 | 18±0.04 | 21±0.03 | 30±0.67 | ND |
| *P. aeruginosa* | 14±0.03 | 15±0.02 | 16±0.01 | 17±0.01 | 18±0.01 | 28±0.83 | ND |
| *Kleb. Aerogenes* | 12±0.02 | 14±0.03 | 16±0.01 | 17±0.02 | 18±0.01 | 20±0.65 | ND |
| *B. subtilis* | 11±0.01 | 12±0.02 | 13±0.01 | 14±0.02 | 15±0.01 | 34±0.42 | ND |
| *S. aureus* | 18±0.02 | 20±0.01 | 22±0.03 | 23±0.01 | 24±0.02 | 32±0.53 | ND |
| *C. albican* | G | G | G | 12±0.01 | 13±0.01 | ND | 24±0.23 |
| *C. parasilopsis* | G | G | G | 13±0.03 | 12±0.01 | ND | 22±0.45 |

Values are expressed as mean ± SEM, (G) indicates no inhibition zone, (ND) indicates Not Determined, (Cp) indicates ciprofloxacin $P<0.05$, (Nys) indicates Nystatin $P<0.05$

**Table: 7: Antimicrobial activity of aqueous fraction of the stem bark of *A. zygia***

| Organism | Diameter of zones of inhibition (mm) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Aqueous fraction (mg/ml) | | | | | Cp (µg/ml) | Nys (µg/ml) |
| | 20 | 40 | 60 | 80 | 100 | 10 | 10 |
| *E.coli* | G | G | G | G | G | 30±0.22 | ND |
| *P.aeruginosa* | G | G | G | G | G | 34±0.21 | ND |
| *Kleb. Aerogenes* | G | G | G | G | G | 26±0.12 | ND |
| *B.subtilis* | G | G | G | G | G | 28±0.31 | ND |
| *S.aureus* | G | G | G | G | G | 32±0.33 | ND |
| *C.albican* | G | G | G | G | G | ND | 26±0.02 |
| *C.parasilopsis* | G | G | G | G | G | ND | 24±0.03 |

Values are expressed as mean ± SEM, (G) indicates no inhibition zone, (ND) indicates Not Determined, (Cp) indicates ciprofloxacin, (Nys) indicates Nystatin.

The inhibition of growth of the organisms by the crude extract and fractions, except for the aqueous fraction can be attributed to the presence of biologically active complex organic chemicals (secondary plant metabolites) in the tissues. Traditional healers do not use the water as a solvent system administering *A. zygia*. The crude extract demonstrated broad spectrum of activity, justifying its usage in the management of infectious diseases, including oral thrush, candidiasis in which Candida albicans and Candida parasilopsis are implicated

## Conclusion

The results obtained from this study showed that *Albizia zygia* Benth possesses unique Pharmacognostic parameters that can aid its standardization and quality control. It has also been shown that it could be effective in the management of infectious diseases.

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