

ORIGINAL RESEARCH ARTICLE

Antifungal Activity of Aqueous and Ethanolic Extracts of *Bryophyllum Pinnatum* Against Pathogenic Fungi in Sokoto State, Nigeria

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ABSTRACT

This study evaluates the antifungal activity of the aqueous and ethanolic extracts of the leaves and stems of *Bryophyllum pinnatum* against *Aspergillus niger*, *Aspergillus flavus*, and *Fusarium oxysporum*. Ethanolic extracts exhibit higher antifungal activity against *F. oxysporum* (38.67mm at 200mg/mL), corresponding to the control drug's activity (hexaconazole, 38.33mm at 10µg/mL). In contrast, *A. Flavus* demonstrated a complete resistance to all aqueous extracts with lower susceptibility to ethanolic extracts. The statistical analysis showed significant differences ($p < 0.05$) between concentrations and extracts, while MIC confirmed the highest efficacy of ethanolic extract, with the lowest MIC (60 mg/mL) observed against *F. oxysporum* and *A. niger*. This study offers new insights into the antifungal properties of *Bryophyllum pinnatum* and underlines its significance as a locally accessible antifungal drug in resource-poor areas. Future work needs to isolate bioactive compounds in this plant and compare efficacy with traditional treatments.

KEYWORDS

Antifungal, *Bryophyllum pinnatum*, *Aspergillus niger*, *Aspergillus flavus*, *Fusarium oxysporum*.



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INTRODUCTION

Medicinal herbs are used broadly to cure and treat several diseases (Grover *et al.*, 2002). The World Health Organization (WHO) recognizes the importance of medicinal plants as a valuable source of potential therapeutic drugs. Medicinal herbs have been utilized since long ago for the treatment of different ailments in multiple traditional medical systems such as Ayurveda, Traditional Chinese Medicine, and various indigenous healing systems (Mekuria *et al.*, 2017; Ekor, 2014; Bahmani *et al.*, 2014). From the beginning of history, man has searched for ways to remedy common illnesses and diseases. Such knowledge has resulted in significant advances in using plants (leaves, roots, stem barks, pods, and flowers) with therapeutic properties for treating prevalent diseases (Petrovska, 2012). Traditional herbal medicine has received great acceptance over the decades, with about 80% of the world population using the system as the only form of treatment (Dorine *et al.*, 2021). The ease of access, availability, effectiveness, and affordability of medicinal plants make them acceptable for treating common ailments (Patwardhan *et al.*, 2005). Moreover, secondary metabolites of various plant origins have shown considerable activity against multiple microbes (Abreu *et al.*, 2012; Chandra *et al.*, 2017). This has been recognized by the World Organization for Health (WHO), which considers traditional medicine a fundamental

component of the health system with excellent results for its users (Palhares *et al.*, 2020).

Bryophyllum pinnatum is a member of the family Crassulaceae, also known as the family of sprouting leaves. A widely-used carrot plant in traditional medicine in Africa, China, Australia, Tropical America and India. It is a perennial herbaceous plant that grows in the wild. It is a member of the family of Crassulaceae and is bred as a decorative house plant on stone and in gardens (Obaid *et al.*, 2019). It holds various names like air plant, canterbury bell, cathedral bell, life plant, and resurrection plant (Shrestha *et al.*, 2019). It is known as Eru-odundun (Yoruba), Odaopue or Alupu (Igbo), and Abomoda (Hausa). It is common worldwide due to its ease of cultivation and widespread use in herbal medicine. Deep green and scalloped edges, many trimmed in red, make up its lush foliage. It flowers from November to March and fruits in April. The leaves and bark are a bitter tonic, astringent to the bowels, analgesic, carminative, and beneficial in diarrhoea, ulcers, and vomiting (Khan *et al.*, 2009). They are also recognized for their haemostatic and wound-healing capabilities (Aleksandra *et al.*, 2019). It is gaining great attention for its medicinal properties and is used in folk and modern medicine (Ogidi *et al.*, 2009). It is a good source of vitamins and includes alkaloids,

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flavonoids, saponins, triterpenes, steroids, glycosides, and tannins as bioactive substances (Uchegbu *et al.*, 2017). The plant has shown anti-leishmanial activity Afzal *et al.* (2012), hepato- and nephro-protective effects Anosike *et al.*, (2019), and analgesics (Aprioku and Igbe, 2017). Antihypertensive is one suggested action of the leaf extract (Orelien *et al.*, 2014) and anti-allergic activity (Parra *et al.*, 2019).

Fungal infections pose significant challenges in healthcare and agriculture, particularly in regions with scarce commercial antifungal drugs. In circumstances such as Sokoto State, Nigeria, where high temperature and humidity foster the Prevalence of fungal infections, the problems are worsening due to antifungal resistance. *Bryophyllum pinnatum* has been traditionally used for wound healing and antimicrobial treatments, yet its antifungal potential remains underexplored. This study evaluates the antifungal properties of its aqueous and ethanolic extracts against key fungal pathogens to assess its potential as a cost-effective alternative to synthetic antifungals.

MATERIALS AND METHOD

Study Area

Usmanu Danfodiyo University Laboratory of Mycology, Sokoto, Nigeria. Sokoto is in the north westerly extreme of Nigeria at the confluence of River Sokoto and River Rima (latitude 13°N, longitude 5°E, and 350m above sea level).

Plant Material Collection

Fresh and matured leaves and stems of *Bryophyllum pinnatum* (Miracle Leaf) were collected from the Biological Garden of Usmanu Danfodiyo University, Sokoto, in May 2023. Herbarium of the Plant Science Department, Usmanu Danfodiyo University, Sokoto, Nigeria, confirmed the plant's identity.

Preparation of Plant Material

The collected leaves and stems were kept separately, washed thoroughly with tap water to remove any visible impurities, and then air dried at room temperature to avoid active compound loss (Girish and Satish, 2000). Stems and leaves were dried for three and four weeks, respectively, due to differences in thickness. The dried parts of the plant material were ground with a mortar and pestle, sieved through 2mm mesh (British Standard), and kept in a sterile container until ready for experiments.

Preparation of the Aqueous and Ethanolic Extracts

Extraction was performed as described by (Harborne, 1973; Aliyu *et al.*, 2009). The plant powder weighing one hundred grams (100g) was soaked in 1 litre of distilled water or ethanol for a day. The resulting mixtures were filtered and evaporated in a hot, dry oven at 40°C; the dried residues were stored and used for sensitivity tests.

Source of the Fungal Isolates

Pure cultures of *A. flavus*, *A. niger*, and *F. oxysporum* were obtained from the Mycology Laboratory of Usmanu Danfodiyo University, Sokoto.

Systematic Preparation of Fungal Inoculum

The fungal inoculum was prepared by growing the test organisms in potato dextrose broth for 24 hours at room temperature. 0.2 ml of the different fungal broth cultures was used for the antifungal study (Okpoho *et al.*, 2018).

Preparation of Media

Potato dextrose agar (PDA) was prepared by dissolving 39g of PDA powder in 1 liter of distilled water (thoroughly stirred to dissolve) and was subsequently sterilized by autoclaving at 121°C for 15 min. After it was cooled to room temperature, 30mL of the media was dispensed into sterile Petri dishes. Stock solutions of extracts (60, 140, and 200 mg/mL) were prepared, and the test organisms were introduced at 5ml per plate. Control plates contained 15 mL of sterile media to monitor fungal growth.

Antifungal Activity Screening

Wells of 6mm diameter were made in the PDA media using a sterile cork borer. 0.1 mL of the extract prepared at concentrations (60, 140, and 200 mg/mL) were used to fill each well using a micropipette. A separate well was used to fill the standard antifungal drug hexaconazole as a control. Assign a horizontal line beneath the horizontal line, center under this line, and the relative line between the horizontal line with the Zonal inhibition (the area where the fungus does not grow around the disk) was measured after 48 hours of incubation at 28°C.

Minimum Inhibitory Concentration (MIC)

MIC was determined at the lowest concentration (60, 140, and 200 mg/mL) of the extracts that inhibited visible signs of fungal growth (EUCAST, 2024)

Statistical Analysis

SPSS version 23.0 was used for data analysis. All the results were presented as mean \pm standard errors, and statistically significant differences among parameters were determined ($p < 0.05$) using one-way ANOVA (Ogbeibu, 2015).

RESULTS

Tables 1 and 2 show the antifungal activity of *Bryophyllum pinnatum* extracts obtained from ethanol and aqueous solvents. Compared to the aqueous extract, ethanolic extracts showed much higher antifungal activity for all test organisms. Higher concentrations had greater zones of inhibition, indicating concentration-dependent activity.

Fusarium oxysporum was the most susceptible pathogen to the ethanolic leaf extract (38.67 mm at 200 mg/mL, and this activity was equal to that of the control antifungal drug (hexaconazole, 38.33 mm at 10 µg/mL). All concentrations of aqueous extracts and the lowest (60 mg/mL) of ethanolic extracts showed resistance against *Aspergillus flavus*. It was moderate susceptibility with ethanolic extracts of *Aspergillus* species exhibiting the inhibition zones ranging from 11.33 mm - 22.00 mm, while the aqueous extract showed the least activity.

Likewise, the antifungal activity of ethanolic stem extracts was greater than that of aqueous extracts, and *Fusarium oxysporum* was highly susceptible (38.67 mm at 200 mg/mL). Some activity was observed for *A. flavus*;

however, the aqueous stem extracts did not inhibit *A. flavus* at all concentrations.

The MIC values (Table 3) further confirm the superior efficacy of the ethanolic extracts, particularly the leaves, which exhibited lower MIC values against all tested organisms. This suggests that bioactive compounds responsible for antifungal activity are more effectively extracted in ethanol than water.

The scatter plots (Figure 1, 2, 3 &4) show the relationship between extract concentration and antifungal activity. A strong positive correlation was observed for almost the test organisms, except for *A. flavus* which show no correlation on aqueous extract.

Table 1. Antifungal activity of leaves extract of *Bryophyllum pinnatum* and control (hexaconazole) on test microorganisms.

Extracts	Concentration (mg/mL)	<i>A. Flavus</i> (mm)	<i>A. Niger</i> (mm)	<i>F. oxy.</i> (mm)
Ethanolic extract	60	0.00	11.33 ^b	19.00 ^a
	140	7.67 ^a	16.00 ^a	32.00 ^b
	200	8.67 ^a	22.00 ^c	38.67 ^c
Aqueous Extract	60	0.00	0.00	0.00
	140	0.00	5.33 ^a	7.67 ^a
	200	0.00	5.00 ^a	9.00 ^b
Control (hexaconazole)	10µg	0.00	24.00 ^b	38.33 ^c

Values with different superscripts (a, b, c) within the same column are significantly different at p < 0.05, based on comparisons between concentrations of the same extracting solvent.

Table 2. Antifungal activity of Stem extract of *Bryophyllum pinnatum* on test microorganisms

Extract	Concentrations (mg/ml)	<i>A. Flavus</i> (mm)	<i>A. niger</i> (mm)	<i>F. oxy.</i> (mm)
Ethanolic extract	60	6.33 ^a	15.00 ^a	30.67 ^a
	120	12.33 ^b	18.00 ^b	31.33 ^a
	200	15.00 ^c	19.00 ^c	38.67 ^b
Aqueous extract	60	0.00	5.67 ^a	0.00
	120	0.00	9.33 ^b	16.00 ^a
	200	0.00	9.33 ^b	16.67 ^a

Values with different superscripts (a, b, c) within the same column are significantly different at p < 0.05, based on comparisons between concentrations of the same extracting solvent.

Table 3. Minimum inhibitory concentration of stem and leaves of *Bryophyllum pinnatum*.

Micro-organisms	AL	AS	EL	ES
<i>A. flavus</i>	-	-	140	60
<i>A. niger</i>	140	60	60	60
<i>F. oxysporum</i>	140	140	60	60

AL: Leaves aqueous extract AS: Stem aqueous extract EL: Leaves ethanolic extract ES: Stem ethanolic extract.

DISCUSSION

Medicinal plants and their secondary metabolites are widely known to be potential sources of new antimicrobial agents to treat various pathogens (Dorine et al., 2021). Phytochemicals, including alkaloids, flavonoids, and saponins, are associated with plants' therapeutic efficacy and have fewer side effects than synthetic drugs (Kennedy and Wightman, 2013).

The antifungal activity of ethanolic and aqueous extracts of *Bryophyllum pinnatum* leaves and stems against *Aspergillus flavus*, *Aspergillus niger*, and *Fusarium oxysporum* was

evaluated in this study. Ethanolic extracts were more potent than aqueous extracts, and leaves were more effective than stems. The ethanolic leaf extract at 200 mg/ml had a zone of inhibition of 38 mm against *Fusarium oxysporum*, similar to the control antifungal drug 10 µg/ml. This supports prior reports that organic solvent, such as ethanol, will usually extract more of the bioactive component of the plant compared to aqueous solvent, hence the reason for higher antifungal activity in the ethanolic fraction of the extracts (Okpoho et al., 2018).

A. flavus exhibited resistance to the aqueous extracts and moderate susceptibility to ethanolic extracts, with

inhibition zones ranging from 11.33 mm to 22.00 mm. This resistance may be attributed to the thick, rich cell wall of *A. flavus*, which limits the permeability of bioactive compounds present in water-soluble extracts, as observed in previous studies (Okpoho *et al.*, 2018). An example is *Fusarium oxysporum*, whose cell wall is more permeable, which makes it more attackable by ethanolic extracts that

are rich in active phytochemicals (Ammara *et al.*, 2009). This phenomenon has been observed in other fungal species, suggesting that the cell wall structure plays a crucial role in fungal resistance. Future studies should investigate the synergistic effects of *B. pinnatum* extracts in combination with conventional antifungal agents to overcome this resistance.

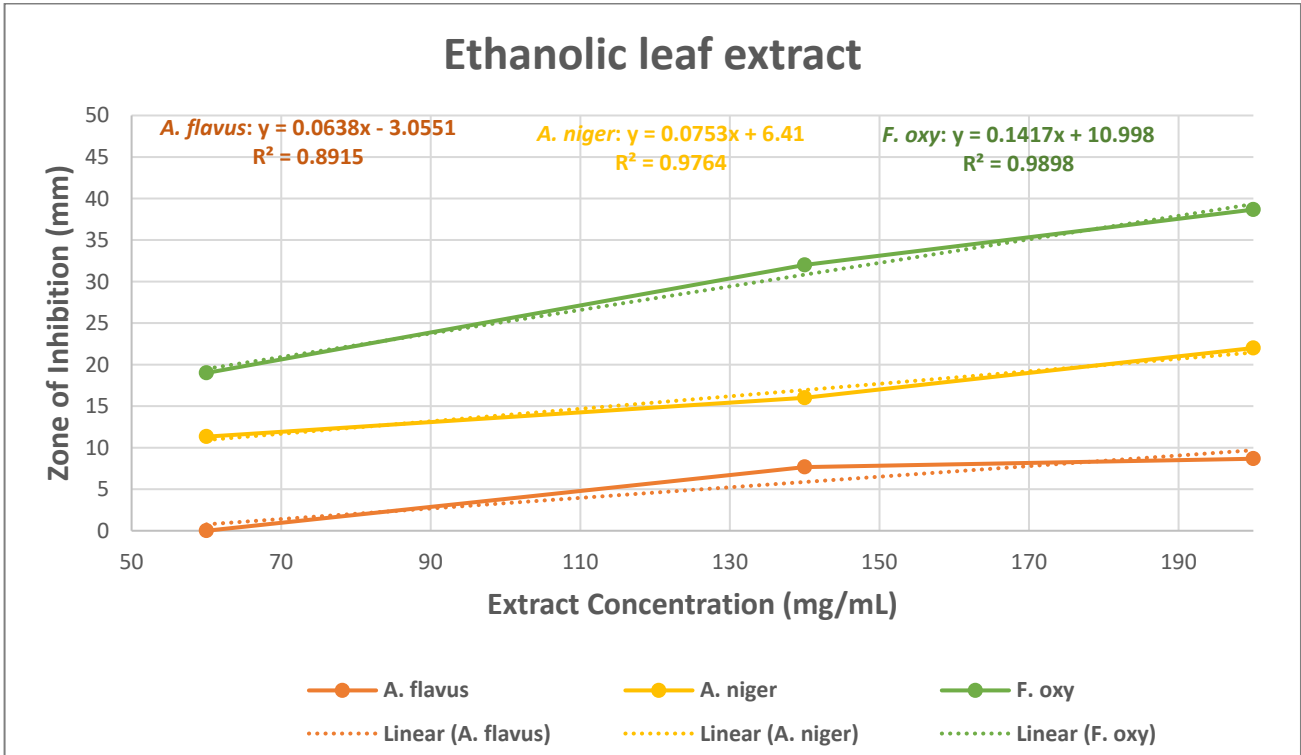


Figure 1: Scatter plot showing correlation between extract concentration(mg/mL) and zone of inhibition (mm) for ethanolic leaf extract against *A. flavus*, *A. niger*, and *F. oxysporum*

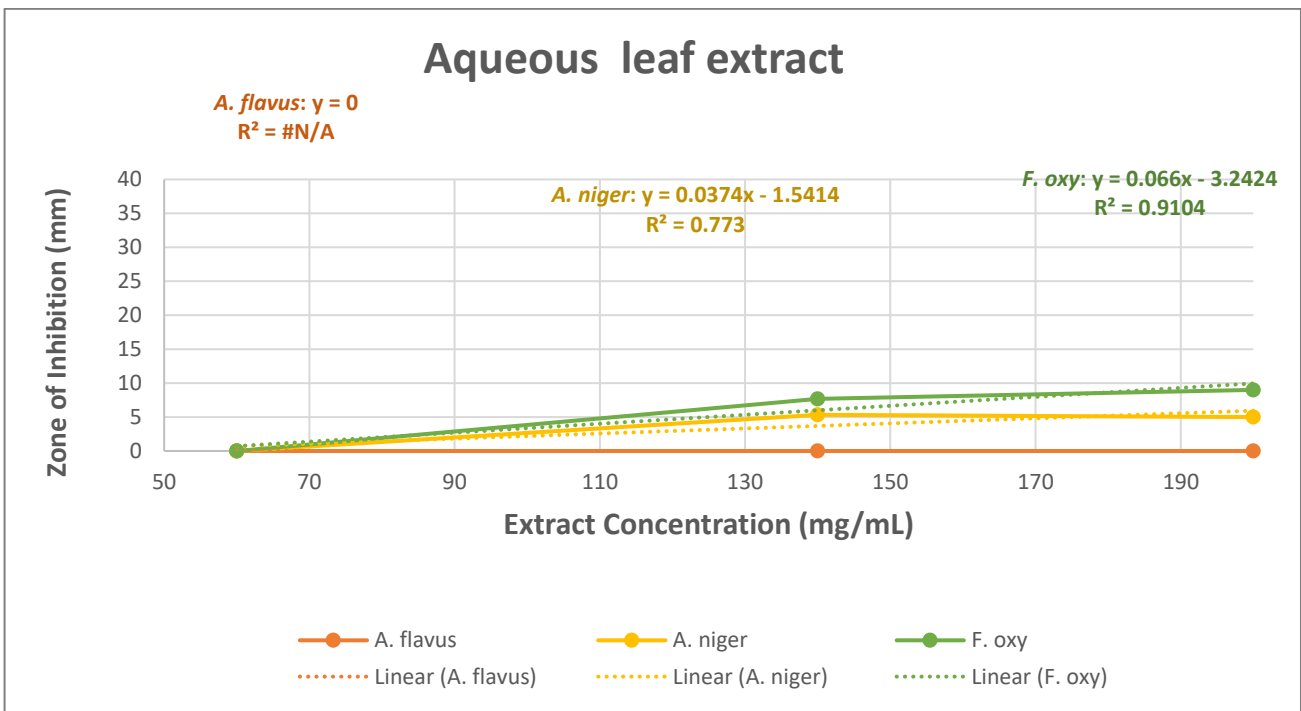


Figure 2: Scatter plot showing correlation between extract concentration(mg/mL) and zone of inhibition (mm) for aqueous leaf extract against *A. flavus*, *A. niger* and *F. oxysporum*

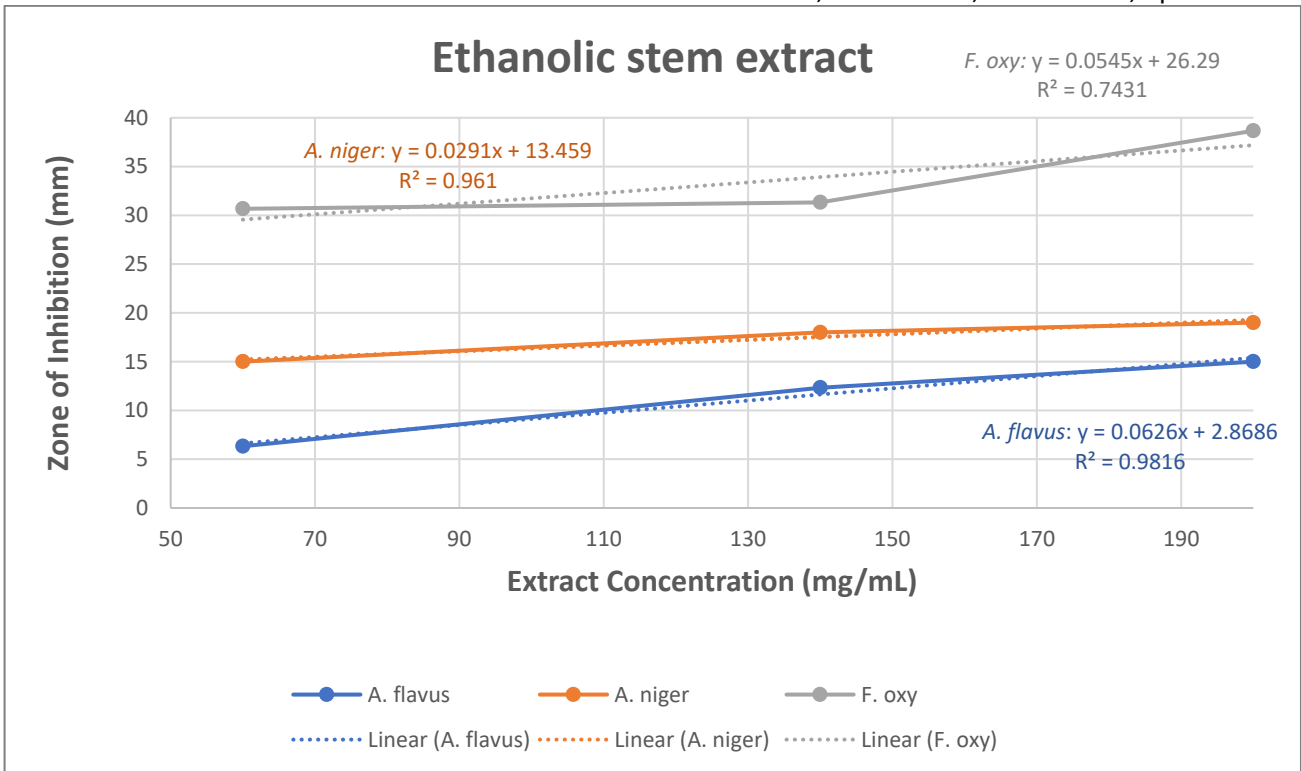


Figure 3: Scatter plot showing correlation between extract concentration(mg/mL) and zone of inhibition (mm) for ethanolic stem extract against *A. flavus*, *A. niger*, and *F. oxysporum*

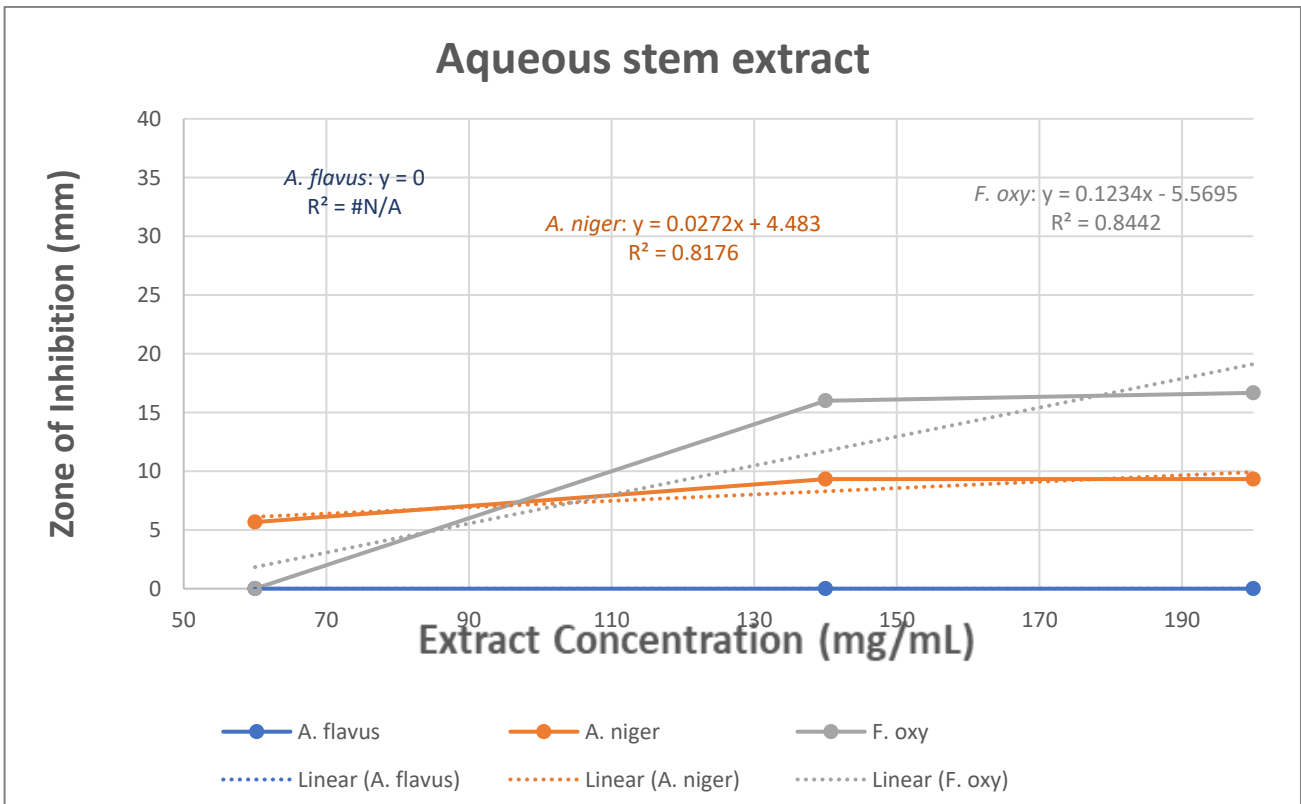


Figure 4: Scatter plot showing correlation between extract concentration(mg/mL) and zone of inhibition (mm) for aqueous stem extract against *A. flavus*, *A. niger*, and *F. oxysporum*

These variations observed in this study may have resulted from various fungal strains, extraction techniques, or environmental factors in which Akinnibosun and Edionwe (2015) reported a related finding where several fungal species, including *C. albicans*, were resistant to extracts of *Bryophyllum pinnatum*.

In the case of MIC, the Concentration of ethanolic extracts had lower results compared to those of the aqueous extracts (therefore more potent) on the basis of the MIC values. The ethanolic leaf extract showed inhibition at 60 mg/ml against most of the test organisms as opposed to the aqueous extracts that needed higher

concentration for the same effect; this is in line with the work of Okpoho *et al.* (2018), which was also observed in their studies. These results also highlight the utility of ethanol as a solvent to yield active antifungal constituents for the extract of *Bryophyllum pinnatum*.

Correlation analysis was conducted between extract concentration and fungal inhibition. A clear concentration-dependent relationship was observed across all tested extracts. Regression analysis of the data revealed that higher concentrations resulted in larger zones of inhibition, confirming that the antifungal effect of *B. pinnatum* extracts is concentration-dependent, with most of the organisms having R^2 close to 1. Figures 1 and 3 demonstrate a strong correlation, supporting the efficacy of higher concentrations for antifungal activity.

Given the effectiveness of *B. pinnatum* ethanolic extract against *F. oxysporum* and *A. niger*, it could be developed as a botanical fungicide to protect crops from fungal diseases. Using plant-based antifungals is particularly beneficial for organic farming, reducing reliance on synthetic chemicals. Furthermore, in regions like Sokoto State, where access to commercial antifungal drugs is limited, *B. pinnatum* may serve as an alternative treatment for fungal infections, pending further toxicological evaluations.

CONCLUSION

This study confirms the antifungal potential of *Bryophyllum pinnatum*, particularly its ethanolic extracts, against *F. oxysporum* and *A. niger*. The results highlight the choice of solvent and plant parts as the main aspects for maximizing efficacy since ethanolic leaf extracts presented the highest activity. However, the resistance observed in *A. flavus* suggests the need for further studies on its mechanism of action. Future studies should also isolate and characterize the specific bioactive compounds that contribute to the antifungal effects observed and compare their effects with conventional antifungal agents.

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