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Evaluation of *Heliotropium indicum* Extracts Against Fungal Pathogens of Snake Gourd Tomato (*Trichosanthes cucumerina*)

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ABSTRACT

Background and Objective: Snake Gourd Tomato, is a crop of great agronomic, nutritional and medicinal importance. This study investigates fungal pathogens affecting (*Trichosanthes cucumerina*) and evaluates the antifungal potential of *Heliotropium indicum* extracts. The objective was to explore eco-friendly alternatives for managing fungal diseases in this crop. **Materials and Methods:** In 2023, a study was conducted to isolate and identify fungal pathogens from samples. Fungal isolates were identified and the antifungal activity of aqueous and ethanol extracts of *Heliotropium indicum* was evaluated at concentrations of 2.5, 5.0 and 7.5% using a mycelial growth inhibition assay. Phytochemical screening of the extracts was also performed. Statistical analysis was done using ANOVA with significant differences determined at $p<0.05$. **Results:** *Colletotrichum* spp. was the most prevalent pathogen (29.11%), followed by *Fusarium* spp. and *Curvularia lunata* (18.99% each). Both aqueous and ethanol extracts of *H. indicum* inhibited fungal mycelial growth, with ethanol extracts showing higher efficacy. At 5.0% concentration, ethanol extracts achieved up to 94.99% inhibition for several fungal species. Phytochemical analysis revealed high levels of tannins (11.52 mg/g) and saponins (7.85 mg/g), which may contribute to the antifungal activity. Agronomic evaluation of accessions showed that 'Ijero 2' had the tallest plants and highest number of leaves, while 'NHS10-6' had the highest fruit yield. **Conclusion:** *Heliotropium indicum* extracts exhibit significant antifungal activity, particularly ethanol extracts and can be integrated into disease management strategies for Snake Gourd Tomato. This approach supports sustainable crop protection and enhances productivity.

KEYWORDS

Snake gourd tomato, fungal pathogens, *Heliotropium indicum* extracts, antifungal activity, phytochemical analysis

INTRODUCTION

Snake Gourd Tomato (*Trichosanthes cucumerina* L.), a member of the Cucurbitaceae family, is a significant alternative to Solanum tomatoes in West Africa. It plays a vital role in regional food security and the staple food economy of Sub-Saharan Africa[1]. This annual climbing plant is cultivated widely across Asia and has

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emerged as a major source of tomato puree in Nigeria, contributing to food security and self-sufficiency, particularly in low-income, food-deficit countries[2,3]. In Nigeria, particularly in Ekiti State, where the humid lowland tropics are ideal for its growth, cultivation is prevalent[3]. However, production is challenged by various biotic and abiotic factors. Among the biotic challenges, Anthracnose disease, caused by the filamentous ascomycete *Colletotrichum lagenarium*, is a major concern. This disease affects both the foliage and fruits, manifesting as fruit blight, rotten fruit spots and leaf spots[4,5]. Anthracnose can significantly impact yields from the seedling stage through to maturity, particularly during the leaf spot phase between seedling and late flowering stages [6]. Chemical fungicides have been employed to manage these diseases and enhance yields. However, their repeated use has led to resistance development in target pathogens, posing a significant problem for sustainable disease management[7]. In response, there is growing interest in alternative control methods, such as plant extracts, which have shown promise in disease management across various crops[8-11]. *Heliotropium indicum* (L.), a member of the Boraginaceae family, is traditionally used in managing various ailments, including conjunctivitis[12]. Scientific investigations have revealed its diverse biological activities, including gastroprotective effects[13], wound healing[14], anti-inflammatory properties[15], antituberculosis effects[16], antiproliferative activities and immunostimulant effects[17]. Notably, phytochemical analyses have identified key compounds such as saponins, tannins, phenols, flavonoids and alkaloids in *H. indicum*, with saponins and tannins being predominant[18]. Despite these findings, there is limited research on the antimicrobial properties of *H. indicum* in Nigeria. This study aims to fill this gap by investigating the fungal pathogens associated with diseased samples in Ara, Ijero and evaluating the antifungal potential of *H. indicum* extracts.

MATERIALS AND METHODS

Collection of diseased plant samples: Infected plants were collected from five farms within Ara Ijero Local Government Area, Ekiti State, Nigeria, during the cropping season of May 2023 to August 2023. Plants showing symptoms of anthracnose, including leaf spots, fruit rot, seed discoloration, malformed seeds and leaf lesions, were selected. The samples were transported in sterile bags to the Central Laboratory at the National Horticultural Research Institute, Idi-Ishin, Oyo State, Nigeria, for subsequent fungal isolation.

Collection of plant materials: The 950 g of healthy leaves of India Heliotrope (*Heliotropium indicum* L.) were sourced from the Teaching and Research Farms at the Federal College of Agriculture, Ibadan, Oyo State, Nigeria.

Isolation of fungi from infected plants: Infected plant tissues (leaves, stems, seeds and ripe fruit) were cut into 2x2 mm pieces from the transition zones between infected and healthy tissues. These were surface-sterilized using 1% sodium hypochlorite for 1 min, rinsed in sterile distilled water and dried on sterile tissue paper. The tissues were then plated on Potato Dextrose Agar (PDA) amended with 0.3 mL streptomycin. Five replicate pieces from each type of infected tissue were plated. Plates were incubated at 28-30°C for 5 days. Fungal colonies were observed and identified based on structural features compared to descriptions in standard fungal manuals[19].

Determination of fungal incidence: The incidence of fungal isolates was calculated as the percentage of diseased plants in which each pathogen was detected[20]. The formula used was:

$$\text{Disease incidence (\%)} = \frac{T}{N} \times 100$$

Where:

N = Total number of diseased plants collected

T = Number of diseased plants from which the pathogen was isolated[20]

Preparation of plant extracts: Fresh leaves of *H. indicum* were washed, surface sterilized in 1% sodium hypochlorite for 2 min and rinsed with sterile distilled water. They were then dried at 28-30°C for 10-12 days until crispy. Extracts were prepared by blending 25, 50 and 75 g of dried leaves with 100 mL of sterile distilled water for aqueous extracts and with 100 mL of 70% ethanol for ethanol extracts. The mixtures were allowed to settle for 2 hrs, then filtered through sterile cheesecloth to obtain extract concentrations of 2.5, 5.0 and 7.5%[20].

Determination of mycelial growth inhibition: Mycelial growth inhibition was assessed using the method by Kolawole and Kolawole[21]. The PDA was mixed with 1 mL of each extract concentration and allowed to solidify. A 4 mm diameter disc of a 7-day old fungal culture was placed at the center of each plate. Negative controls consisted of PDA without extracts, while positive controls used Mancozeb fungicide (0.5 g in 100 mL sterile distilled water). Plates were incubated at 2°C for 5 days and radial growth was measured daily. The percentage inhibition of fungal[22] growth was calculated using:

$$\text{Inhibition (\%)} = \frac{\text{Growth in control - Growth in treatment}}{\text{Growth in control}} \times 100$$

Phytochemical screening of *Heliotropium indicum* extracts: The phytochemical analysis was conducted on dried, powdered leaves of *Heliotropium indicum* to determine the content of various bioactive compounds using established methods.

Tannin content: Quantified using the Folin-Denis reagent method with modifications and expressed as mg of tannic acid equivalents per 100 g of dried sample. The tannin content was found to be 11.52 mg/g, indicating a significant presence of tannins in the leaves[23].

Steroid content: Measured by a colorimetric assay involving chloroform and acetic anhydride, with absorbance readings taken at 420 nm. The steroid content was determined to be 1.09 mg/g, reflecting a relatively low concentration of steroids in the plant[24].

Saponin content: Extracted using a Soxhlet Extractor Model S300, LABCO Nuremberg, Germany Borosilicate glass 250 mL (Borosilicate glass capacity: 250 mL thermostat: Integrated heating mantle extraction time: Adjustable from 1 to 24 hrs) features: Reflux condenser, glass extraction chamber and efficient solvent recovery system with acetone and methanol and quantified following the method outlined by Pandey *et al*[23], AOAC and Helrich[24]. The saponin content was recorded at 7.85 mg/g, indicating a moderate presence of saponins.

Phenol content: Extracted with methanol and analyzed using the Folin-Ciocalteu method[25] with absorbance measured at 540 nm. The phenol content was not specified in the provided data but is typically reported in mg/g.

Alkaloid content: Determined through gravimetric analysis after extraction with 10% acetic acid and precipitation with ammonium hydroxide[26]. The alkaloid content was not specified in the provided data but would generally be reported in mg/g.

Flavonoid content: Extracted with 80% aqueous methanol and quantified by difference after evaporation[27] .The flavonoid content was not specified in the provided data but is typically reported in mg/g.

Cyanogenic glycosides: Measured using the alkaline picrate method[28]with absorbance read at 490 nm[29]. The cyanogenic glycosides content was not specified in the provided data but is typically reported in mg/g.

Statistical analysis: Data were analyzed using a Completely Randomized Design (CRD) with three replications. Analysis of Variance (ANOVA) was performed using Minitab Version 17, with means separated by Tukey's test at $p \leq 0.05$.

RESULTS

Incidence of fungal isolates on accessions: The commonly cultivated accessions included 'Ijero 1,' 'Ijero 2-45' and 'Ara' (Locals). The incidence of fungal isolates on the four accessions in 2023 exhibited considerable variation (Table 1). Anthracnose symptoms observed on farms included leaf lesions, brown spots, leaf spots, fruit rot, seed discoloration and malformed seeds as shown in (Fig. 1a-d). The fungi isolated from diseased plants and fruit included *Colletotrichum* spp. Which was the most frequently isolated fungus, that appeared 23 times and constituting 29.11% of the total fungal isolated. *Fusarium* spp. and *Curvularia lunata* each accounted for 18.99% of the isolates, with 15 occurrences each. *Cercospora* spp. was isolated 10 times (12.36%), *Rhizoctonia solani* 9 times (11.39%) and *Phytophthora* spp. was the least frequent, with 7 occurrences (8.86%).

Mycelial growth inhibition by *Heliotropium indicum* extracts: Both aqueous and ethanol extracts of *Heliotropium indicum* significantly inhibited the mycelial growth of various fungal isolates (Table 2). At a 2.5% concentration: The aqueous extract provided the highest inhibition for *Colletotrichum* spp. and *Cercospora* spp. (43.50% each). The least inhibition was observed for *Rhizoctonia solani* (12.50%). The ethanol extracts similarly showed the highest inhibition for *Colletotrichum* spp. and *Cercospora* spp.

Table 1: Effect of incidence of fungal isolates performance of the four accessions in 2023

Isolated fungi	Number of occurrences	Occurrence (%)
Collectrotricum spp.	23	29.11
Phytophthora spp.	7	8.86
Fusarium spp.	15	18.99
Rhizotonia solani.	9	11.39
Cercospora spp.	l0	12.36
Curvularia lunata	15	18.99

Table 2: Mycelial growth inhibition of fungal species by *Heliotropium indicum* etracts

Fungal pthogens isolated	2.5% aqueous extract (%)	2.5% ethanol extract (%)	5.0% aqueous extract (%)	5.0% ethanol extract (%)	7.5% aqueous extract (%)	7.5% ethanol extract (%)
Collectrotricum spp.	43.50	48.79	61.48	64.50	73.25	94.99
Curvularia lunata	21.23	15.34	16.65	22.12	29.78	59.17
Phytophthora spp.	35.75	40.10	44.83	49.25	65.90	79.25
Fusarium spp.	29.75	37.83	39.00	42.40	61.35	72.40
Rhizotonia solani.	12.50	22.83	32.67	34.80	39.17	44.80
Cercospora spp.	43.50	48.79	61.48	73.25	64.50	92.50

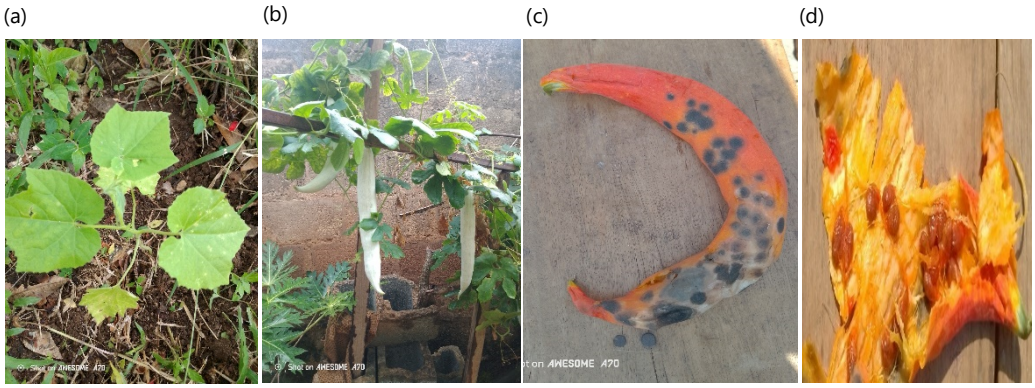

Fig. 1(a-d): Diseased snake gourd plant, leaves, fruit and seeds observed on the field, (a) Diseased plant, (b) Diseased leaves, (c) Diseased fruit and (d) Diseased seeds

Table 3: Quantitative analysis of phytochemical constituents in *Heliotropium indicum* leaves

Phytochemical constituent	Content (mg/g)
Tannin	11.52
Saponin	7.85
Steroid	1.09
Phenol	Not specified
Alkaloid	Not specified
Flavonoid	Not specified
Cyanogenic gycosides	Not specified

Table 4: Mean performance of agronomic characters evaluated in the three accessions of snake gourd tomato in 2023

Accession	Plant height (cm)	Primary branches	Days 50% flowering	Number of leaves	Leaf length (cm)	Leaf width (cm)	Fruit diameter (mm)	Fruit length (mm)	Number of fruit	1000-Seed weight (g)	Fruit yield/ plant(g)
Ijero -1	44.67^{f}	3.47^{bc}	62.67^{c}	46.53^{c}	7.30^{cd}	5.20^{de}	48.74^{a}	42.57^{c}	5.33^{b}	0.33^{d}	183.93^{de}
Ijero 2	95.67^{ab}	5.67^{a}	81.33^{a}	185.67^{a}	19.97^{a}	14.50^{a}	28.67^{c}	19.33^{d}	126.33^{ab}	2.80^{b}	145.7^{e}
Ara	83.30^{bcd}	6.33^{a}	81.33^{a}	125.00^{ab}	18.33^{ab}	13.77^{ab}	26.20^{c}	16.57^{d}	41.33^{b}	2.20^{bc}	202.8^{e}
NHS10-6	60.13^{ef}	6.30^{a}	68.67^{bc}	42.60^{c}	6.30^{d}	4.17^{e}	42.49^{b}	124.11^{a}	7.00^{b}	0.32^{d}	481.77^{c}

Means with the same letter along the column is not significantly ($p<0.05$) different

(48.79% each), while *Rhizoctonia solani* had the lowest inhibition (22.83%). At a 5.0% concentration: The aqueous extract continued to show the highest inhibition for *Colletotrichum* spp. and *Cercospora* spp. (61.48% each). *Rhizoctonia solani* showed the lowest inhibition (32.67%). The ethanol extract demonstrated a broader range of inhibition across all fungal species, with the inhibition ranging from 39.17 to 94.99%. At a 7.5% concentration: The inhibition patterns were consistent with those observed at lower concentrations, with ethanol extracts showing enhanced inhibition for all fungal species. *Colletotrichum* spp. and *Cercospora* spp. exhibited the highest inhibition (94.99 and 92.50%) respectively, while *Rhizoctonia solani* had the least inhibition (44.80%).

The content of tannins, saponins and steroids as determined in the study. The results (Table 3) indicated that tannins were the most abundant constituent, with a concentration of 11.52 mg/g. Saponins followed with a content of 7.85 mg/g, while steroids were present in the lowest concentration at 1.09 mg/g. Other phytochemical constituents, such as phenols, alkaloids, flavonoids and cyanogenic glycosides, were not quantified in the analysis.

Agronomic performance of Snake Gourd Tomato accessions in 2023: The agronomic performance of the three accessions, including 'Ijero-1,' 'Ijero 2,' 'Ara' and 'NHS10-6' was evaluated for various traits in 2023. The results are summarized in Table 4. Plant height: 'Ijero 2' exhibited the tallest plants (95.67 cm), significantly higher than 'Ijero-1' and 'NHS10-6'. Primary branches: 'Ara' and 'NHS10-6' had the highest number of primary branches (6.33 and 6.30, respectively), while 'Ijero-1' had the fewest (3.47). Days to 50% flowering: Both 'Ijero 2' and 'Ara' took the longest to reach 50% flowering (81.33 days), whereas 'Ijero-1' reached it the earliest (62.67 days). Number of leaves: 'Ijero 2' had the highest number of leaves (185.67), significantly more than the other accessions. Leaf dimensions: 'Ijero 2' also had the largest leaves, with a mean length of 19.97 cm and width of 14.50 cm. Fruit characteristics: 'NHS10-6' had the largest fruit length (124.11 mm), while 'Ijero-1' had the largest fruit diameter (48.74 mm). Number of fruits: 'Ara' produced the highest number of fruits (41.33), significantly more than 'Ijero-1' and 'NHS10-6'.1000-Seed weight: 'Ijero 2' had the heaviest seeds (2.80 g), while 'Ijero-1' had the lightest (0.33 g). Fruit yield per plant: 'NHS10-6' had the highest fruit yield per plant (481.77 g), significantly outperforming the other accessions.

DISCUSSION

In this study, diseased (*Trichosanthes cucumerina*) accessions were evaluated for Anthracnose disease symptoms that provided insights into the incidence of fungal pathogens that caused the disease conditions on the (*Trichosanthes cucumerina*) accessions in relation to their agronomic

performance which revealed that the three accessions, including 'Ijero-1,' 'Ijero 2,' 'Ara,'locals and 'NHS10-6,(exotic)' was susceptible to different fungi attack and the antifungal potential of *Heliotropium indicum e*xtracts was evaluated for it biofungistatic action[30] as the findings highlighted the prevalence of *Colletotrichum* spp. as the dominant fungi pathogen, followed by *Fusarium* spp. and *Curvularia lunata*. These were all implicated in this study an attempt to modify this pathogen's alternative method of control was adopted using botanical extracts within this context is the utilization of plant extracts which are natural sources of antimicrobial substances regarded as safe and biodegradable. The result of *H. indicum* extract inhibition recorded for the fungal pathogen as compared with the control experiment varied across each of the fungal pathogens and the efficacy of *H. indicum* extracts against these pathogens underscored the potential of botanical extracts in integrated disease management (IDM) strategies. The prevalence of *Colletotrichum* spp. as the most common fungal pathogen with an incidence rate of 29.11%, aligns with the report from other studies on cucurbit crops, where *Colletotrichum* species have been identified as major pathogens[31]. The presence of *Fusarium* spp. and *Curvularia lunata*, each accounting for 18.99% of the isolates, corroborated the findings from similar research on fungal pathogens affecting and related crops[32].

The lower incidence of *Rhizoctonia solani* and *Phytophthora* spp. suggested that these pathogens, while present, are less significant in the current context which may be due to climatic variabilities such as temperature rain and relative humidity. However, the occurrence and severity of a disease in an individual plant could be a result of the derivation of each climatic variables within the optimal range for disease development thus climate affects all life stages of fungal pathogens and the host that could decrease the vigour and activeness in their rate of spore germination, multiplication and sporulation[32]. The high frequency of *Colletotrichum* spp. and its associated symptoms, such as fruit rot and leaf spots, were consistent with the impact of Anthracnose disease in Snake Gourd Tomatoes which could be due to high rainfall and temperature coupled with the humid weather must have contributed to the greater synergistic disease development during the period of experimentation as described[33].

This underscores the need for effective management strategies to control this pathogen, which has been identified as a significant threat to yields. The significant inhibitory effects of both aqueous and ethanol extracts of *Heliotropium indicum* on fungal growth are noteworthy which thus reflected similar reports on studies evaluating plant extracts for antifungal activity[33,34]. The enhanced activity of ethanol extracts compared to aqueous extracts was consistent with research works that reported that ethanol often extracts more potent antifungal compounds from plants[35].

The phytochemical analysis conducted in this experiment revealed high concentrations of tannins, saponins and steroids in *H. indicum* extracts. Tannins and saponins, in particular, have been associated with antifungal properties in various plant studies[36]. The presence of these compounds in *H. indicum* extracts largely contributed to the observed antifungal activity. Tannins have been documented for their ability to form complexes with proteins that aided the disruption of the fungal cell walls and inhibited growth[37]. Similarly, saponins had been reported to possess antifungal properties that are very potent in the disruption of fungal cell membranes[37,38]. The comparative analysis of *H. indicum* extracts with synthetic fungicides had highlighted the potential of these extracts as an eco-friendly alternative since synthetic fungicides have long been used to manage fungal diseases, but their repeated use has led to resistance issues and environmental concerns[39]. In contrast, plant extracts such as those from *H. indicum* offer a sustainable approach to disease management. This study has shown that plant extracts can be effective in controlling fungal pathogens to reduce the overreliance on synthetic chemicals[40]. While the results are promising, further research is necessary to validate these findings in field conditions. *In vivo* trials are essential to assess the efficacy of *H. indicum* extracts under natural environmental conditions and to optimize formulation strategies for practical use. Future studies should also explore the synergistic effects of *H. indicum* extracts in combination with other natural or synthetic agents to enhance disease control[41,42]. Additionally, the expansion of the phytochemical analysis to include phenols, alkaloids, flavonoids and

cyanogenic glycosides could provide a more comprehensive understanding of the active compounds responsible for antifungal activity. This could lead to the development of more targeted and effective botanical formulations for plant disease management[43].

This study has highlighted *Heliotropium indicum* extracts as a promising, eco-friendly alternative to synthetic fungicides for managing fungal diseases in Snake Gourd Tomatoes, thus, promoting sustainable agriculture. *Heliotropium indicum* extracts can be integrated into disease management strategies and developed into biopesticides for agronomic evaluation that can aid in the selection and breeding of resistant varieties. Therefore, field trials should be carried out to validate the efficacy of *H. indicum* extracts under natural conditions to improve the stability and effectiveness of the extract; explore synergistic combinations and the phytochemical analysis should include additional compounds like phenols, alkaloids and flavonoids for a comprehensive and complete understanding of their antifungal activities.

CONCLUSION

This study underscores the potential of *Heliotropium indicum* extracts as an effective and sustainable alternative for managing fungal pathogens in varieties. The significant antifungal activity observed, combined with the eco-friendly nature of plant extracts, supported their incorporation into integrated disease management strategies to enhance crop protection and sustainability. Future research should focus on optimizing the application methods and concentrations of *Heliotropium indicum* extracts in field conditions, as well as the evaluation of their long-term effects on crop health and yield stability across different agro ecoogical regions.

SIGNIFICANCE STATEMENT

This study highlights the potential of *Heliotropium indicum* extracts in managing fungal diseases of (*Trichosanthes cucumerina*), particularly against the prevalent pathogen *Colletotrichum* spp. The objective was to explore the antifungal efficacy of *H. indicum* extracts as an eco-friendly alternative to synthetic fungicides. Results revealed significant antifungal activity, supporting the use of these plant-based extracts in integrated disease management strategies. The study also evaluated the agronomic performance of different accessions, aiding in the selection of disease-resistant varieties. These findings promote sustainable crop protection practices and offer future prospects for reducing chemical inputs in agriculture, improving both environmental health and crop productivity.

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