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Phytochemical composition and antifungal activity of *Balanites aegyptiaca* seed extracts against *Colletotrichum capsici* causing cowpea anthracnose

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ABSTRACT

Anthracnose is a major disease of cowpea caused by *Colletotrichum capsici*, responsible for yield losses of up to 85% in the field. Although synthetic fungicides are commonly used to control this disease and are generally effective, their use has detrimental effects on the environment and human health. Therefore, plant extracts represent a promising alternative. This study aimed to investigate the antifungal activity of phytochemical compounds present in *Balanites aegyptiaca* (L.) Del. seed extracts, as identified by GC-MS, against *C. capsici*. Phytochemical screening and GC-MS analyses were carried out, and in vitro inhibition tests were conducted using aqueous and organic (methanolic and acetonic) extracts of *B. aegyptiaca* seeds at concentrations of 12.5, 25 and 50 µL·mL^{-1}, compared with a control (0 µL·mL^{-1}) and a commercial fungicide containing 80% Maneb at 3.33 g·L^{-1}. The tests were performed against two isolates of *C. capsici* collected from the Akonolinga locality. Mycelial growth, as well as the minimal inhibitory concentrations (MIC_{50} and MIC_{90}), were evaluated. The results showed that phytochemical screening and GC-MS analysis revealed the presence of several secondary metabolites and bioactive compounds in *B. aegyptiaca* seed extracts with known antifungal properties. The most abundant compounds were pyridine (52.05%) in the aqueous extract and 9-octadecenamide (29.79%) in the organic extracts. Both aqueous and organic extracts at 50 µL·mL^{-1} inhibited up to 93% of mycelial growth in the two *C. capsici* isolates. The lowest MIC_{50} values were obtained with the aqueous extract (5.81 µL·mL^{-1} and 9.21 µL·mL^{-1}) for isolates CAK01 and CAK05, respectively. Based on these results, *B. aegyptiaca* seed extract appears to be a promising natural antifungal source, warranting further field trials to confirm its potential efficacy under natural conditions.

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INTRODUCTION

Cowpea (*Vigna unguiculata* L. Walp.) is one of the most important legumes cultivated in the hot, arid, and semi-arid regions of sub-Saharan Africa, where other crops often fail due to their poor adaptation to drought, high temperatures, and poor soils (Lalsaga and Drabo, 2017; Hamidou *et al*., 2018). Rich in protein (23–25%) and carbohydrates (64%), cowpea grain also contains essential nutrients such as thiamine, niacin and riboflavin (Jackson, 2009; Modu *et al*., 2010). It is an important source of protein and essential amino acids including lysine, tryptophan, phenylalanine, valine, threonine and methionine in the human diet (USDA, 2004; Néya *et al*., 2019). Cowpea is also recognized for its ability to fix atmospheric nitrogen, up to 240 kg/ha, through a symbiotic association with Rhizobium bacteria present in the nodules of its roots, thus contributing to improved soil fertility (Kaboré, 2004; Husson *et al*., 2010).

Despite its many roles, the cultivation of this legume remains subject to various biotic and abiotic stresses. Among these, fungal diseases represent a major constraint, leading to significant economic losses in terms of yield (Chowdappa *et al*., 2013). Anthracnose, caused by *Colletotrichum capsici* (Syd.) Butler & Bisby, is one of the most devastating diseases of cowpea, especially during periods of high humidity, potentially causing up to 85% yield losses in the field without appropriate phytosanitary measures (Alabi, 1994). To limit the damage caused by this disease, producers resort to synthetic fungicides; however, these have toxic effects on the environment and human health and promote the emergence of resistant pathogenic strains (Adam *et al*., 2010). In this context, biopesticides appear as a promising alternative, as they are locally available, biodegradable, and non-toxic to humans and the environment (Faye, 2010; Sané *et al*., 2018; Traoré *et al*., 2019). Numerous studies have also highlighted the biopesticidal efficacy of plant extracts rich in natural bioactive compounds (Djeugap *et al*., 2023; Dida *et al*., 2024; Bolie *et al*., 2025).

Plant extracts rich in secondary metabolites such as terpenoids, phenols, tannins, and nitrogen compounds have attracted increasing attention in recent years as alternative strategies for controlling plant diseases and pests. These extracts contain bioactive compounds capable of triggering plant defense mechanisms and inducing systemic resistance against phytopathogenic fungi (Desoky *et al*., 2019).

Balanites aegyptiaca (L.) Del., belonging to the family Balanitaceae, is widely distributed across southern Asia and Africa. It is a multi-branched, thorny tree adapted to arid and semi-arid environments. This species is valued in traditional medicine and used as a source of food, oil, and fodder. Moreover, it serves as a potential agroforestry and windbreak species (Elfeel and Warrag, 2011). Several studies have reported its insecticidal (Elamin and Satti, 2013), fungicidal (Haruna *et al*., 2020; Toka *et al*., 2023), antimicrobial (Emad *et al*., 2012), vermifugal and anthelmintic (Dwivedi *et al*., 2009), as well as fasciolicidal (Al Ashaal *et al*., 2010) properties. The antiparasitic activity of *B. aegyptiaca* bark, root, and seed extracts has also been extensively documented. The present study aimed to evaluate the antifungal activity of phytochemical compounds detected in *B. aegyptiaca* (L.) Del. seed extracts by GC-MS against *Colletotrichum capsici*.

MATERIALS AND METHODS

Isolation and identification of *Colletotrichum capsici*

The plant material consists of cowpea pods harvested from the experimental field not treated with synthetic fungicide. The field is located in Akonolinga, Nyong et Mfoumou Department in the Centre Region of Cameroon (N 03°48', E 012°15'). Isolates were obtained using the method used by Photita *et al.* (2005) and coded (CAK01 and CAK05). Cowpea pods of the symptomatic plants were cut (small fragments of 3 mm) and washed with distilled water, followed by blotting of excess moisture and disinfected with sodium hypochlorite (10%) solution for 2 min. These fragments were deposited on Potato dextrose agar (PDA) amended with ampicillin (250 mg/L) and streptomycine (200 mg/L) for fungus isolation and Petri dishes were incubated for 6 days at 27 °C. The mycelium that emerged was subcultured on a PDA medium to obtain a pure explant (Hibar *et al.,* 2007). The identification of the different pure isolates was made by observing the different cultural and microscopic characteristics of *Colletotrichum capsici* and their comparison to a reference *C. capsici* isolate. In culture on PDA culture medium, *C. capsici* has a gray mycelial mass characterized by an absence of sclerotia. Under the microscope, the acervuli are made up of conidiophores producing conidia and numerous long, black-brown to black bristles protruding from the conidial mass. The conidia are unicellular, hyaline, fusoid with rounded and slightly hooked ends, most often falcate (Sérémé *et al*., 2001)

Preparation of *Balanites aegyptiaca* seeds extracts

The mature fruits of *B. aegyptiaca* were collected from Bibemi district, Northern Region of Cameroon (N 04°12', E 11°24', then the identification was confirmed at a National Herbarium. The seeds kernels were grounded using manual hand mill grinder. Acetone and methanol extracts were prepared by macerate 500 g of powder in 2 liters of each solvent for 72 hours. After filtration using filter paper, the solutions were transferred to the rotary vapor (Büchi R-200 Rotary Evaporator at 60 °C), for the separation of the solvent from the extractable compounds (Gayathri and Sahu, 2015; Zhang *et al*., 2018; Haruna *et al*., 2020). The extract obtained after evaporation was stored in refrigerator at 4 °C until use. The aqueous extracts were obtained by maceration of 100 g of powder in 200 mL of distilled water and filtered through of fine muslin (Zhang *et al*., 2018).

Phytochemical screening of extracts

The classes of secondary metabolites present in the aqueous, methanol and acetone extracts of *B. aegyptiaca* seeds were determined by adapting standard procedures described by Alhassan *et al*. (2018). Tannins and polyphenols were identified by the $FeCl_3$ test and Stiasny's reagent; flavonoids by the cyanidin reaction; saponosides by the foam test; quinones by the Bornträger test; triterpenes and steroids by the Liebermann-Burchard test and finally alkaloids by the Mayer and Dragendorf tests (Koffi *et al*., 2015). These techniques are based on the turbidity, precipitation and foam of the extracts in the presence of different reagents characterizing each class of secondary metabolites.

Extracts analysis Through Gas Chromatography-Mass Spectrometry (GC-MS) Method

Balanites aegyptiaca seed extracts (aqueous, methanol and acetone) were analyzed by gas chromatography coupled with mass spectrometry (CG-MS), using an Autosystem XL gas chromatograph (Agilent GC 7890A) equipped with a split mode vaporization injector (1:50) interfaced with a Turbomass Perkin-Elmer mass spectrometer (Agilent 5975 C TAD VL MSD). The analytical parameters were helium as the carrier gas with the column flow rate of 1.21 mL/min. The oven temperature program was 40 °C for 3 min, then increased at 5 °C/min to 180 °C, followed by 15 °C/min to 240 °C and finally 10 °C/min to 300 °C (isotherm 15 min). A fused silica capillary column, 30 × 25 mm internal diameter and 30 × 32 mm (DB-1; 100 % di-15099 Methylpolysiloxane) was used.

The ion source and transfer line were maintained at 200 and 280 ºC, respectively. Electron ionization mass spectra in the 40-500 Da range were recorded at an electron energy of 70 eV. The sweep time was 1 ms, the multiplier potential 430 V and the source pressure 10 Torr. A computer recorded all the data and the compounds were identified by comparison with the spectral database of the Wiley and NIST libraries. The fraction previously evaporated and re-suspended in dichloromethane was analyzed twice (1 mL; hot needle) and for semi-quantitative purposes the mean percentage composition was calculated from the normalized peak areas without using correction factors (Nayak and Padhy, 2017; Isam *et al*., 2019).

In vitro evaluation of the antifungal activities of *Balanites eagyptiaca* seeds extracts

A stock solution of 500 µL/mL was prepared by mixing 50 mL of each extract (with 100 mL of solvent (acetone and methanol). Concentrations of 12.5; 25 and 50 µL.mL-1 of aqueous extract (AqE), acetone extract (AcE) and methanol extract (ME) were then prepared by taking successively 0.75 ; 1.5 and 3 mL of the stock solution and adding 59.25 ; 58.5 et 57 mL PDA, respectively, to give a final volume of 60 mL each. The mixture was poured into 90 mm Petri dishes at a rate of 20 mL per dish. These volumes were obtained using the formula (CiVi = CfVf) (Gatagonçalves, 2001). The preparation of the medium enriched with the synthetic fungicide (Monchamp) with active ingredient 80 % maneb was used at 3.33 g/L concentration recommended.

For the control, a solution of 20 mL of PDA medium was poured directly into a Petri dish. There were three replications of each treatment. The mycelial growth of *C. capsici* isolates was evaluated from the second day after inoculation and every day until the

control dish was completely colonized by the pathogen, by measuring the two perpendicular diameters of the culture according to the formula of Singh *et al*. (1993).

$$RG = (d1 + d2)/2 – d0$$

Where: RG = radial growth; d1 and d2 = diameters of the culture; d0 = explant diameter.

The inhibition percentage (IP) of mycelial growth related to the control was calculated for all concentrations of each extract using the following formula.

$$IP = (MGc-MGt)/MGc \times 100$$

Where: MGc = mycelial growth in the control; MGt = mycelial growth in the treatment (Sharma and Rana, 2018).

Determination of the minimal inhibition concentration (MIC) of the different extracts

The minimum inhibiting concentration 50 and 90 % (MIC50 and MIC90) was determined by comparing the values of the percentage inhibition (PI) with those of the natural logarithm of the corresponding concentrations (Ci): PI = f (ln Ci). The linear regression line of the type Y = ax + b from the function I = f (ln Ci) thus made it possible to determine the MIC50 and MIC90 (Griffin *et al*., 2000).

Where: Y = inhibition rate (%), a = slope of the line, b = constant

Statistical analysis

The data obtained from mycelial growth were subjected to one-way analysis of variance (ANOVA) using the R software version 4.0.4 (R development Core Team 2022). Means were separated by Tukey's multiple range test (HSD) when the significance level was assessed at the 5 % threshold.

RESULTS

Morphological characterization of *Colletotrichum capsici* isolates

Two isolates were obtained using infected cowpea pods on PDA medium. Morphological characterization was used to identify fungus. Macroscopic observation showed white to grey mycelium colonies, sparse and aerial (Fig. 1A). Microscopic observation of the isolates showed falcate conidia (Fig. 1B). These morphological characteristics of the isolates belong to *Colletotrichum capsici.* Two of the five isolates that rapidly filled the Petri dishes (5 days after incubation) were used for the *in vitro* test.

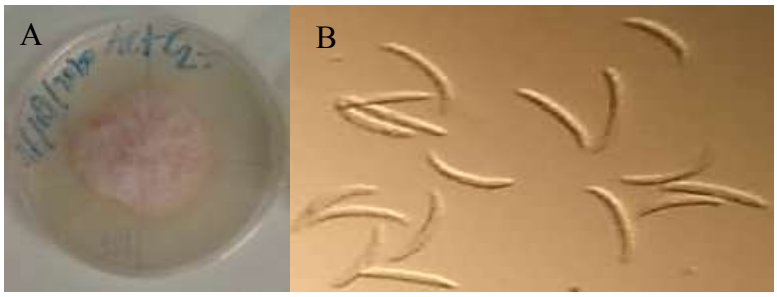

Fig. 1. Macroscopic and microscopic observation of *Colletotrichum capsici* conidia; A: Pure culture; B: Conidia of isolate (magnification X 40).

Phytochemical screening of *Balanites aegyptiaca* extracts

Phytochemical screening test of *B. aegyptiaca* seed extracts revealed the presence of several secondary metabolites. Alkaloids, phenols, steroids, saponins and terpenoids were present in aqueous, methanol and acetone extracts. Anthraquinones, flavonoids and tannins were absent in methanol extract. The biological activities of these secondary metabolites are described in the table 1.

Table 1. Families of chemical compounds identified in *Balanites aegyptiaca* seed extracts with their biological activities

Secondary Metabolites	Extracts of *Balanites aegyptiaca*		
	AcE	AqE	MeE
Alkaloids	+++	++	+
Anthraquinones	-	+	-
Flavonoids	+++	+	-
Glycosides	+	+++	++
Phenols	++	++	+
Steroids	+	+	+ +
Saponins	++	+++	+
Terpenoids	+	++	++
Tannins	++	+	-

(+++) abundant; (++) moderate abundant; (+) present; (-) absent AcE: Acetone extract; MeE: Methanol extract; AqE: Aqueous extract.;

GC-MS analysis of *Balanites aegyptiaca* seed extracts

The biochemical profile of the aqueous, methanolic, and acetone extracts reveals the presence of numerous compounds distributed as ultra-minor, minor, and major (Fig. 2 A, B, and C). The aqueous extract contains 20 biochemical compounds, the most abundant of which are 9-Octadecenamide (29.79%), 3-O-Methyl-D-glucose (21.03%), and 13-Docosenamide (Z)- (20.51%) (Table 2). The acetone extract contains 12 chemical compounds dominated by pyridine (52.04%) and 1,3,5-Trimethyl-1H-pyrazol-4-amine (10.62%) (Table 3). Similarly, the methanolic extract comprises 12 compounds, among which pyridine (52.05%), N-Methoxymethane sulfonamide (22.83%) and (3,7-Dimethyl-1-phenyl-octa-2,6-dienyl)-trimethylsilane (2.21%) predominate (Table 4).

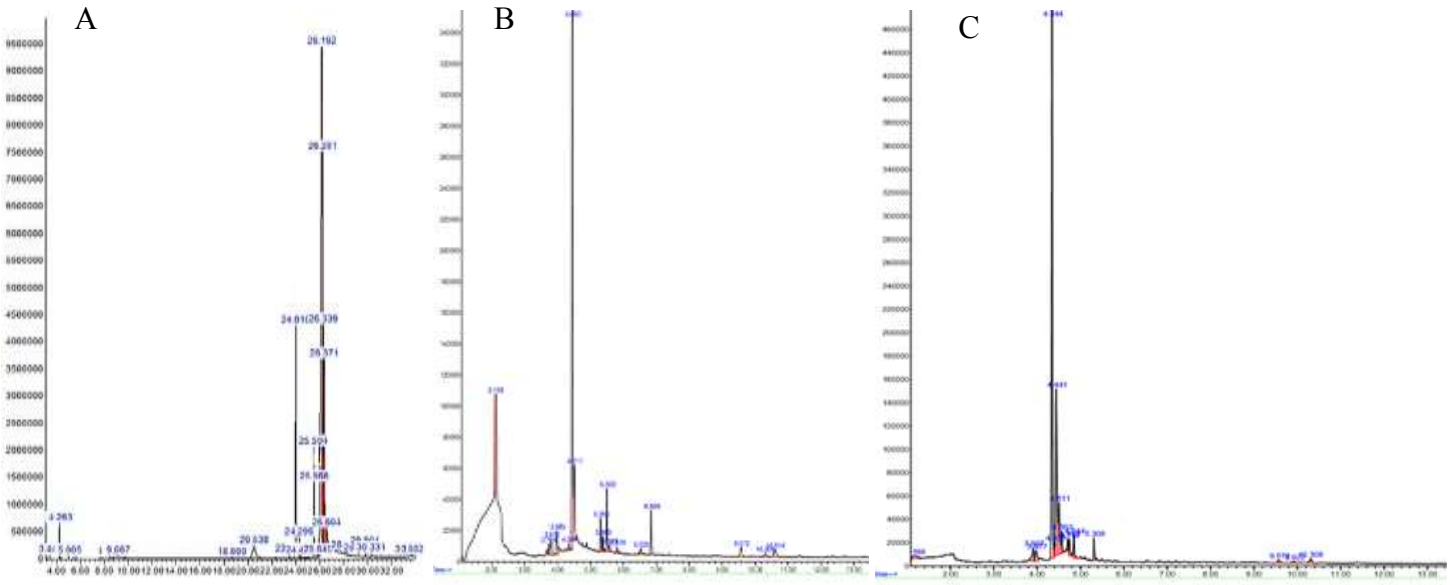

Fig. 2. Gas chromatographic mass spectrometry profile of the aqueous (A), acetone (B) and methanol (C) extract of *Balanites aegyptiaca* seeds

Table 2. Biochemical compounds identified in the aqueous extract of *Balanites aegyptiaca* seeds by GC-MS

Peaks	Rt (min.)	Area (%)	Formula	MW (g /mol)	Compounds names
01	5.731	0.41	$C_{16}H_{22}O_2$	246.34	Cyclohexane carboxylic acid, 3-phenylpropyl ester
02	7.058	0.47	$C_{19}H_{40}$	268.5	Octadecane, 6-methyl-
03	8.191	0.32	$C_{12}H_{13}N_7$	255.28	3-[N'-(3H-Indol-3-yl methylene)-hydrazino]-5-methyl-[1,2,4]triazol-4-ylamine
04	11.029	0.32	$C_{14}H_{20}O_2$	220.31	Methyl 2,4-tridecadiynoate
05	13.33	6.61	$C_{18}H_{32}O_{16}$	504.42	Melezitose
06	16.419	21.03	$C_7H_{14}O_6$	194.18	3-O-Methyl-d-glucose
07	17.301	0.57	$C_{17}H_{34}O_2$	270.5	Hexadecanoic acid, methyl ester
08	17.884	0.32	$C_{28}H_{58}O_9$	538.8	Octaethylene glycol monododecyl ether
09	18.931	0.77	$C_{19}H_{34}O_2$	294.5	9,12-Octadecadienoic acid (Z, Z) - methyl ester
10	18.983	0.72	$C_{19}H_{36}O_2$	296.5	11-Octadecenoic acid, methyl ester
11	19.206	0.42	$C_{19}H_{38}O_2$	298.5	Heptadecanoic acid, 16-methyl-, methyl ester
12	19.75	4.68	$C_{16}H_{33}NO$	255.44	Hexadecanamide
13	21.329	29.79	$C_{18}H_{35}NO$	281.5	9-Octadecenamide
14	21.501	3.42	$C_{18}H_{37}NO$	283.5	Octadecanamide
15	22.376	0.74	$C_{19}H_{34}O_6$	358.5	Dodecanoic acid, 2,3-bis(acetyloxy)propyl ester
16	22.462	2.55	$C_{19}H_{38}O_4$	330.5	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester
17	23.847	2.28	$C_{21}H_{38}O_3$	338.5	Glycidyl oleate
18	24.007	3.46	$C_{21}H_{42}O_4$	358.6	Octadecanoicacid,2-hydroxy-(hydroxymethyl)ethyl ester
19	24.51	20.51	$C_{22}H_{43}NO$	337.6	13-Docosenamide, (Z)-
20	31.394	0.6	$C_{29}H_{50}O$	414.7	Gamma-Sitosterol

Rt: Retention time; MW: Molecular weigh

Table 3. Chemical compounds identified in the acetone extract of *Balanites aegyptiaca* seeds by GC-MS

Peaks	Rt (min)	Area (%)	Formula	MW (g /mol)	Compounds names
01	2.10	52.05	C_5H_5N	79.10	Pyridine
02	3.71	1.59	$C_8H_{15}N$	125.21	2-(1-Cyclohexenyl) ethylamine
03	3.81	10.62	$C_2H_7NO_3S$	125.15	N-(Methylsulfonyl)-O-methylhydroxylamine
04	3.98	4.15	C_6H_7NO	109.13	5-methyl-2(1H-pyridinone
05	3.98	4.15	C_6H_7NS	125.19	3-Aminobenzenethiol
06	3.98	4.15	C_4H_7NO	85.10	Cyclopropane carboxamide
07	4.51	10.62	$C_6H_{11}N_3$	125.17	1,3,5-Trimethyl-1H-pyrazol-4-amine
08	5.37	1.13	$C_3H_8Cl_4Si_2$	242.1	Trichloro-[[chloro(dimethyl) silyl] methyl] silane
09	5.82	1.83	$C_{17}H_{34}O_3$	286.45	Methoxyacetic acid Tetradecyl ester
10	6.52	0.89	$C_7H_{11}NO$	125.17	Dicyclopropyl ketoxime
11	9.57	1.83	$C_7H_{11}NO$	125.17	2-Acetyl-3,4,5,6-tetrahydropyridine
12	10.61	2.03	$C_8H_{15}N$	125.21	1-Azabicyclo [3.2.1] octane, 6-methyl-, exo-

Rt: Retention time; MW: Molecular weigh

Table 4. Chemical compounds identified in the methanol extract of *Balanites aegyptiaca* seeds by GC-MS

Peaks	Rt (min.)	Area (%)	Formula	MW (g /mol)	Compounds names
01	1.16	1.59	$C_8H_6Cl_2O_3$	221.04	2,4-Dichlorophenoxyacetic acid, 2-chloroethyl ester
02	1.16	1.59	C_2H_6FN	63.07	Dimethylfluoroamine
03	1.16	1.59	$C_4H_7Cl_3O_4S_2$	289.6	3,3,5-Trichloro-2,4-dithiahexane 2,2,4,4-tetroxide
04	3.91	1.97	C_6H_7NS	125.19	3-Aminobenzenethiol
05	3.91	2.21	C_6H_7NO	109.13	2-(1H)-Pyridinethione, 5-methyl-
06	3.91	2.21	C_4H_7NO	85.11	Cyclopropanecarboxamide
07	3.98	2.21	$C_{13}H_{26}Si$	210.43	(3,7-Dimethyl-1-phenyl-octa-2,6-dienyl)-trimethyl-silane
08	4.34	22.83	$C_2H_7NO_3S$	125.15	N-(Methylsulfonyl)-O-methylhydroxylamine
09	4.34	52.05	C_5H_5N	79.10	Pyridine
10	4.41	1.11	C_6H_7NS	125.19	2(1H)-Pyridinethione, 3-methyl-
11	9.57	1.17	$C_3H_8Cl_4Si_2$	242.1	Trichloro-[[chloro(dimethyl) silyl] methyl] silane
12	9.92	0.78	$C_{17}H_{34}O_3$	63.07	Methoxyacetic acid Tétradecyl ester

Rt: Retention time; MW: Molecular weigh

In vitro antifungal activity of *Balanites aegyptiaca* seeds extract on *Colletotrichum capsici* isolates

A significant difference ($P < 0.05$) was observed in the mycelial growth of the two *C. capsici* isolates (Fig. 3A and 3B). The inhibition percentages of the *C. capsici* isolates increased with the concentration of the *B. aegyptiaca* seed extracts. At a concentration C3 = 50 µL/mL, the inhibition percentage recorded for isolate CAK01 was 93.14% with the aqueous extract, 81.63% with the acetone extract, and 73.39% with the methanolic extract (Fig. 3A). As for the CAK05 isolate, at the same concentration (C3 = 50 µL/mL), the recorded inhibition was 71.06% with the acetone extract, 53.16% with the methanolic extract and 78.87% with the aqueous extract (Fig. 3B).

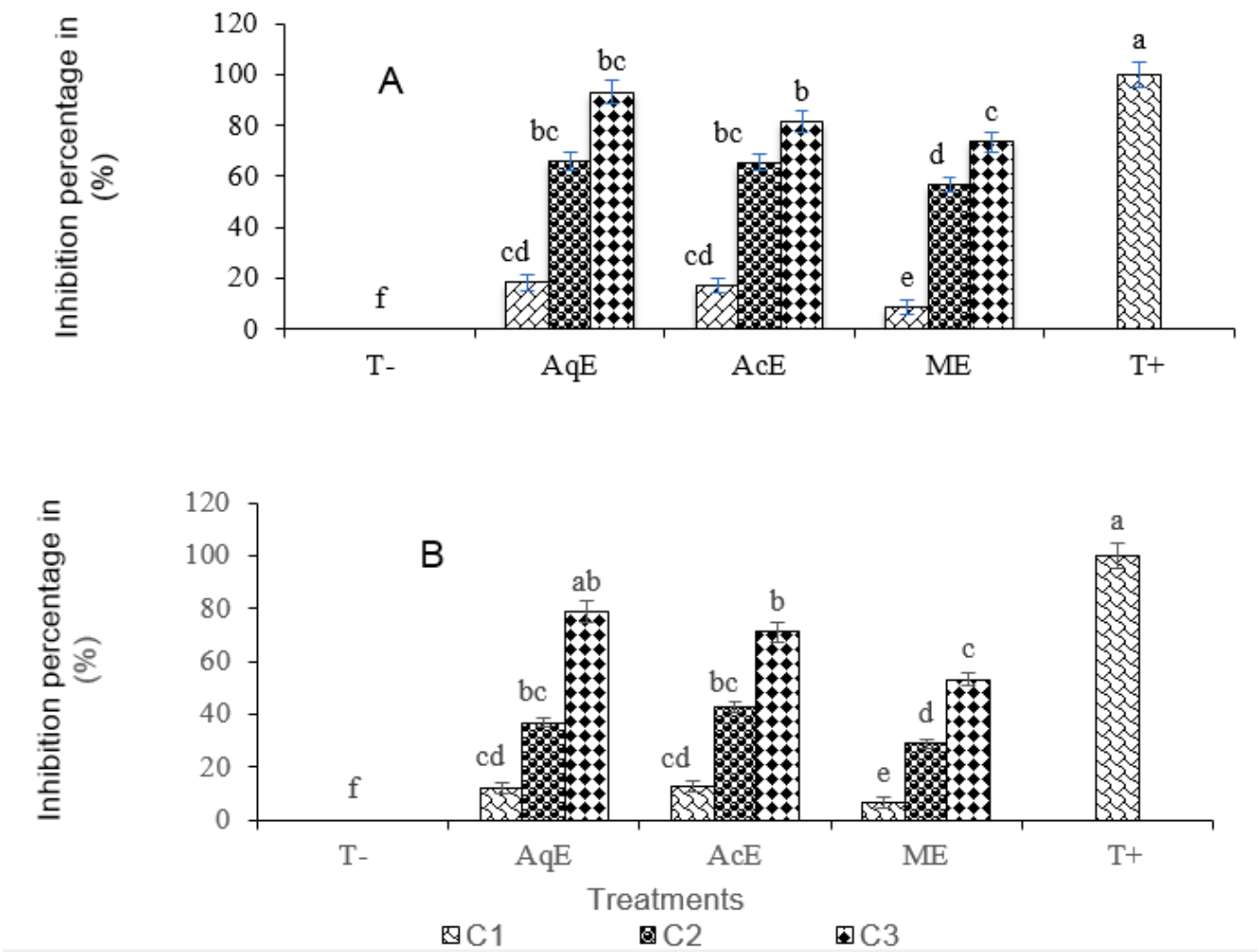

Fig. 3. Inhibition percentage of mycelial growth in A: CAK01 and B: CAK05 isolates of *C. capsici;* where (T-): Negative control; (C1): 12.5 µLmL^{-1}; (C2): 25 µL. mL^{-1}; (C3): 50 µL.mL^{-1}; (T+): Positive control ; AcE: Extracted with Acetone; MeE: Extracted with Methanol; AqE: Aqueous Extract. The histograms surmounted by the same letters are not significantly different at the 5 % level.

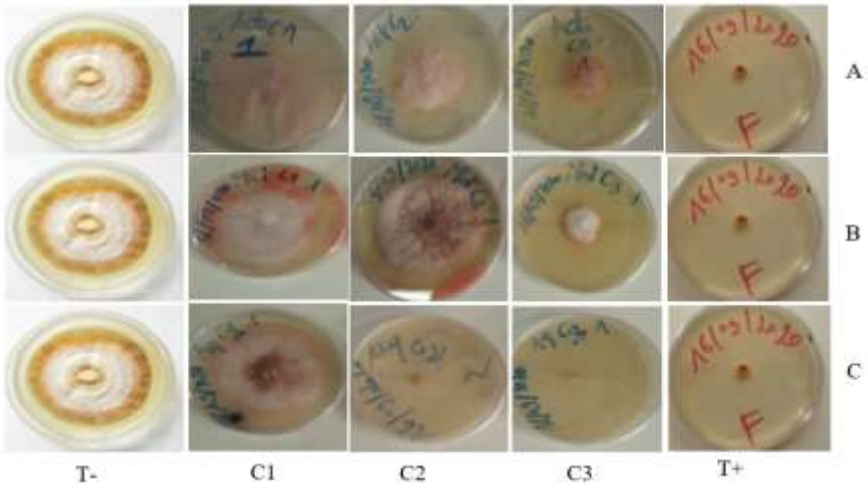

Fig. 4. *In vitro* inhibitory activity of aqueous and organic extracts of *B. eagyptiaca* seeds on the mycelial growth of CAK01 isolate after 6 days of incubation on PDA medium. (T-): negative control; (C1): 12.5 µL.mL^{-1}; (C2): 25 µL.mL^{-1}; (C3): 50 µL.mL^{-1}and (T+) : 3.33 g.L^{-1} of fungicide; A= Acetone extract; B = Methanol extract; C = aqueous extract

Minimum inhibitory concentrations of *Balanites aegyptiaca* seed extract

Minimal inhibitory concentrations (MIC) were determined using linear regression for the acetone extracts (y = 32.19x - 9.8433 and y = 29.13x - 16.173), methanol extracts (y = 32.37x - 18.477 and y = 23.415x - 17.443), and aqueous extracts (y = 37.45x - 15.78 and y = 33.31x - 24.043), respectively for the CAK01 and CAK05 isolates. For CAK01, the lowest MIC values were observed with the aqueous extract (5.81 and 16.78 µL·mL^{-1}), followed by the acetone extract

(6.42 and 22.20 µL·mL⁻¹), while the highest MIC values were recorded with the methanol extract (8.33 and 28.50 µL·mL⁻¹), corresponding to MIC_{50} and MIC_{90}, respectively (Table 5). Regarding CAK05, the lowest MIC values were also obtained with the aqueous extract (9.21 and 30.57 µL·mL⁻¹), followed by the acetone extract (9.68 and 37.34 µL·mL⁻¹), whereas the methanol extract exhibited the highest values (17.81 and 98.50 µL·mL⁻¹) for MIC_{50} and MIC_{90}, respectively (Table 5).

Table 5. Minimal inhibition concentration (MIC) of the mycelial growth of two isolates of *C. capsici* in (µL.mL^{-1}). *Colletotrichum* isolates 01 & 05 (CAK01 & CAK05) from the Akonolinga locality

Extracts	CAK01		CAK05	
	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
Acetone extract	6.42	22.20	9.68	37.34
Methanol extract	8.33	28.50	17.81	98.49
Aqueous extract	5.81	16.78	9.21	30.57

Fungicidal or fungistatic activity of the extracts

The antifungal activity of *B. aegyptiaca* seed extracts against *Colletotrichum capsici* is presented in Table 6. At the concentration C3 (50 µL·mL⁻¹), the extracts inhibited 81.63% and 93% of the mycelial growth of the CAK01 isolate, exhibiting fungicidal effects for the acetone and aqueous extracts, and fungistatic activity for the methanolic extract. For the CAK05 isolate, the concentration of 50 µL·mL⁻¹ showed fungistatic effects with the organic extracts and fungicidal effects with the aqueous extract.

Table 6. Fungicidal and fungistatic effect of *Balanites aegyptiaca* seed extracts

Isolates	Extracts (50 µL. mL^{-1})	Effect
CAK01	Acetone extract	Fungicidal
	Methanol extract	Fungistatic
	Aqueous extract	Fungicidal
CAK05	Acetone extract	Fungistatic
	Methanol extract	Fungistatic
	Aqueous extract	Fungicidal

DISCUSSION

Anthracnose affects the plant throughout its entire developmental cycle. Although the infected plant does not die at the seedling stage, secondary symptoms appear as it grows, affecting stems, leaves, and pods (Adegbite and Amusa, 2008). Initially brownish-black, the lesions gradually turn straw-yellow on the stems, often leading to complete flower abortion or deformation of immature pods. Pods may be infected at any stage of development, showing brown lesions with variable shading. Under favorable humidity conditions, the fungus can fructify on dried pods (Sérémé and Mathur, 1996). To protect cowpea crops, farmers commonly resort to synthetic fungicides, which are toxic to both humans and the environment. Given these concerns, it is essential to identify environmentally friendly alternatives that safeguard human health. The present study therefore investigates the antifungal potential of phytochemical compounds from *Balanites aegyptiaca* (L.) Del. seed extracts, as identified by GC-MS analysis, against *Colletotrichum capsici*

The morphological characteristics of the two collected *C. capsici* isolates were characterized by white to gray, aerial mycelial colonies, sometimes exhibiting beige spore masses. Microscopic observation of the isolates revealed falcate conidia. According to Sérémé *et al*. (2001), *C. capsici* colonies are white to gray and display diurnal zonation, alternating between areas of high and low mycelial density, with aerial mycelium and occasionally beige spore masses.

Phytochemical screening of the seed extracts revealed the presence of several families of secondary metabolites belonging to various classes, including alkaloids, saponins, terpenoids, steroids, phenols, flavonoids, glycosides, anthraquinones, and tannins. All these secondary metabolites were detected in the aqueous extract, while in the methanolic extract, anthraquinones, flavonoids, and tannins were absent. Among the identified compounds, phenolic compounds are known for their diverse pharmacological and biological activities (Mhya *et al*., 2016).

Phytochemical analyses performed by GC-MS revealed the presence of several biochemical compounds in the acetone, methanol, and aqueous extracts, such as (2,4-dichlorophenoxy)acetic acid, 2-chloroethyl ester, pyridine, 2-(1-cyclohexenyl)ethylamine, N-methoxymethanesulfonamide,5methyl2(1H)pyridinone ,3aminobenzenethiol,cyclopropanecarboxamide,1,3,5trime1Hpyrazol4amine,4(1trichlorosilyl3,3dimethylbutyl)cyclopentane,tetradecylmethoxyacetate,dicyclopropyl ketoxime,1-azabicyclo[3.2.1]octane,6-methyl,exo-,9,12-methyloctadecadienoate (Z,Z) and methyl 11-octadecenoate, known for their fungicidal and insecticidal properties.

GC-MS analysis of aqueous and methanolic extracts of *Balanites aegyptiaca* (L.) Del. seed oil, conducted by Mokhtar *et al*. (2021), revealed the presence of various phytochemicals, with phenolic

compounds and fatty acids being the most abundant in different parts of the plant. The aqueous extract was found to be the richest in natural bioactive substances. Indeed, according to Emad *et al*. (2012) and Haruna *et al*. (2020), GC-MS analysis of *B. aegyptiaca* seeds showed that the aqueous extract contained a wide variety of phytochemicals.

B. aegyptiaca extracts inhibited the growth of *C. capsici*. For the highest concentration (C3 = 50 $\mu L \cdot mL^{-1}$), the inhibition rates observed after six days of incubation were 93.14%, 81.63%, and 73.39% for the aqueous, acetone, and methanol extracts of isolate CAK01 (*Colletotrchum* from Akonolinga localaity number 01), respectively. For isolate CAK05 (*Colletotrchum* from Akonolinga localaity number 05), the inhibition rates were 78.87% with the aqueous extract and 71.06% with the acetone extract.

This inhibitory activity of *B. aegyptiaca* seed extracts is attributed to the presence of phytochemicals with fungicidal activity, such as n-hexadecanoic acid, 9,12-octadecadienoic acid (Z,Z), methyl hexadecanoate, cis-13-octadecenoic acid, 1-pentene-3-ol, 4-methylpentane, 2,2,4-trimethyl, and octadecanoic acid. Among these compounds, several fatty acids, particularly n-hexadecanoic acid, are known for their strong biological activities, including antimicrobial, anti-inflammatory, and antioxidant activity (Natarajan *et al*., 2014; Okolie *et al*., 2019).

Several researchers have reported that n-hexadecanoic acid exhibits strong antifungal potential against *Alternaria solani*, *Aspergillus fumigatus*, *Penicillium chrysogenum*, and *Colletotrichum gloeosporioides* (Karim *et al*., 2017). Meanwhile, Djeugap *et al*. (2023), Koné *et al*. (2018), Bolie *et al*. (2021), Manga *et al*. (2021), and Toka *et al*. (2023) demonstrated that the plant extracts used possess significant antifungal activity against *Cercospora malayensis*, *Sclerotinia sclerotiorum*, *Fusarium oxysporum*, and *Phytophthora infestans*. This activity is attributed to the chemical compounds present in the various plant extracts studied.

The bioactive compounds identified in *Balanites aegyptiaca* seed extracts have been described in several studies as having antifungal, antimicrobial, insecticidal, and antibacterial properties (Rubila and Ranganathan, 2014). Furthermore, Habieballa *et al*. (2021) showed that *B. aegyptiaca* seed extracts also possess antimicrobial, antibacterial, larvicidal, antidiabetic, and antifungal activities. Although the precise mechanism of action of the chemical compounds in the plant extracts remains poorly understood, it is likely that these molecules form complexes with polysaccharides and proteins in the outer layer of fungal cells, causing destabilization of the cell membrane and, consequently, the death of the pathogen (Rongai *et al*., 2017).

The inhibition percentages obtained allowed the determination of the minimum inhibitory concentrations (MIC_{50} and MIC_{90}), corresponding to a 50% and 90% reduction in mycelial growth, respectively. The lowest inhibitory concentrations were recorded with the aqueous extracts (AqE), namely 5.81 and 16.78 $\mu L \cdot mL^{-1}$ for MIC_{50} and MIC_{90}, respectively, followed by the acetone extracts (AcE) with values of 6.42 and 22.20 $\mu L \cdot mL^{-1}$ for the CAK01 strain. Regarding strain CAK05, the lowest inhibitory concentrations were also observed with aqueous extracts (AqE), namely 9.21 and 30.57 $\mu L \cdot mL^{-1}$ for MIC_{50} and MIC_{90}, respectively, followed by acetone extracts (AcE), showing values of 9.68 and 37.34 $\mu L \cdot mL^{-1}$ for MIC_{50} and MIC_{90}, respectively. These results highlight the effectiveness of the extracts on the mycelial growth of the pathogen, consistent with the observations of Doumbouya *et al*. (2012), who also reported strong inhibition of phytopathogenic fungal development by *Ocimum gratissimum* extracts at low minimum inhibitory concentrations.

CONCLUSION

The general objective of this work is to evaluate the antifungal activity of phytochemical compounds from extracts of *Balanites aegyptiaca* (L.) Del. seeds, identified by gas chromatography-mass spectrometry (GC-MS), against *Colletotrichum capsici*. GC-MS analysis revealed the presence of several bioactive compounds, including cyclohexanecarboxylic acid, a 3-phenylpropyl ester known for its fungicidal properties. At a concentration of 50 µL/mL, the aqueous and acetone extracts inhibited the mycelial growth of *C. capsici* by 93.14% and 81.63%, respectively, values comparable to those obtained with the synthetic fungicide (100% inhibition). These results highlight the significant antifungal potential of aqueous and organic extracts from *B. aegyptiaca* seeds and suggest their use as biological alternatives to chemical products in the integrated pest management of cowpea anthracnose. Looking ahead, further studies on the isolation and characterization of active molecules, as well as their mechanisms of action *in vitro* and in planta, could promote the development of more effective and environmentally sustainable biofungicide formulations.

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Authors' Contribution

Conceptualization: BN Methodology, data collection: TT, KT, HB; Data Analysis: PZN, SLLD; Original draft preparation: TT; Writing –Review-Editing: LBT, SLLD, CTT, EBN, JPA, HB.

Conflict of interest

The authors declare no conflict of interest

Abbreviations

AcE :	Acetone Extracted
AqE :	Aqueous Extract
CAK 01 :	*Colletotrchum* from Akonolinga localaity 01
CAK 05 :	*Colletotrchum* from Akonolinga localaity 05
Fig :	Figure
GC-MS :	Gas Chromatography coupled with Mass Spectrometry
IP :	Inhibition Percentage
MeE :	Methanol Extracted
MGc :	Mycelial Growth in the control
MGt :	Mycelial Growth in the treatment
MIC :	Minimum Inhibitory Concentrations
MINADER :	Ministry of Agriculture and Rural Development
MW :	Molecular Weight
PDA :	Potato Dextrose Agar
RG :	Radial Growth
Rt :	Retention time

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