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Preliminary Standardization and Antifungal Potential of the Ethanol Extract of *Emilia praetermissa* leaf on *Candida albicans* and *Aspergillus niger*

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ABSTRACT

Fungal infections caused by *Candida albicans* and *Aspergillus niger* has become a rampant global health challenge, particularly among immunosuppressed individuals. Conventional antifungal drugs are available with setbacks such as drug resistance and adverse effects, prompting the need for alternative therapies. This research aims to evaluate the antifungal efficacy of Ethanol Extract of *Emilia praetermissa* leaf against *Candida albicans* and *Aspergillus niger*. In this study, *Emilia Praetermissa* leaf was extracted using cold maceration technique. Phytochemical and Physicochemical tests was evaluated using standard procedures. The antifungal effect of the plant extract was evaluated against laboratory isolates of *Candida albicans* and *Aspergillus niger*. Standard antifungal tests using microdilution method were performed to determine the minimum inhibitory concentrations (MICs). To ascertain the zones of inhibition, agar well diffusion method was employed. The ethanol extract of *Emilia praetermissa* leaf demonstrated significant antifungal activity against both *Candida albicans* and *Aspergillus niger*. For *Candida albicans*, the highest zone of inhibition was seen at 300 mg/mL and the least at 18.75 mg/mL. However, when compared, the positive control (Fluconazole) had a higher zone of inhibition. For *Aspergillus niger*, the least zone of inhibition was at 18.75 mg/mL while its highest was at 300 mg/mL. In contrast, the control (Fluconazole) had no effect. The MIC for both *Candida albicans* and *Aspergillus niger* was 37.5 mg/mL. This study therefore, suggests that Ethanol Extract of *Emilia praetermissa* leaf possesses antifungal activity against tested isolates.

Keywords: *Emilia praetermissa*, *Candida albicans*, *Aspergillus niger*, Phytochemical, Physicochemical

1. INTRODUCTION

The insurgence of fungal infections, particularly those caused by *Candida albicans* and *Aspergillus niger*, has become a rampant global health challenge. *Candida albicans*

exhibits commensalism and lives within the human microbiota. It can transition to a pathogenic state, causing infections ranging from superficial mucocutaneous infections to severe systemic diseases. The prevalence of *Candida albicans* infections is often exacerbated in cases of immunodeficiency arising from medical disorders or drug therapies (Bertolini and Dongari-Bagtzoglou, 2019). About 75% of women will experience at least one episode of vulvovaginal Candidiasis during their lifespan. Factually, 70-75% of healthy adult women experience an episode of vulvovaginal candidiasis during their reproductive years, and 50% of women in colleges would have been be diagnosed of vulvovaginal candidiasis by age twenty-five (25) (Arya and Naureen, 2023).

Aspergillus niger is a filamentous fungus commonly found in soil and decaying organic matter. The microbe is known for its black spore-producing structures and its significant role in industrial processes, such as the production of citric acid and enzymes (Klich, 2009). However, *A. niger* can also be a potent pathogen, particularly in immunocompromised individuals, where it causes a variety of infections collectively known as aspergillosis. *A. niger* infections can manifest in different forms, ranging from non-invasive conditions like allergic bronchopulmonary aspergillosis (ABPA) to more severe invasive aspergillosis, affecting organs such as the brain, lungs etc (Klich, 2009). Another life-threatening form of aspergillosis is Invasive aspergillosis, which is usually associated with a high incidence of mortality and morbidity in immunosuppressed patients, especially in patients undergoing chemotherapy, organ transplants, those suffering from long-term granulomatous diseases etc (De Francesco, 2023)

Aspergillus niger is ubiquitous in nature, thus the spores has high tendency of being inhaled by humans (Centre for Disease Control, 2022). They mainly affect those with impaired immune systems or pre-existing lung diseases, such as cystic fibrosis and asthma. The types of aspergillosis include allergic bronchopulmonary aspergillosis (ABPA), allergic aspergillus sinusitis, azole-resistant aspergillus fumigatus, cutaneous (skin) aspergillosis, and chronic pulmonary aspergillosis (Centre for Disease Control, 2021). Forty (40) of the one hundred and eighty (180) species of aspergillus molds are mainly pathogenic in immunosuppressed individuals (Centre for Disease Control, 2021). Among horticultural workers who frequently breathe peat dust, which can be rich in *Aspergillus niger* spores, aspergillosis is very common.

Emilia praetermissa, a plant with a history of traditional medicinal use, has been employed in folk medicine for its diverse medicinal properties (Afolayan *et al*., 2017). The aim of this research was to systematically evaluate the antifungal potentials of *Emilia praetermissa* leaf extracts against *Candida albicans* and *Aspergillus niger*, shedding light on its possible application as a good antifungal agent of natural origin.

Conventional anti-fungal drugs are associated with setbacks such as drug resistance and adverse effects. This has triggered the recent surge in exploration of alternative and complementary medicines such as medicinal plants usage. Most people in developing countries find plants more appealing as medicinal agents due to their availability and affordability in comparison with orthodox medications (Sharma *et al*., 2015)

Due to the alarming rate of fungal infections in Delta State of Nigeria, understanding the antifungal potential of indigenous plants like *Emilia praetermissa* becomes a paramount step towards addressing these challenges. Frequent incidence of antifungal resistance justifies the urgency of exploring novel sources of antifungal agents. This research aims to bridge the knowledge gap by evaluating the antifungal potentials of *Emilia praetermissa* extracts against *C. albicans* and *A. niger* thereby providing valuable information that will aid development of antifungal agents.

Standardization can be defined as measures taken to establish consistent biological activity, and/or chemical profile, or simply a quality assurance program that must be complied with, during the production of herbal drugs (Patra et al., 2010). On that note, this research attempts to standardize the crude drug using preliminary parameters such as moisture content, ash value and extractive value. This present study aligns with global efforts to come up with novel therapeutic compounds of natural origin, elucidating their vitality in accumulating evidence-based knowledge on herbal remedies while advancing scientific understanding. The outcomes of this study hold the potential to enhance our arsenal against *C. albicans* and *A. niger* infections and pave the way for future developments in natural antifungal therapies.

2. MATERIALS AND METHODS

Materials

Laboratory Materials

Foil paper, syringe, test-tube, test-tube racks, beakers, measuring cylinder, Pasteur pipette, spirit lamp, 6mm cork borer, swab stick, cotton wool, metre rule, Nichrome wire loop, fluconazole infusion (2 mg), Petri dish, analytical and mechanical weighing balances, whatmann No 42 filter paper, rotary evaporator.

Chemicals and Reagents

Hydrochloric acid (concentrated and dilute) (BASF, Germany), Sodium hydroxide (AkzoNobel, China), Ferric chloride (BASF, Germany), Sulphuric acid (BDH, China), Mayer's reagent (SHC, China), Dragendorff's reagent (SHC, China), Wagner's reagent (SHC, China), Lead acetate, saboraund dextrose agar, Nutrient broth and Saboraund dextrose broth (HK, Ltd., China).

Solvents

The following analytical grade solvents were used for the experiment; Acetone (INEOS, China), methanol (BASF/YPC, Germany/China), Dichloromethane (AkzoNobel, China), H_2O/chloroform, Ethanol (ADM, China).

Collection of Plant Materials

The plant *Emilia praetermissa* was collected in March 2024 at Abraka community, Delta State. Authentication of plant sample was done by Prof. Henry Adewale Akinnibosun, a taxonomist in the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin. A unique identification number, UBH-E407 was assigned to the voucher specimen.

Preparation of Plant Extract

The leaves was dried to remove moisture for at least two weeks. The dried leaves were pulverized using a milling machine. The pulverized dried leaves was weighed and about 400g of the smooth plant material was removed for extraction and the remaining was stored in an air tight container for physicochemical screening. Cold maceration technique was used to extract the plant material. After weighing, four hundred grams (400 g) of the pulverized leaf was carefully weighed and macerated in 1,600 ml of ethanol in a container and was allowed to stand for 72 hours with occasional agitation for proper extraction. The filtrate was obtained after 72 hours and concentrated using the rotary evaporator.

Phytochemical Screening

Phytochemical screening was conducted according to standard procedures of Shaikh et al., (2020) to detect the presence or absence of phytochemicals such as saponins, tannins, flavonoids, alkaloids, terpenoids in the *Emilia praetermissa* leaf extract.

Physicochemical Screening

The quality of the plant extract was ascertained following established procedures of Krishna *et al*., (2014). The following parameters were used for physicochemical screening

1. Moisture content
2. Ash value
3. Extractive value
4. Acid-insoluble and water-soluble ash

Determination of Moisture Content

The porcelain dish was weighed and the weight was noted correctly, 2 g of powdered sample was added to the porcelain dish, the weight of the powdered sample and the porcelain dish was also noted. The porcelain dish containing the powdered sample was placed in hot air oven. The temperature of the hot air oven was set at 105 degrees centigrade using the temperature setting knob. After 15 minutes, the porcelain dish containing the dried extract was brought out and placed in a desiccator to cool and prevent re-absorption of

moisture by the dried powdered plant sample. The dish containing the dried extract was weighed after cooling for 15minutes in the desiccator. This process of drying, cooling and weighing was repeated until a constant weight was gotten. The moisture content for each plant extract was the difference between the initial weight of the porcelain dish and the powdered plant and the final weight of the porcelain dish and the dried powdered plant. Percentage moisture content was calculated as the moisture content divided by the initial weight of the powdered plant multiplied by 100 (Krishna *et al*., 2014).

Determination of Total Ash Value

An empty crucible was dried to remove any trace of moisture using hot air oven at a temperature of 105°C. The crucible was removed from the hot air oven after 20 minutes and cooled in a desiccator. The weight of the crucible was carefully noted. 2 g of each powdered sample was measured into both crucible and the crucible covered with a lid. Both crucibles containing the powdered sample were placed inside the muffle furnace which was turned on and the temperature set at 700 degrees centigrade. The samples were allowed to stay in the furnace for 2 hrs. After 2hrs, the muffle furnace was turned off and a stainless steel locking tong was used to take out the crucible which was transferred to a desiccator for cooling. After cooling, the final weight of the crucible containing ash was noted and the percentage ash was also calculated.

Determination of Acid-insoluble Ash

HCl (40%) was prepared by adding 60 mL of water to 40 mL of HCl. The crucible was placed on a weighing balance and 2 g of the powdered sample was measured correctly into the crucible. The crucible was covered with a lid and placed in a muffle furnace. The powdered sample was burned at 550 degrees centigrade for 30 minutes. After 30 minutes, the crucible was ejected from the furnace, the lid was removed and the ash was observed carefully, few drops of distilled water was added to the ash content in the crucible to check for any possible development of black colour which usually indicates that the ash still has a lot of carbon. Upon addition of the distilled water, a black colour was observed and this prompted the need to burn again. Before placing the crucible in a furnace to burn again, the crucible was placed on a hot plate to dry the moist ash completely. The crucible containing the dried ash was returned to the furnace for another 40 minutes after which it was observed that it was carbon free after addition of distilled water failed to produce any black colour.

After confirming that the ash was carbon free, 25 mL of the 40% HCl solution was measured and emptied into the crucible containing ash. The ash content in the HCl solution was boiled for 5 minutes by placing the crucible on the hot plate. After boiling, a filter system was prepared using an ashless filter paper, the ash solution was filtered while it was still warm. The residues trapped on the filter paper after filtration was noted as the acid-insoluble ash. The ashless filter paper was gently rinsed with hot water to ensure no trace of the acid was left within the filter paper.

To remove any trace of moisture, the crucible was subjected to drying in a hot air oven for 30 minutes. After drying, it was cooled in a desiccator for 10 minutes. The weight of the crucible was noted, then the acid-insoluble ash trapped on the filter paper was immersed in the crucible, covered with lid and pushed into the furnace set at a temperature of 550 degrees centigrade for 90 minutes. The crucible was taken from the furnace after 90 minutes and allowed to cool. At this point, the crucible containing the acid-insoluble ash was weighed and the percentage acid-insoluble ash was calculated. (Krishna *et al*., 2014).

Determination of Water-soluble Ash

With the aid of hot air oven, an empty crucible was dried. After cooling, the empty crucible and lid was weighed and the weight noted. 2 g of the powdered sample was measured into the crucible and then the weight of the crucible, lid and the crude drug was also noted. At this point, the crucible containing the crude drug was placed in the muffle furnace until it becomes red hot, then it was switched off and the crucible containing the ash was removed and kept in the desiccator to cool for 20 minutes. The weight of the crucible, lid and ash was noted and 25 mL of distilled water was added to dissolve the ash. The mixture of the ash and the distilled water was emptied into a beaker and subjected to heating for 15minutes. This was subjected to filtration using ashless filter paper, after filtration, the filter paper retains the water insoluble ash while the filtrate contains the water-soluble ash. The insoluble ash retrieved, placed in a crucible and kept in an incinerator until became red hot, then it was carefully ejected from the incinerator and placed in a desiccator to allow cooling. The water-soluble ash was the difference between the water insoluble ash and the total ash. Finally, the percentage water-soluble ash was calculated accordingly (Krishna *et al*., 2014).

Determination of Ethanol Soluble Extractives

Powdered drug weighing 5g was added into a glass container and transferred into a dry 250 mL conical flask. A 100 mL graduated flask was filled with 90% ethanol and emptied into the conical flask containing the powdered drug. The conical flask was corked and subjected to constant agitations within the first 6hrs, then allowed to stand for the remaining 18hrs. Filtration was done after 24 hours, when sufficient filtrate has been collected. 25 mL of each filtrate was transferred to a weighed, thin porcelain dish. The filtrate in the porcelain dish was evaporated to dryness on a water bath, followed by complete drying in a hot air oven at 100°C. The dried extract was then transferred to a desiccator to ensure cooling. The weight of the cooled dried extract was noted. The percentage w/w of the extractives with reference to the air-dried drug was calculated accordingly (Krishna *et al*., 2014).

Determination of Water-soluble Extractives

A powdered drug weighing 5g was added into a glass container and transferred into a dry 250 mL conical flask. A 100 mL graduated flask was filled with 99% distilled water (99 mL water and 1 mL chloroform). The chloroform acts as a preservative. The mixture was emptied into the conical flask containing the powdered drug. The conical flask was corked and subjected to constant agitations within the first 6 hrs, then allowed to stand for the remaining 18 hrs. Filtration was done after 24hours, when sufficient filtrate has been collected. 25 mL of each filtrate was transferred to an already weighed porcelain dish and evaporated to dryness on a water bath. Complete drying was done using hot air oven. The dried extract was then transferred to a desiccator to ensure cooling. This cooled dried extract was weighed and the percentage $^{w}/_{w}$ was calculated with reference to the air-dried drug.

Determination of Acetone, Dichloromethane and methanol Soluble Extractives

Five gram (5g) of each powdered drugs was weighed into a weighing bottle and transferred into a dry 250 mL conical flask. 100 mL graduated flask was filled with acetone, dichloromethane and methanol respectively. This was emptied into the conical flask containing each powdered drug. The conical flask was corked and subjected to constant agitations within the first 6hrs, then allowed to stand for the remaining 18 hrs. Filtration was done after 24 hours, when sufficient filtrate has been collected, 25 mL of each filtrate was transferred to an already weighed porcelain dish and evaporated to dryness on a water bath. Further drying was done in a hot air oven at a temperature of 100°C. The dried extract was then transferred to a desiccator to ensure cooling. This cooled dried extract in the porcelain dish was weighed and the percentage w/w with reference to the air-dried drug was calculated accordingly.

Antifungal Sensitivity Test

The plants extract's antifungal activity against *Candida albicans* and *Aspergillus niger* was assessed and the degree of effectiveness was compared. (Balouiri *et al*., 2016)

Preparation of the Organism

The microorganisms *C. albicans* and *Aspergillus niger* were obtained from the Pharmaceutical Microbiology Laboratory. The microorganisms were selected because of their prevalence and clinical importance. The Saboraund dextrose broth was prepared and inoculated with the microorganisms and were incubated for 24 hours before antifungal test was evaluated.

Preparation of Culture Media

The Sabouraund dextrose agar was prepared according to the manufacturer's instructions and was autoclaved for 20 minutes at 121 °C. The media was allowed to cool for 10 minutes. Thereafter 20 mL of the agar was measured using a sterile measuring cylinder and was poured aseptically into the labelled Petri dish and was allowed to solidify. The broth of the test organism was spread on the agar plate under aseptic conditions using a sterile swab stick. This procedure was repeated for each of the test microorganisms.

Preparation of Well Inoculation

The agar well diffusion method was employed. A flame-sterilized 6 mm cork borer was used to bore 4 holes in each freshly prepared Sabouraund dextrose agar for both microorganisms. There were five Petri dishes representing different concentrations with positive control in each of the Petri dishes.

Application of Plant Extract

A 1.5 mL of the plant extract concentration prepared using serial dilution (300, 150, 75, 37.5 and 18.75 mg/mL) was introduced into the various well using the Pasteur pipette. Fluconazole 2 mg/mL was used as a positive control.

Incubation of Plates

The plates were left undisturbed for 20 minutes after application of plant extracts. These plates were subjected to incubation for 48 hours at a temperature of 37°C.

Measurement of Zones of Inhibition

A clear zone around each well indicates inhibition and each zone were measured with the aid of a meter rule in millimeters (mm). The diameter of the well was subtracted from the zone of inhibition and the results was recorded.

Determination of Minimum Inhibitory Concentration (MIC)

The agar well diffusion method was used to ascertain the minimum inhibitory concentration (Magaldi *et al*., 2014). The Sabouraund dextrose agar were prepared according to the manufacturer's instruction. The agar was autoclaved at 121 °C for 20 minutes and was allowed to cool for 10minutes. Thereafter, 2 mL of the extract was prepared with serial dilution for each concentration (300,150, 75, 37.5, 18.75), then the agar was added to each of the extract and poured into the Petri dish, it was swerved to ensure proper mixing. After solidifying a sterile wire loop was used to pick up the organism from the innoculums and streak the surface of the solid agar. The agar plate was incubated at 37 °C for 48 hours. The results were recorded for both microorganisms.

Statistical Analysis

The data obtained were evaluated using Graphpad Prism 7.0. One way analysis of variance (ANOVA) was used in the data analysis and was represented as mean Standard Error of Mean (SEM).

3. RESULTS

Percentage yield of Extraction

A 400g of the powdered *Emilia praetermissa* leaf yielded 70.9 g extract. The percentage yield was 17.73%.

Physicochemical evaluation of *E. praetermissa*

The results of the physicochemical evaluation done on pulverized *E. praetermissa* leaf reveals the moisture content, Total Ash value, Acid-insoluble ash, and water-soluble ash obtained as 6.17±0.73%, 4.56 ±0.12%, 1.40±0.02% and 0.67±0.23% respectively (Table 1). The extractive values using ethanol, water, acetone, dichloromethane and methanol was 6.00±0.12%, 10.67±0.87%, 4.73±0.64%, 5.27±1.37% and 8.00±1.15% respectively.

Table 1: Results from physicochemical evaluation of *E. praetermissa* ethanol leaf extract

Physicochemical parameters	**Yield (%)**
Moisture Content	6.17 ± 0.73
Total Ash Value	4.56 ± 0.12
Acid-insoluble ash	1.40 ± 0.02
Water Insoluble Ash	0.67 ± 0.23
Ethanol soluble extractives	6.00 ± 0.12
Water-soluble extractives	10.67 ± 0.87
Acetone soluble extractives	4.73 ± 0.64
Dichloromethane soluble extractives	5.27 ± 1.37
Methanol soluble extractives	8.00 ± 1.15

Mean ± SEM (Standard Error for Mean) where n=3

Phytochemical evaluation of *E. praetermissa*

The results of the qualitative phytochemical screening of the Ethanol Extract of *Emilia praetermissa* leaf revealed presence of Tannins, Saponins, Steroids, Terpenoids and Alkaloids (Table 2).

Table 2: Results from phytochemical evaluation of *E. praetermissa*

Phytoconstituents	***E. praetermissa* Ethanol Leaf Extract**
Tannins	+
Saponins	+
Flavonoids	-
Alkaloids	+
Cardiac Glycosides	-
Steroids	+
Reducing Sugars	-
Terpenoids	+

Key: - = Absent; + = Present

Results for antifungal activities of ethanol leaf extract of *Emilia praetermissa*

Ethanol Extract of *Emilia praetermissa* leaf showed moderate antifungal activity against *Candida albicans* (Table 3). The highest concentration (300 mg/mL) produced the most significant inhibition (9.67 ± 0.67 mm), which decreased as the concentration decreased with the lowest concentration (18.75 mg/mL) having the lowest inhibition (7.00 ± 0.58 mm). The control (fluconazole) showed a significantly higher zone of inhibition (20.00 ± 0.58 mm) compared to the extract indicating that while the extract was effective, it was less potent than the standard antifungal drug.

The Ethanol Extract of *Emilia praetermissa* leaf also showed moderate antifungal activity against *Aspergillus niger*. The highest observed inhibition was at 150 mg/mL (6.67 ± 0.67 mm), while the lowest was at 18.75 mg/mL (4.00 ± 1.00 mm). Interestingly, the control showed no inhibition against *Aspergillus niger*, indicating that fluconazole at this concentration (2 mg/mL) was ineffective against this fungus.

Table 3: Antifungal activity of ethanol extract of *Emilia praetermissa* leaf

Organism	**Concentrations (mg/mL)/Zones of inhibition (mm)**					
	300	**150**	**75**	**37.5**	**18.75**	**Control**
Candida albicans	9.67 ± 0.67	7.67 ± 1.33	8.00±1.15	8.33 ± 0.67	7.00±0.58	20 ± 0.58
Aspergillus niger	6.67 ± 0.67	7.33 ± 1.86	4.33±0.33	4.33 ± 0.88	4.00±1.00	Nil

Key: Control; Fluconazole 2 mg/mL. Mean±SEM (Standard Error for Mean) where n = 3

Results for Minimum Inhibitory Concentration of Ethanol Extract of *Emilia praetermissa* on both microorganisms

The Minimum Inhibitory Concentration (MIC) of the Ethanol Extract of *Emilia praetermissa* leaf against *Candida albicans* and *Aspergillus niger* are presented as follows (Table 4, Fig.1 & Fig.2):

For *Candida albicans*, at higher concentrations (300 mg/mL, 150 mg/mL, 75 mg/mL and 37.5 mg/mL), the ethanol extract effectively inhibits the growth of *Candida albicans* while at 18.75 mg/mL, there was growth indicating that the Minimum Inhibitory Concentration (MIC) of the ethanol extract of *Emilia praetermissa* on *Candida albicans* is 37.5 mg/mL.

For *Aspergillus niger*, at higher concentrations (300 mg/mL, 150 mg/mL, 75 mg/mL and 37.5 mg/mL), the Ethanol Extract effectively inhibits the growth of *Aspergillus niger* while at lower concentrations (18.75 mg/mL and 9.375 mg/mL), there was growth indicating that the Minimum Inhibitory Concentration (MIC) of the Ethanol Extract of *Emilia praetermissa* on *Aspergillus niger* was 37.5 mg/mL.

Table 4: Minimum Inhibitory Concentration of Ethanol Extract of *Emilia praetermissa* leaf against *Candida albicans* and *Aspergillus niger*

Organism	Concentrations (mg/mL)					
	300	150	75	37.5	18.75	9.375
Candida albicans	-	-	-	-	+	+
Aspergillus niger	-	-	-	-	+	+

Key: - = No growth; + = Growth

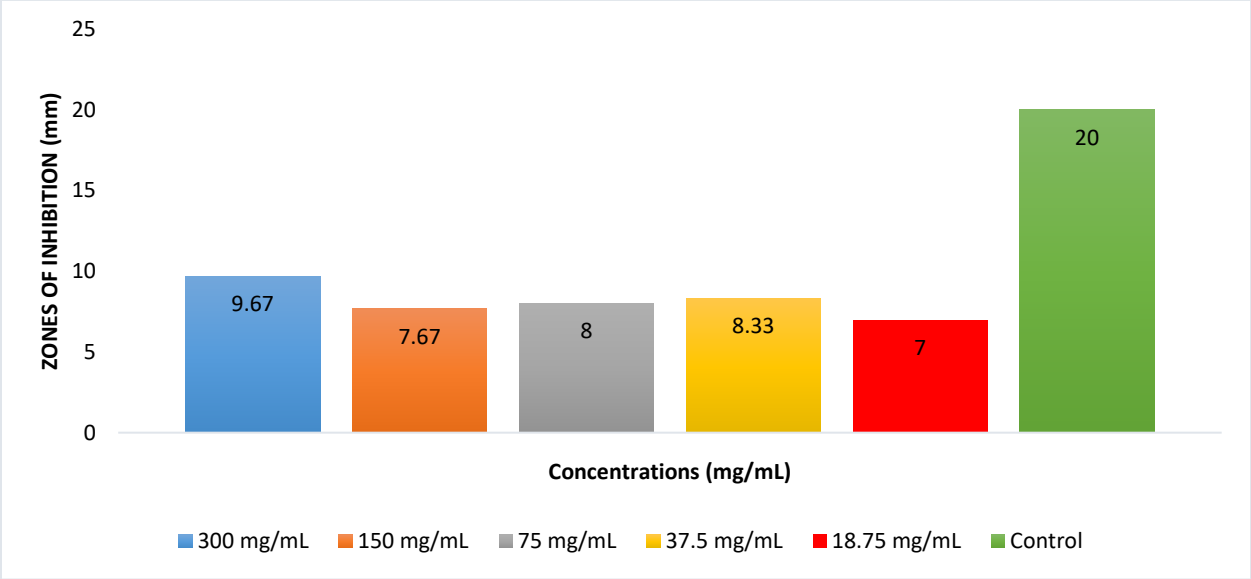

Figure 1: Antifungal activity of Ethanol Extract of *Emilia praetermissa* leaf against *Candida albicans*

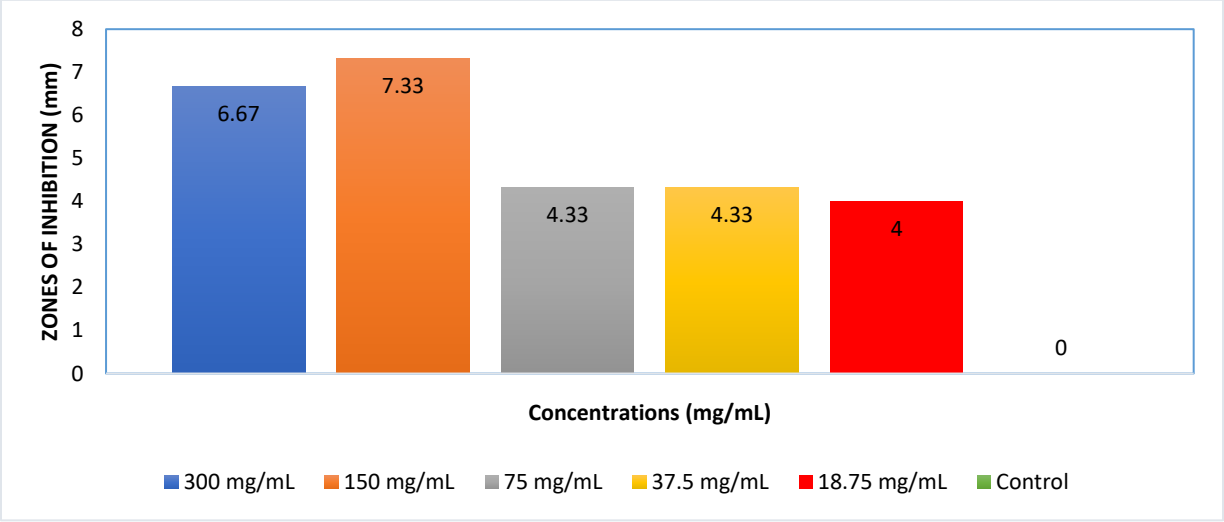

Figure 2: Antifungal activity of Ethanol Extract of *Emilia praetermissa* leaf against *Aspergillus niger*

4. DISCUSSION

The physicochemical properties of *Emilia praetermissa* leaf discloses information about purity, identity and quality of the plant material. The moisture content of *Emilia praetermissa* leaf was 6.17 ± 0.73. Moisture content is crucial as high moisture levels can promote microbial growth, leading to spoilage (Smith, 2024). Therefore, the observed value suggests that the *Emilia praetermissa* leaf has a relatively low moisture content, thereby suggesting low risk of microbial contamination and an extended shelf life.

The total ash value, which measures the total amount of inorganic material, was 4.56 ± 0.12%. Ash values are essential for determining purity and detecting possible adulterants in the plant material (Sharma, 2022). The acid-insoluble ash value (1.40 ± 0.02%) and water-soluble ash value (0.67 ± 0.23%) suggests that the crude drug has minimal level of silica and phosphate contamination.

The extractive values signifies the amount of active constituents extracted using different solvents (Sharma, 2022). Ethanol-soluble extractives were 6.00 ± 0.12%, indicating that many of the plant's active compounds are ethanol-soluble. The water-soluble extractives (10.67 ± 0.87%) suggest that more compounds can be extracted with water, which could be advantageous for formulations requiring aqueous extracts. Acetone, dichloromethane, and methanol soluble extractives were 4.73 ± 0.64%, 5.27 ± 1.37%, and 8.00 ± 1.15%, respectively, showing a varied solubility profile of the plant's constituents across different solvents.

Evaluation of the phytoconstituents of the Ethanol Extract of *Emilia praetermissa* leaf revealed the presence of secondary metabolites such as Alkaloids, tannins, saponins, steroids, and terpenoids. Similarly, a study by Ikezu (2023) revealed the presence of tannins, saponins, steroids, terpenoids and alkaloids in the phytochemical screening of *Emilia praetermissa* ethanol leaf extract.

The presence of these secondary metabolites suggests potential therapeutic benefits. Tannins have astringent properties and can protect against microbial infections. Saponins possess antimicrobial and anti-inflammatory properties, while steroids are known for their anti-inflammatory and analgesic effects. Reducing sugars are essential for metabolic activities, and terpenoids are known for their broad spectrum of biological activities, including antimicrobial and anti-inflammatory effects.

The antifungal effect of the ethanol extract of *Emilia praetermissa* leaf was tested against two fungal strains: *Candida albicans* and *Aspergillus niger*. The results indicated moderate antifungal activity, with the highest inhibition observed at 300 mg/mL for both fungi and the Minimum Inhibitory Concentration (MIC) was 37.5 mg/mL. A study by Erhabor *et al*., (2013) on Antimicrobial Activity of the Methanol and Aqueous Leaf Extracts of *Emilia coccinea* (Sims) G. Don showed that the highest zone of inhibition for Methanol and Aqueous Leaf extract of *Emilia coccinea* on both *Candida albicans* and *Aspergillus niger* was 35 mg/mL (which was the highest concentration used in the study). The Minimum Inhibitory Concentration (MIC) for Methanol Extract on both *Candida albicans* and *Aspergillus niger* was 35 mg/mL and 5 mg/mL respectively while the Minimum Inhibitory Concentration (MIC) for Aqueous Leaf Extract on both *Candida albicans* and *Aspergillus niger* was 35 mg/mL and 25 mg/mL respectively. This study shows similarity to the current research as the highest Zone of inhibition recorded in this study for *Emilia praetermissa* was also at the highest concentration used therefore displaying its potent antifungal activity.

5. CONCLUSION

This study suggests that the Ethanol Extract of *Emilia praetermissa* leaf possesses antifungal activity against the tested isolates. Comparatively, the standard drug (fluconazole) exerted a better antifungal effect against the tested fungal isolates. The physicochemical analysis buttressed the quality of the crude drug. Hence, the results obtained can be used as a reference tool for further standardization of the crude drug.

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Author Contributions

The design of the study, research and manuscript writing is by Lawrence U Nwankwo and Uyoyo M Ukpe. The analysis, interpretation of result and discussion is by Uyoyo M. Ukpe and Christiana O. Akpo. Proof reading is by Christiana O. Akpo and Lawrence U. Nwankwo.

Ethical approval & declaration

In this article, as per the plant regulations followed in the Department of Pharmacognosy and Traditional Medicine, Faculty of Pharmacy, Delta State Unversity, Abraka, Nigeria; the authors observed the antifungal Potential of the Ethanol Extract of *Emilia praetermissa* leaf on *Candida albicans* and *Aspergillus niger*. The plant *Emilia praetermissa* was collected in March 2024 at Delta State and authenticated by Prof. Henry Adewale Akinnibosun, a taxonomist in the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin. A unique identification number, UBH-E407 was assigned to the voucher specimen. The ethical guidelines for plants & plant materials are followed in the study for species observation, identification & experimentation.

Informed Consent

Not applicable

Conflict of Interest

The authors declare that there are no conflicts of interests

Funding

The study has not received any external funding

Data and materials availability

All data associated with this study are present in the paper

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