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Antifungal effects of botanical extracts against sweet potato root tuber rot fungal pathogens

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ABSTRACT

Sweet potato (*Ipomoea batatas*) is an important root tuber crop prone to post-harvest losses, mainly due to rot. Among several factors, fungal pathogens are a major cause of sweet potato root tuber rot. The study evaluated the efficacy of aqueous and ethanol extracts of bitter leaf (*Vernonia amygdalina*), lemongrass (*Cymbopogon citratus*), and holy basil (*Ocimum sanctum*) in managing sweet potato root tuber rot fungi. The extracts were screened for phytochemical constituents. Sweet potato root tuber rot fungal pathogens were isolated, and their virulence was determined by inoculating them into healthy sweet potato root tubers. The resulting infection lesions were then measured. The antifungal activities of the plant extracts against the mycelial growth of isolated sweet potato root tuber rot fungal pathogens were determined *in vitro* using the food poison method. The phytochemicals alkaloids, flavonoids, saponins, and phytosterols were detected in the plant extracts. Among the isolated fungal pathogens, *Aspergillus niger*, *Fusarium oxysporum, Lasiodiplodia theobromae, Trichoderma harzianum*, and *Rhizopus stolonifera*, *A. niger* caused significantly higher (P<0.05) infection lesions on the root tubers of the orange flesh (1.33±0.02 cm²) and white flesh (1.14±0.01 cm²) sweet potato varieties compared to the other pathogens. Each plant extract significantly inhibited (P<0.05) the mycelial growth of the isolated fungal pathogens compared to their respective negative controls. The aqueous and ethanol extracts of *V. amygdalina*, *C. citratus*, and *O. sanctum* exhibited antifungal activities against sweet potato root tuber rot pathogens *A. niger, F. oxysporum, L. theobromae, T. harzianum, and R. stolonifera*.

Keywords; Phytochemical, *Vernonia amygdalina, Cymbopogon citratus, Ocimum sanctum*, Sweet potato

INTRODUCTION

Sweet potato (*Ipomoea batatas* L. Lam) is one of the world's most important root crops (Paul *et al*., 2021), with a projected production of 89.49 million tonnes (Balasubramanian, 2023). It is the seventh most important crop cultivated globally (Edun *et al*., 2019). Asia is the largest producer, accounting for almost 66% of the projected world production, followed by Africa at 28.30% (Cartabiano-Leite *et al*., 2020). The top five sweet potato-producing countries are China, Tanzania, Nigeria, Uganda, and Indonesia (Sugri *et al.,* 2017). Sweet potato serves as a rich source of carbohydrates and vitamin A, which is lacking in many children under six years old in Sub-Saharan African countries (Low *et al*., 2017; Gelaye, 2024). It is a prominent food security crop due to its drought tolerance and as an income-earning root tuber crop that thrives in the Guinea savanna region of Ghana. However, in a tropical country like Ghana, sweet potato root tubers are susceptible to microbial attacks, especially fungi, after harvest, leading to losses for farmers.

Sweet potato root tubers are vulnerable to attack when wounds and cuts occur on the skin during harvest or transportation (Ndanyi *et al*., 2021). Several fungi have been reported to induce rots in sweet potato root tubers either in the field or in storage. The most notable fungal pathogens causing these rots in the tropics include *Aspergillus* spp., *Botryodiplodia theobromae, Fusarium* spp., *Ceratocystis fimbriata*, *Macrophomina phaseolina*, and *Rhizopus stolonifer* (Beckley and Awoyemi, 2021). The use of botanicals in managing fungal pathogens is gaining momentum due to the shift away from synthetic chemicals, which have health implications for consumers (Ganiyu *et al*., 2023).

With this perspective, researchers have adopted plant-driven extracts as safer alternatives for controlling root tuber rots (Amadioha, 2003 and Gwinn, 2018). Botanical extracts have a lethal or inhibitory effect on rot fungi, limiting their ability to cause infection (Chahal *et al*., 2021). The antifungal activities of botanical extracts arise from various mechanisms such as disruption of the fungal cell membrane, interference with metabolic processes, and prevention of spore germination (Chen *et al*., 2023). Plant extracts have been exploited in the management of rot fungi. For instance, (Tariq *et al*., 2024) reported managing sweet potato soft rot caused by *R. stolonifer* using *Azadirachta indica* and *Moringa oleifera* extracts. The study evaluates the phytochemical properties of aqueous and ethanol extracts of bitter leaf (*Vernonia amygdalina*), holy basil (*Ocimum sanctum*), and lemongrass (*Cymbopogon citratus*) and assesses the fungitoxicity of these extracts on the mycelial growth of pathogenic fungi isolated from sweet potato root tuber rot.

MATERIALS AND METHODS

Geographic area of study:

The study was conducted at the Nyankpala Campus, University for Development Studies, in the Microbiology Laboratory of the Spanish Laboratory. The area is in the Tolon District (Longitude 0˚ 58" 42' W and Latitude 9˚ 25" 41' N), Ghana, at 1057 meters above sea level. The area experiences unimodal rainfall of about 1,500 mm per annum with a relative humidity of 60% in the Guinea Savanna Ecological Zone of Ghana.

Source of sweet potato root tubers:

Root tubers of white and orange flesh sweet potatoes were obtained from farmers in the Tolon district. The tubers were washed with piped water to remove debris. They were then preserved openly on benches at room temperature until needed for the study.

Preparation of Potato Dextrose agar (PDA):

Thirty-nine grams (39 g) of PDA powder were weighed into a 1-liter media bottle. To inhibit bacterial growth, 250 mg of amoxicillin was added. One litter of distilled water was added and then kept in a heated water bath to dissolve the solute, after which it was autoclaved at a pressure of 1.03 kg/cm² and a temperature of 121°C for 15 minutes. Into a 9.00 cm diameter Petri dish, 20 mL of the autoclaved molten PDA was dispensed and allowed to solidify.

Isolation and determination of percentage occurrence of fungi:

Sweet potato root tubers of the white and orange flesh varieties, each with rot symptoms, were washed under running tap water. Small tissues containing both healthy and diseased parts of the rotten tubers were excised. These were soaked in 75% alcohol for 5 seconds, then washed with sterile distilled water and blotted dry with sterile tissue paper in a laminar airflow chamber. Five tissues from each of the white and orange flesh sweet potato varieties were plated separately at equal distances on solidified PDA in a Petri dish. This was then incubated at room temperature (27 ± 2 ℃) for six days. The percentage occurrence of each fungus was determined using the formula (Mshelia *et al*., 2024);

$$\text{Occurrence (\%)} = \frac{\text{Number of tissues on which fungus occurred}}{\text{Number of tissues in the Petri dish}} \times 100$$

The grown mycelia were sub-cultured until a pure culture of each fungal isolate was obtained. The isolated fungal pathogens were identified with a light microscope using their cultural and morphological characteristics as documented by (Campbell and Johnson, 2013 and Barnett and Hunter, 1998).

Pathogenicity test:

The method of (Beckley and Awoyemi, 2021) was used for the pathogenicity test. Fresh and healthy white flesh and orange flesh varieties of sweet potato root tubers were washed with piped water and then surface sterilized with 75% alcohol. A 5 mm cork borer was used to create a cylindrical hole in each tuber by removing the tissue core. A six-day-old 3 mm mycelial plug from each isolated fungal pure culture was placed into each hole at a depth of 5 mm, covered with the cylindrical tissue core, and sealed with Vaseline to prevent drying of the mycelial plug. The inoculated tubers were placed on cotton wool moistened with 20 ml of distilled water in relative humidity chambers. These chambers were created by inserting a binding wire frame measuring 100 cm × 50 cm × 30 cm, providing an area of 150,000 cm³, into a double-layered white polyethylene bag. The chambers were sealed and left for 14 days at a room temperature of 27 ± 2 ℃. Controls involved PDA plugs inserted into the tubers. After 14 days of incubation, the tubers were assessed for disease development before re-isolating the pathogens on freshly prepared PDA to confirm Koch's postulates.

Virulence of test fungal pathogens:

Fresh and healthy root tubers of white and orange flesh sweet potato varieties were washed with piped water and then surface sterilized with 75% alcohol. A 5 mm cork borer was used to create a cylindrical hole in each tuber, removing the tissue core. A six-day-old pure culture of *F. oxysporum, L. theobromae, T. harzianum, R stolonifer*, and *A. niger* was placed separately into the holes created in the tubers. The holes were then covered with the cylindrical tissue core before sealing completely with Vaseline to prevent drying of the mycelial plug. The inoculated tubers were placed on cotton moistened with 20 ml of distilled water in relative humidity chambers created by inserting a binding wire frame into a 150 cm white polyethylene. The chambers were sealed and left for 14 days at a room temperature of 27 ± 2 ℃. For the control, a 5 mm PDA disc was inserted into the cylindrical hole created on the tuber. The lesion size created by each test fungal pathogen on the sweet potato root tubers was determined according to the method of (Scruggs and Quesada-Ocampo, 2016) with some modifications. The tubers were cut in a transverse section across the point of mycelia inoculation, and the lesion area was determined by multiplying the width and height of the lesion.

Preparation of plant extracts:

O. sanctum and *C. citratus* were obtained in the Nyankpala community. *Vernonia amygdalina* was also obtained from Damango, about 125 km from Nyankpala. Each botanical was washed under running tap water and then air-dried on tables in a shaded environment for 14 days. The dried samples were sent to the Savanna Agricultural Research Institute (SARI) of the Council for Scientific and Industrial Research (CSIR) Food Laboratory in Nyankpala for grinding. They were ground into powder using a grinding mill fitted with a 2 mm sieve. Each extract was prepared by suspending 100 g of dried powder in 1 L of solvent in an Erlenmeyer flask. This mixture was agitated for 8 hours on a shaker, after which the extracts were filtered using a vacuum system. A rotary evaporator was then used to evaporate the filtrate to dryness. A stock solution (100% extract concentration) was obtained by diluting each of the dried aqueous and ethanol filtrates in a measuring cylinder to the 1000 ml mark. Part of the stock solution was diluted to a 50% extract concentration by adding an equal quantity of sterile distilled water.

Phytochemical analysis of plant extracts:

The qualitative phytochemical analysis of the aqueous and ethanol plant extracts of *V. amygdalina, C. citratus*, and *O. sanctum* was conducted according to the following protocols.

Alkaloids

Wagner's Test: Two drops of Wagner's reagent were added to 2 ml of the plant extract. The formation of a reddish colour indicates the presence of alkaloids (De *et al*., 2010).

Saponins

Froth Test: The extract was diluted to 20 ml with distilled water and shaken in a graduated cylinder for 15 minutes. The presence of saponins was indicated by the formation of a 1 cm layer of foam (Tyagi and Agarwal, 2017).

Phytosterols

Salkowski's Test: The extract was treated with chloroform and then filtered. Three drops of concentrated sulfuric acid were added to the filtrate, agitated, and allowed to stand. The appearance of a golden yellow tint indicates the presence of triterpenes (Khalid *et al*., 2018).

Phenols

Ferric Chloride Test: Three to four drops of ferric chloride solution were applied to the extracts. The presence of phenols was indicated by the creation of a bluish-black coloration (Pandey and Tripathi, 2014).

Flavonoids.

Lead Acetate Test: To the extract, two drops of lead acetate solution were added. The formation of a yellow precipitate indicated the presence of flavonoids (Bhandary *et al*., 2012).

Effect of plant extracts against isolated sweet potato root rot fungal pathogens:

The poisoned food technique was used to assess the antifungal activities of 50% and 100% aqueous and ethanol extracts of *V. amygdalina, C. citratus*, and *O. sanctum* on the mycelial growth of the test fungal pathogens. Five millilitres of the plant extract were dispensed into a Petri dish. Twenty millilitres (20 ml) of molten PDA were added and then swirled for uniform mixing of the content. This was allowed to solidify, after which a 5 mm mycelial disc of the test fungal pathogen was placed in the middle of the media at a point where two perpendicular lines drawn under the bottom dish meet. The radial growth of the test fungal pathogen was determined on the fifth day along a

perpendicular line drawn under the Petri dish. Each treatment was replicated three times. The positive and negative controls were PDA mixed with the fungicide (mancozeb 640g/kg + metalaxyl 80g/kg WP) prepared at a rate of 2g/L of water and sterile distilled water, respectively. The inhibition of mycelial growth was computed using the formula (Amadioha, 2003);

$$Growth\ inhibition\ (\%) = \frac{RC - RT}{RC} \times 100$$

Where RC = Average radius of control, RT = Average radius of a fungal colony with treatment.

Experimental design and data analysis:

The experimental design was a Completely Randomized Design (CRD) with three replications for each treatment. Data were analyzed using Analysis of Variance (ANOVA) with the 12th edition of GenStat Statistical Software. Square root transformation √(x+1) was performed on fungal percentage occurrence and mycelium growth inhibition (Özer *et al*., 2024).

RESULTS

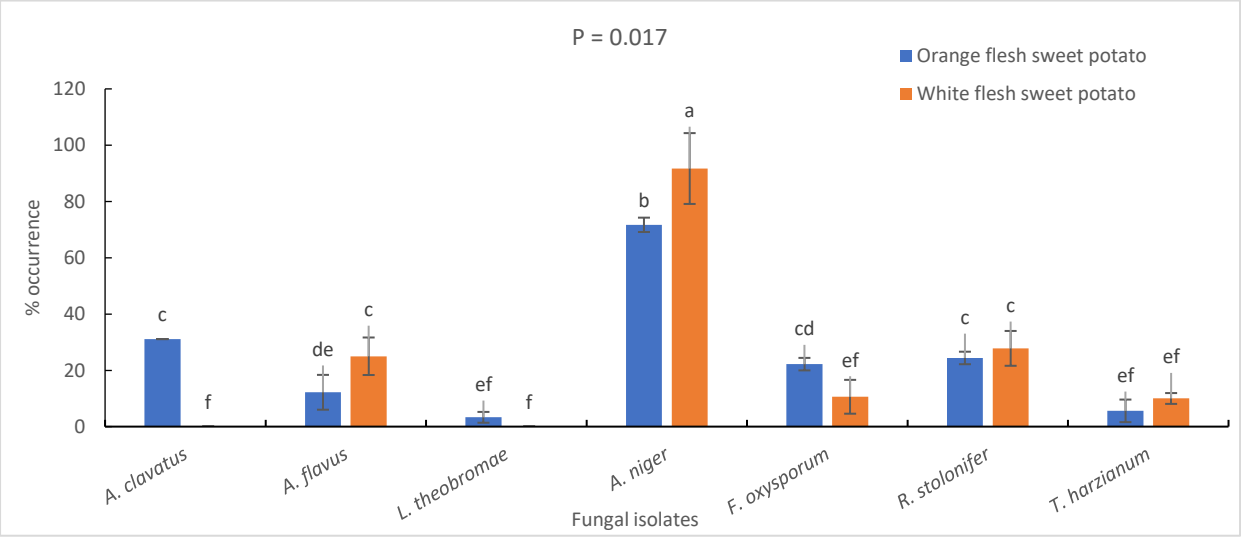
Fungal isolates and pathogenicity test:

Seven fungal species were isolated from both the diseased white flesh and orange flesh sweet potato root tuber varieties. The fungal isolates were *Aspergillus clavatus, A. flavus, A. niger, L. theobromae, F. oxysporum, R. stolonifer*, and *T. harzianum*. Of these, *A. niger, F. oxysporum, L. theobromae, R. stolonifer*, and *T. harzianum* were pathogenic on both varieties of sweet potato root tubers, while *A. flavus* and *A. clavatus* were not pathogenic after being subjected to Koch's postulate.

Occurrence of fungi causing sweet potato root tuber rot:

Aspergillus niger was the most frequently occurring fungus in both the white flesh (92.00%) and orange flesh (72.00%) sweet potato root tubers (Fig. 1). Generally, at *P*<0.05, *A. niger* recorded a significantly higher occurrence than the other fungal isolates (Fig. 1). There was no significant difference at *P*<0.05 in *A. niger* occurrences between the white flesh (92.00%) and orange flesh (72.00%) sweet potato varieties (Fig. 1). *L. theobromae* recorded the least occurrence of 3.30%, followed by *T. harzianum* (5.60%) in the orange flesh sweet potato root tuber variety (Fig. 1). For the white flesh sweet potato, *R. stolonifer* (27.00%), *A. flavus* (25.00%), *F. oxysporum* (10.60%), and *T. harzianum* (10.00%) showed a decreasing pattern in occurrence, while *A. clavatus* and *L. theobromae* were not encountered in the white flesh variety (Fig. 1).

Fig. 1. Occurrence of fungal isolates from white-flesh and orange-flesh sweet potato root tubers varieties.



Bars indicate Standard Errors of Means. Different letter(s) indicate significant (*P* <0.05) differences.

Virulence of isolated fungal pathogens:

The necrotic area recorded for the orange flesh sweet potato root tubers ranged from 1.08 ± 0.02 to 1.33 ± 0.018 cm² for *L. theobromae* and *A. niger* inoculated root tubers, respectively (Table 1). The white flesh sweet potato root tubers recorded the lowest necrotic area (1.09 ± 0.02 cm²) for those inoculated with *T. harzianum* and the highest for both *L. theobromae* (1.19 ± 0.01 cm²) and *R. stolonifer* (1.19 ± 0.02 cm²) inoculated root tubers (Table 1). For the orange flesh sweet potato root tubers, the necrotic area recorded for the *A. niger* inoculated tuber (1.33 ± 0.018 cm²) was significantly higher (P< 0.05) than those inoculated with *F. oxysporum* (1.15 ± 0.01 cm²), *L. theobromae* (1.08 ± 0.02 cm²), *T. harzianum* (1.13 ± 0.03 cm²), and *R. stolonifer* (1.11 ± 0.04 cm²) (Table 1). The necrotic area recorded for the white flesh sweet potato tubers inoculated with *L. theobromae* (1.19 ± 0.01 cm²) and *R. stolonifer* (1.19 ± 0.02 cm²) was significantly higher (P < 0.05) than those inoculated with *F. oxysporum* (1.10 ± 0.01 cm²), *T. harzianum* (1.09 ± 0.02 cm²), and *A. niger* (1.14 ± 0.01 cm²) (Table 1). Additionally, the white flesh sweet potato inoculated with *A. niger* recorded a significantly higher (P < 0.05) necrotic area (1.14 ± 0.01 cm²) than those inoculated with *F. oxysporum* (1.10 ± 0.01 cm²) and *T. harzianum* (1.09 ± 0.02 cm²) (Table 1). No necrotic area was recorded for the control treatments (Table 1).

Table 1. Necrotic area caused by fungal pathogens on sweet potato varieties

Fungal pathogen	Type of sweet potato and Lesion size (cm²)	
	Orange Flesh	**White Flesh**
Aspergillus niger	1.33±0.02 a	1.14±0.01 b
Fusarium oxysporum	1.15±0.01 b	1.10±0.01 c
Trichoderma harzianum	1.13±0.03 b	1.09±0.02 c
Rhizopus stolonifera	1.11±0.04 b	1.19±0.02 a
Lasiodiplodia theobromae	1.08±0.02 b	1.19±0.01 a
Control	0.00±0.00 c	0.00±0.00 d
P value (*P*<0.05)	<0.001	<0.001
LSD	0.081	0.028
CV %	1.600	1.800

Means± Standard Errors of Means in the same column with different letter(s) indicate significant (P <0.05) differences.

Phytochemical constituents of plant extracts:

The screening of plant extracts for phytochemical constituents in the aqueous extract detected the presence of flavonoids, saponins, and phytosterols in *V. amygdalina* (Table 2). Alkaloids and flavonoids were detected in the aqueous extract of *O. sanctum*, while only flavonoids were detected in the aqueous extract of *C citratus* (Table 2). The ethanol extract detected flavonoids, saponins, and phytosterols in *V. amygdalina* extracts; alkaloids and flavonoids in the extract of *O. sanctum*, while *C. citratus* only detected the phytochemical flavonoids (Table 2).

Table 2. Phytochemical constituent of aqueous and ethanol extracts of *V. amygdalina, C. citratus* and *O. sanctum*

Phytochemical Constituent	Aqueous			Ethanol		
	V. amygdalina	***C. citratus***	***O. sanctum***	***V. amygdalina***	***C. citratus***	***O. sanctum***
Alkaloids	-	-	+	-	-	+
Flavonoids	+	+	+	+	+	+
Saponins	+	-	-	+	-	-
Phenolics	-	-	-	-	-	-
Phytosterol	+	-	-	+	-	-

The plus sign (+) indicates the presence of phytochemical while the minus sign (-) indicates the absence of phytochemical

Antifungal effect of plant extracts on mycelia growth of fungal pathogens:

The 50 and 100% aqueous and ethanol extract concentrations of *V.amygdalina*, *C. citratus*, and *O. sanctum* had varied effects on the mycelial growth of the isolated fungal pathogens *L. theobromae, R. stolonifera, F. oxysporum, T. harzianum*, and *A. niger* (Table 3). Significant differences (P < 0.05) were observed among the mycelial growth recorded for these fungal pathogens at both 50 and 100% concentration levels for the various plant extract treatments (Table 3). The least mycelial growth of 2.33 ± 0.08 mm was recorded for the treatments with 100% concentrations of aqueous *C. citratus* and *F. oxysporum*, and ethanol *V. amygdalina* and *F. oxysporum* (Table 3). Among the plant extract treatments, the highest mycelial growth (14.83 ± 0.22 mm) was recorded for the treatment with a 50% ethanol concentration of *O. sanctum* and *R. stolonifer* (Table 3). The mycelial growth recorded for each negative control was significantly higher (P< 0.05) than their corresponding aqueous and ethanol plant extract

treatments at both 50 and 100% concentration levels, with *R. stolonifer* recording the highest mycelial growth (19.33 ± 0.36 mm) and *F. oxysporum* recording the least (8.00 ± 0.14 mm) (Table 3). The positive control treatments completely inhibited the mycelial growth of all the fungal pathogen isolates (Table 3).

Table 3. Plant extracts antifungal activity against fungal isolates at 100 and 50 % concentration levels

Treatment	Mycelia growth (mm)			
	Aqueous		Ethanol	
	100%	50%	100%	50%
R –VE	19.33±0.36 a	19.33±0.36 a	19.33±0.36 a	19.33±0.36 a
T –VE	14.08±0.22 b	14.08±0.22 b	14.08±0.22 b	14.08±0.22 c
A –VE	12.83±0.08 c	12.83±0.08 c	12.83±0.08 c	12.83±0.08 d
L –VE	12.67±0.33 c	12.67±0.33 c	12.67±0.33 c	12.67±0.33 d
CCR	11.00±0.38 d	13.67±0.17 b	9.17±0.68 de	11.50±0.25 ef
OSR	10.17±0.36 e	12.75±0.14 c	12.83±0.22 c	14.83±0.22 b
CCT	9.25±0.25 f	9.18±0.12 g	6.08±0.17 hi	11.00±0.25 fg
VAR	9.00±0.25 f	12.00±0.14 d	8.17±0.46 fg	12.58±0.08 d
CCA	8.25±0.29 g	11.58±0.08 d	9.33±0.08 d	10.58±0.08 gh
F -VE	8.00±0.14 gh	8.00±0.14 i	8.00±0.14 g	8.00±0.14 k
VAA	7.42±0.58 h	11.58±0.08 d	8.83±0.08 def	12.33±0.08 d
OSA	6.17±0.08 i	10.25±0.14 e	8.25±0.27 fg	11.58±0.17 e
OST	6.08±0.08 i	10.25±0.14 e	9.25±0.50 d	11.08±0.08 efg
VAT	5.92±0.44 i	9.17±0.22 g	8.17±0.22 fg	8.58±0.08 j
OSL	5.08±0.17 j	9.67±0.08 f	8.42±0.17 efg	10.33±0.17 h
CCL	4.67±0.08 jk	10.42±0.17 e	6.42±0.22 h	9.67±0.30 i
VAL	4.67±0.08 jk	8.67±0.17 h	5.58±0.08 ij	8.67±0.22 j
OSF	4.08±0.17 kl	5.67±0.22 k	5.00±0.14 jk	7.08±0.08 l
VAF	3.55±0.05 l	7.67±0.17 i	2.33±0.08 l	7.08±0.08 l
CCF	2.33±0.08 m	6.33±0.08 j	4.58±0.08 k	8.83±0.30 j
F +VE	0.00±0.00 n	0.00±0.00 l	0.00±0.00 m	0.00±0.00 m
L +VE	0.00±0.00 n	0.00±0.00 l	0.00±0.00 m	0.00±0.00 m
T +VE	0.00±0.00 n	0.00±0.00 l	0.00±0.00 m	0.00±0.00 m
R +VE	0.00±0.00 n	0.00±0.00 l	0.00±0.00 m	0.00±0.00 m
A +VE	0.00±0.00 n	0.00±0.00 l	0.00±0.00 m	0.00±0.00 m
P value <0.05)	<0.001	<0.001	<0.001	<0.001
LSD	0.688	0.45	0.731	0.51
CV %	0.900	0.800	0.300	0.700

Means± Standard Errors of Means in the same column with different letter(s) indicate significant (*P* <0.05) differences.
Key: *F. oxysporum*(F), *L. theobromae*(L), *T. harzianum*(T), *R. stolonifera* (R), and *A. niger* (A) treated with plant extracts; *V. amygdalina* and *F. oxysporum* (VAF), *C. citratus* and *F. oxysporum* (CCF), *O. sanctum* and *F. oxysporum* (OSF), *V. amygdalina* and *L. theobromae* (VAL), *C. citratus* and *L. theobromae* (CCL), *O. sanctum* and *L. theobromae* (OSL), *V. amygdalina* and *T. harzianum* (VAT), *C. citratus* and *T. harzianum* (CCT), *O. sanctum* and *T. harzianum* (OST), *V. amygdalina* and *R. stoloniferm*(VAR), *C citratus* and *R. stolonifer* (CCR), *O. sanctum* and *R. stolonifer* (OSR), *V. amygdalina* and *A. niger* (VAA), *C. citratus* and *A. niger* (CCA) and *O. sanctum* and *A. niger* (OSA) at 100% and 50% concentration levels respectively. Negative control (–VE), positive control (+VE).

DISCUSSION

The qualitative phytochemical analysis of the aqueous and ethanol extracts of *Vernonia amygdalina, Ocimum sanctum*, and *Cymbopogon citratus* revealed the presence of alkaloids, flavonoids, saponins, and phytosterols. This aligns with the findings of (Mandal *et al*., 2022; Degu *et al*., 2024, and Rahhal *et al*., 2024), who also reported these phytochemicals in *V. amygdalina, C. citratus*, and *O. citratus*. The phytochemicals in the plant extracts were responsible for the antifungal activity against the test fungal pathogens *A. niger, F. oxysporum, T. harzianum, L. theobromae,* and *R. stolonifer*. Each of these phytochemical constituents has its function in inhibiting the isolated fungal pathogen. For instance, saponin has anti-inflammatory and detergent properties (Wijesekara *et al*., 2024). Alkaloids have a heterocyclic ring with a complex structure and tend to be poisonous for the protection of plant species (Thawabteh *et al*., 2021). They are usually produced as metabolic waste products and have been implicated

in the antimicrobial action of many substances (Erasto *et al*., 2007). Among the plant extracts, *Vernonia amygdalina* was found to have more phytochemical constituents in both aqueous and ethanol solvent extractions. This might have resulted in its highest antimicrobial activity on the isolated fungal pathogens compared to *Ocimum sanctum* and *Cymbopogon citratus*. The phytochemical constituents detected in the aqueous and ethanol extracts of *Vernonia amygdalina* (flavonoids, saponins, and phytosterols), *Ocimum sanctum* (alkaloids and flavonoids), and *C. citratus* (flavonoids) were fewer compared to those detected in other studies (Borah and Biswas, 2018; Bhardwaj, 2020 and Eraga and Ijeh, 2021). This could be attributed to factors such as the type of solvent used for extraction, extraction method, age of the plant, time of harvest, and part of the plant harvested (Li *et al*., 2012 and Ramasar *et al*., 2022). It is reported by (Kumar *et al*., 2017 and Moomin *et al*., 2023) that seasons, temperature, rain, and wind patterns affect the phytochemical components of plants. These factors might have influenced the availability of the phytochemical components in the plant extracts, as the plants used were harvested from different locations in both the Northern and Savanna regions of Ghana.

The fungi *A. niger, F. oxysporum, T. harzianum, L. theobromae*, and *R. stolonifer* were confirmed by pathogenicity tests as pathogens of sweet potato root tuber rot. These fungi have been documented by many researchers as the cause of storage root tuber rots in all sweet potato growing regions in the tropics (Opiyo *et al*., 2011; Beckley and Awoyemi, 2021 and Ogero *et al*., 2023). The inability of *A. flavus* to cause sweet potato root tuber rot in this study contradicts the findings of (Gyasi *et al*., 2022), who documented it as a pathogen of sweet potato root tuber rot. Additionally, *A. clavatus*, observed in this research as non-pathogenic in sweet potato root tuber rot, aligns with the report by (Sugri *et al*., 2020), who listed the most common fungal pathogens causing root tuber rots in sweet potatoes in Ghana, which did not include *A. clavatus*. Among the fungal isolates, *A. niger* was the most common root tuber rot pathogen in both white flesh and orange flesh sweet potato varieties. It was also the most virulent in orange flesh sweet potatoes but produced moderate necrotic lesions in the white flesh variety during pathogenicity tests. This aligns with (Gyasi *et al.*, 2022), who reported that *A. niger* was frequently found on sweet potato root tubers in the Eastern region of Ghana, although it produced the smallest lesion diameter of 12.7 mm according to their findings. This could be attributed to the fact that *A. niger* spores are easily dispersed by air currents. Furthermore, the relative humidity in the growing chambers might have played a major role in the ability of the fungal pathogens to produce root tuber rot symptoms. This supports the assertion by Argenta *et al.* (2024) that the rate of fungal infection is related to relative humidity. Necrotically, *R. stolonifer* produced a greater affected area in the white flesh variety compared to *A. niger*. *R. stolonifer* has been reported as a microorganism causing soft rot in sweet potato root tubers in the tropics (Ogero *et al*., 2023). They noted that *R. stolonifer* is best controlled at a temperature of 13 ℃, while complete decay of root tubers occurs at 29 ℃. This could explain why it produced the highest necrotic area with *L. theobromae* in the white-flesh sweet potato variety, as root tubers were stored at a temperature of 27 ± 2 ℃. However, this contradicts the report by (Clark *et al*., 2013) that a greater necrotic lesion is produced at a temperature range between 6 ℃ and 22 ℃.

The small necrotic areas produced by *F. oxysporum* on both white and orange fleshed sweet potato tubers support the findings of (Beckley and Awoyemi, 2021) that *F. oxysporum* causes moderate root tuber rot in sweet potatoes. Scruggs and Quesada-Ocampo (2016) emphasized that the rate of disease infection and progression of *Fusarium* spp. on sweet potato tubers depends on the method of inoculation, which could have been the case in this study and potentially affects the outcome. *L. theobromae* has been reported to cause Java black rot on sweet potato root tubers in Ghana. The fungus was isolated from root tubers of sweet potatoes with a rot lesion diameter of 27.1 mm (Gyasi *et al*., 2022), and this study was no exception, as it produced a 1.19 cm^2 diameter in white flesh sweet potatoes. Okigbo and Emeka (2010) discovered that *L. theobromae* is one of the most virulent pathogens causing root rot in sweet potato tubers during storage. *Trichoderma* spp. have been reported as a pathogen causing green mold disease in sweet potatoes (Yang *et al*., 2021). However, there was no report of *T. harzianum* causing sweet potato root rot in Ghana at the time of this study. Yang *et al*. (2021) reported its presence in Guangdong Province, China, as the cause of green mold disease. *Trichoderma* spp. is well known for the biological control of pathogenic fungal species (Mazrou *et al*., 2020).

The 50 and 100% concentrations of the aqueous and ethanol extracts of *V. amygdalina, C. citratus*, and *O. sanctum* each inhibited the mycelial growth of *F. oxysporum, L. theobromae, T. harzianum, R. stolonifera*, and *A.*

niger. This can be attributed to the antifungal activities of the plant extracts resulting from their phytochemicals. This aligns with report of (Kwodaga *et al*., 2019) that the antifungal activities of plant extracts on fungi are due to secondary metabolites present in plants. Generally, *C. citratus* was highly efficacious on *F. oxysporum* in both aqueous and ethanol extracts. This might result from the highly therapeutic ability of *C. citratus*, possessing flavonoids as seen in the phytochemical analysis. This assertion was reported by (Dong and Thuy, 2021 and Oniha *et al*., 2023), stating that the fungitoxic effect on several fungal strains is due to the presence of flavones, flavonoids, and phenols in C. citratus oil extracts. In their studies, (Helal *et al*., 2007 and Mukarram *et al*., 2021) reported that *C. citratus* extracts can rupture the plasma membrane and disorganize the cell mitochondria, leading to ion leakage. As a result, signal transduction and fungal germination can be adversely affected (Alviano *et al*., 2005). *Vernonia amygdalina* extract performed moderately on the test fungi, with greater inhibition occurring on *F. oxysporum* at both 50 and 100% concentration levels in both solvent extractions, although it contains more phytochemical components than *C. citratus* in both aqueous and ethanol solvents. This could be due to its high therapeutic potential against *F. oxysporum*, indicative of the active secondary metabolites in *C. citratus* that could inhibit the growth of fungi causing sweet potato root rots. John *et al.* (2016) made a similar observation, who reported that extracts of *V. amygdalina* reduced the mycelial growth of *F. oxysporum* and *R. stolonifer* by 32.26% and 22.58%, respectively. This agrees with this study's findings on the phytochemical properties exhibited by *V. amygdalina* in inhibiting the mycelial growth of these fungal pathogens. Among the plant extracts, *O. sanctum* showed the least efficacy against the test fungi, although it significantly inhibited mycelial growth of fungal pathogens. The lower performance could be attributed to factors such as the part of the plant used, extraction method, and age of the plant. A similar observation was made by (Anukwuorji *et al.*, 2013), noting that phytochemical content can be altered by plant age, extraction method, and harvesting time. Regardless, *Ocimum* has been accorded antifungal, antibacterial, antiviral, and antiprotozoal properties across its numerous species (Dixit *et al*., 2021).

In this study, the solvent extraction methods used may have played a major role in the antifungal activities of the plant extracts on the fungal pathogens. In general, the aqueous extracts exhibited greater antifungal activity against the mycelial growth of the fungal pathogens compared to ethanol extracts. This trend contradicts the findings of (Al-Garadi *et al*., 2023), who reported superior antifungal performance with ethanol extracts compared to aqueous extracts. This discrepancy could be attributed to the volatility of some phytochemical constituents during the ethanol extraction process, which does not occur in the aqueous preparation process, thus adversely affecting the full inhibition potential of the ethanolic extracts of the plants. On a contrary note, (Anukwuorji *et al*., 2013) reported that water is an inorganic solvent and hence gave a lower performance on mycelial growth *in vitro* against fungal pathogens causing sweet potato root tuber rots. This resulted from the inability of the aqueous plant extracts to completely dissolve the biologically active properties responsible for antimicrobial activities in these plants. At different concentration levels, there was varying antifungal activity in the plant extracts, with higher fungitoxicity at 100% concentration level in both the aqueous and ethanolic extracts. In their studies, (Ijato *et al*., 2010) reported that the higher the concentration of plant extract, the better the inhibitory performance against pathogens. This suggests that the higher the phytochemical quantity of a plant extract, the higher the antifungal activity of the extract against the test organisms. There may be a direct correlation between a plant extract's concentration and the quantity of secondary metabolites it contains. As a result, raising the concentration of any extract can boost its antifungal effectiveness.

CONCLUSION

The phytochemicals alkaloids, flavonoids, saponins, and phytosterols were detected in the aqueous and ethanol extracts of *Vernonia amygdalina*, *Ocimum sanctum*, and *C. citratus*. Of the seven fungi isolated from the diseased root tubers of the white and orange flesh sweet potato varieties, except for *Aspergillus flavus* and *A. clavatus*, the other isolates, namely *A. niger, F. oxysporum, L. theobromae, R. stolonifera*, and *T. harzianum*, were pathogenic on the root tubers. The 50 and 100% extract concentrations of the aqueous and ethanol extracts of *V. amygdalina*, *O. sanctum*, and *C. citratus* significantly inhibited ($P < 0.05$) the mycelial growth of the sweet potato root tuber rot fungal pathogens *A. niger, L. theobromae, F. oxysporum, T. harzianum*, and *R. stolonifer* compared to their respective negative controls. The aqueous and ethanol extracts of *V. amygdalina, O. sanctum*, and *C. citratus* have exhibited antifungal activities against sweet potato root tuber rot pathogens: *A. niger, F. oxysporum, L. theobromae, R.*

stolonifera, and *T. harzianum*. Further studies should be conducted to develop these extracts into botanical fungicides for managing sweet potato root tuber rot.

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