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Antifungal Activity of *Jatropha curcas* Seed Extracts Analysed by GC-MS against Two Cowpea (*Vigna unguiculata* L.) Pathogenic Fungi

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Cowpea (*Vigna unguiculata* L. Walp.), native to Africa, is one of the most popular and widely grown seed legumes in arid and semi-arid areas. In these areas, fusarium wilt and white rots caused by *Fusarium oxysporum Schl. f.sp. tracheiphilum* and *Sclerotinia sclerotiorum* are the most devastating pathogens of cowpea crops, causing losses of 50-100 %, respectively. This study aims to evaluate

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the antifungal potential of biochemical compounds in *Jatropha curcas* L. seed extracts identified by GC-MS analysis against *F. oxysporum* and *S. sclerotiorum*. *In vitro* tests were carried out using aqueous, acetone and hexane extracts of *J. curcas* seeds at concentrations of 15, 30, 60 and 120 µL/mL and a synthetic fungicide (3.33 g/L). Mycelial growth and Minimal Inhibitory Concentrations (MIC50) were assessed. As a result, *J. curcas* seed extracts are rich in phytochemical molecules such as 9,12-octadecadienoic acid (Z,Z) ; n-Hexadecanoic acid ; Squalene ; D-Limonene ; 9,15-octadecadienoic acid, methy and 1,3-Dioxane, 5-ethyl-2,2-dimethyl, with antifungal activity. Total inhibition (100%) of mycelial growth of *F. oxysporum* and *S. sclerotiorum* was obtained in Petri dishes contaminated with the aqueous and acetone extracts at concentrations C3=60 µL/mL and C4=120 µL/mL, as well as with the synthetic fungicide. The MIC50 obtained with isolates of *S. sclerotiorum* and *F. oxysporum* were 9.2 and 9.96 µL/mL with the aqueous extract, respectively followed by the acetone extract (12.96 and 14.97 µL/mL). All *Jatropha curcas* lethal extracts tested were fungistatic, but the synthetic fungicide was fungicidal. Bioformulation based on *J. curcas* and characterization of pathogen are needed.

Keywords: Jatropha curcas; antifungal activity; GC-MS; Fusarium oxysporum and Sclerotinia sclerotiorum.

1. INTRODUCTION

Cowpea, *Vigna unguiculata* (Linnaeus) Walpers, is one of the most economically important legumes in the traditional sector in Africa and is known as the southern pea [1]. It is rich in minerals, fats, oils and vitamins [2]. Cowpea is found throughout the world, especially in arid and semi-arid areas [3]. In Cameroon, cowpea is the third most produced and consumed legume after groundnut and soybean [4]. Cowpea belongs to the *Fabaceae* family, one of the most important crops for food and nutrition security, and the main source of protein (20-30% content) for millions of people in developing countries [5,6]. Cowpea is grown for its seeds, tender leaves and pods for human consumption [7]. It is also contributes to improving soil fertility through symbiotic nitrogen fixation with nodule bacteria (*Bradyrhizobium spp*.) [8]. However, its cultivation is mostly limited by biotic constraints that significantly reduce cowpea grain yields by 50-100% [9,10,11]. *Fusarium oxysporum* and *Sclerotinia sclerotiorum* are among the major pathogens limiting legumes production [11,12]. *Sclerotinia sclerotiorum* is a cosmopolitan pathogen that causes white mould, also known as white rot. Due to its unique life cycle, the pathogen infects host plants by spores, and infection can occur on leaves, stems and pods at different stages of seedling development [13,14]. Symptoms are manifested by the production of numerous sclerotia, and the presence of cotton-like mycelium covering the infected area of the plant [15]. Cowpea vascular wilt is caused by *Fusarium oxysporum*, which is one of the major constraints for this legume in the field [16]. Symptoms in the early stages of the disease begin with mild chlorosis of young leaves, which turn from green to yellow. *F. oxysporum* is the most common and damaging species of *Fusarium* responsible for root rot of crops in arable fields. It poses a risk to cowpea production and is the most widespread species that can be recovered from most soils [17]. Several methods have been adopted to reduce the damage caused by the pathogen, namely the selection of improved cowpea varieties, crop rotation and the misuse of chemical fungicides. However, synthetic fungicides have negative effects on human health and the environment [18,19]. Several attempts have been made to find alternatives to the use of chemical fungicides to control plant diseases [20]. Plants with pesticidal potential, rich in secondary metabolites with multiple mechanisms of action, could be a viable alternative to chemical pesticides in sustainable agriculture. Their various modes of action are attributed to the phytochemical composition of different plants [21]. Plants with secondary metabolites (alkaloids, terpenes, amines, glucosinolates, cyanogenic glucosides, quinones, phenolic compounds, peptides and polyacetylenes) with bioactive properties for controlling crop diseases and pests have already demonstrated their efficacy [22,23]. Furthermore, plant extracts contain various chemical compounds, including antioxidants and plant hormones, which activate plant defence mechanisms that promote growth and enable plants to withstand abiotic and biotic stresses [24,25,26]. Extracts from the leaves, stems, roots and seeds of *Jatropha curcas* show a variety of properties to control plant pests and diseases [27,28,29,30]. The efficacy of *J. curcas* extracts with phytochemical compounds as biocides has been confirmed in various studies where fungicidal, bactericidal, insecticidal, nematicidal

and molluscicidal activities against pathogenic species have been demonstrated [31,32,33,34,35]. The present work aimed to test the *in vitro* antifungal effect of *Jatropha curcas* (*Euphorbiaceae*) seed extracts using the GC-MS analytical methods (Gas Chromatography and Mass Spectrometry) on the development of *Fusarium oxysporum* and *Sclerotinia sclerotiorum* phytopathogenic fungi of cowpea.

2. MATERIALS AND METHODS

2.1 Isolation and Identification of the Pathogens

Pure isolates of *Sclerotinia sclerotiorum* and *Fusarium oxysporum* were obtained from infected cowpea (leaves, stems and pods) showing typical symptoms of the diseases. These samples were collected from fields in the locality of Akonolinga (N 03°47'32.2'' and E 012°15'48''), kept in sealed envelopes and immediately transferred in laboratory. These infected organs were washed in running tap water to remove adhering dirt particles, then rinsed in distilled water, after which excess moisture was blotted off with sterile blotting paper and disinfected in 2 % alcohol solution for 2 minutes. Finally, the infected organs were cut with a scalpel into small fragments approximately (3 mm) from the pathogen growth zone and incubated in Petri dishes containing PDA culture medium. After three days of incubation in the laboratory at 23 ± 2◦C, mycelial growth around the seeded fragments were removed and transferred to new Petri dishes containing PDA culture medium. This process was repeated several times until pure cultures were obtained. After eight days of incubation, morphological observations, namely macroscopic and microscopic, of the isolates were observed. These observations were charactezeded in *S. sclerotiorum* by the presence of conidia, colour, shape and growth rate of the colony and number of days of sclerotia initiation [36,37] and in *Fusarium oxysporum* by the presence of conidia, colour and shape of the colony and growth rate of the hyphae [38,39]. Slides were prepared using 95% ethanol. Pathogens were identified under the microscope using identification keys [40,41,42].

2.2 Pathogenicity Test

The pathogenicity test was performed on healthy young cowpea leaves placed on blotting paper soaked in distilled water in Petri dishes (Ø = 90 mm) and treated with the spore suspension (10 µL). Petri dishes containing 10-day-old pure isolates of both *S. sclerotiorum* and *F. oxysporum* fungi on PDA medium were selected for the preparation of spore suspensions. Healthy control leaves were treated with sterile distilled water (SDS). Inoculated dishes were sealed and incubated under laboratory conditions at 23°C. Observations were made from day 2 of treatment to day 15 [43].

2.3 Preparation of Various *Jatropha curcas* Seed Extracts

Jatropha curcas seeds were collected in the North Cameroon region. The mature fruits of *J. curcas* were pulped and the seeds obtained were dried at room temperature (23 ± 2°C) in the laboratory for 3 weeks. These seeds were then ground to obtain a powder. 500 g of this powder was weighed using a precision balance and then macerated in 2 L of solvent for 72 hours [44].The solute-solvent mixture was filtered using filter paper (Whatman No. 1), and the filtrate obtained was concentrated using a rotary evaporator (Büchi R 200 rotary evaporator at 60°C). The extract obtained after evaporation was stored in a refrigerator at 4°C until use. The aqueous solution was obtained by maceration of 125 g of *J. curcas* seed powder in 250 mL of sterile distilled water for 48 h. The solution was filtered and used directly.

2.4 GC-MS Analysis of *Jatropha curcas* Seed Extracts

For the quantities of organic extracts in the laboratory, 0.5 mL of extract was collected and completed with its solvent. 0.5 mL of *J. curcas* extract in acetone was made up with 1.5 mL of acetone and 0.5 mL of *J. curcas* extract in hexane was made up with 1.5 mL of hexane. For the aqueous extract, 5 mL of extract was taken with a graduated pipette and added to the graduated test tube. The mixture was made up with 6 mL of acetonitrile solvent. 2.5 g magnesium sulphate and 0.5 g sodium acetate were added to the mixture to absorb water. The mixtures were passed through a vortex mixer for 1 minute to homogenise the mixture. The tubes were then placed in a centrifuge to separate the particles at a speed of 5000 rpm for 3 minutes. The particles were separated into two phases. Approximately 1 mL of each extract was taken with a pipette and transferred to the GC-MS flasks. The GC-MS is a gas chromatograph (Agilent GC 7890A) coupled to a mass

spectrometer detector (Agilent 5975 C TAD VL MSD) equipped with an Elite-1 fused silica capillary column (30 m x 0.25 mm with 0.25 layer thickness) and helium as a carrier gas. An electron ionisation system with an ionisation energy of 70 eV was used to detect the compounds. One microlitre (1 µL) of each preparation was injected into the column. The initial oven temperature was 150°C maintained at 20°C/min for 1 min to a final temperature of 280°C maintained for 9 min. The total GC run time was 13.44 min. The separated components were identified by comparing their spectra with those of the National Institute Standards and Technology (NIST) database, which contains more than 62,000 models [45].

2.5 *In vitro* Evaluation of the Antifungal Activity of *Jatropha curcas* Seed Extracts

A stock solution of 500 µL/mL was prepared by mixing 50 mL of each extract (with 100 mL of solvent (acetone, hexane or water). Concentrations of 15, 30, 60 and 120 µL/mL of aqueous extract (AqE), acetone extract (AE) and hexane extract (HE) were then prepared by taking successively 0.9;1.8; 3.6 and 7.2 mL of the stock solution and adding 29.1; 28.2; 26.4 and 22.8 mL PDA, respectively, to give a final volume of 30 mL each. The mixture was poured into 90 mm Petri dishes at a rate of 10 mL per dish. The synthetic fungicide containing 80 g/kg metalaxyl and 640 g/kg mancozeb was used as a control at the recommended concentration of 3.33 µL/mL. However, the control plates received only the PDA medium. The 7 mm diameter discs of mycelial hyphae from 10-day-old pure cultures of *S. sclerotiorum* and *F. oxysporum* were removed with a sterilised scalpel and placed in the centre of each Petri dish containing the different extracts and the PDA medium spiked with the extracts. The experiment was performed in triplicate. The Petri dishes were sealed with adhesive film and incubated in a culture chamber at 25ºC and the mycelial growth of both pathogens was measured every two days, starting on the second day of incubation and ending when the mycelium had filled the control Petri dish. It was measured in mm from two perpendicular axes drawn on the back of the Petri dish using the formula of Singh et al. [46]. The percentage inhibition of mycelial growth relative to the control was calculated for all concentrations of each fungicide using the formula proposed by [47].

I (%) = (Mc - Mt/Mc) × 100 where: I = percentage inhibition; Mc = control mycelial growth; Mt = mycelial growth in treatment.

2.6 Determination of Minimum Inhibitory Concentrations

The minimum inhibitory concentrations (MIC50 and MIC90) of the different treatments used against *S. sclerotiorum* and *F. oxysporum* were determined by calculating the values (MIC50 and MIC90) using the linear regression equation of the type y= ax + b, where the abscissa is the concentration of the extracts tested and the ordinate is the percentage of inhibition [48].

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

2.7 Fungicidal and Fungistatic Tests of Extracts

At the end of the experiment, mycelial explants of the two fungi tested were collected in Petri dishes in which fungal growth was completely inhibited. These explants were aseptically plated on PDA medium without seed extract or fungicide. After seven days, depending on whether the fungi resumed growth or not, the starting substance (extract) was classified as fungistatic or fungicidal [49].

2.8 Statistical Analysis

The collected data were subjected to one-way analysis of variance (ANOVA) using R software version 4.0.4 (R development Core Team 2022). Significant differences between means were compared using Tukey's HSD test at 0.05. Minimum inhibitory concentrations at 50% and 90% of mycelial growth (MIC50 and MIC90) were evaluated.

3. RESULTS

3.1 Isolation and Identification of the Pathogens *Sclerotinia sclerotiorum* and *Fusarium oxysporum*

Colonies of *S. sclerotiorum* and *F. oxysporum* microorganisms were grown in Petri dishes containing PDA medium (Figs. 1,2). *F. oxysporum* colonies showed a white mycelium with a cottony layer and smooth pink filaments with a slow growth rate compared to *Sclerotinia*. Macroconidia, microconidia and chlamydospores

were observed. In contrast, *S. sclerotiorum* had smooth, brown filaments with sclerotia on one side and an off-white, cotton-like colour on the other. Irregular oval sclerotia were produced from the 6 th day and the number of sclerotia varied between 15 and 25. The sclerotia were located in the centre and at the edge of the Petri dishes. Under the microscope, the hyphae were hyaline and branched. Ascospores were oval and unicellular. The morphological appearance of these fungi identified them as belonging to the families of *Sclerotiniaceae* and *Nectriaceae*, from *S. sclerotiorum* and *F. oxysporum,* respectively.

3.2 Pathogenicity Test

The pathogenicity test revealed the appearance of specific symptoms similar to those of white rot and fusariosis. Healthy young cowpea leaves infected with inocula of both *S. sclerotiorum* and *F. oxysporum* showed symptoms identical to those observed on infected organs in the field, characterized by cottony mycelial growth, sclerotia formation and wilting symptoms (Fig. 3).

3.3 Gas Chromatography and Mass Spectrometry of *Jatropha curcas* seeds extracts

The extracts analyzed in this study show numerous chemical compounds represented by majority, minority and ultra-minority peaks (Fig. 4). The number of compounds identified in the aqueous, acetone and hexane extracts of *J. curcas* seeds was 58 : 35 compounds in the aqueous extract (Fig. 4A), 6 in the acetone extract (Fig. 4B) and 17 in the hexane extract (Fig. 4C). The retention times, structures, area percentage and molecular weights of some compounds derived from this plant show their Anti-fungal properties (Tables 1, 2 and 3).

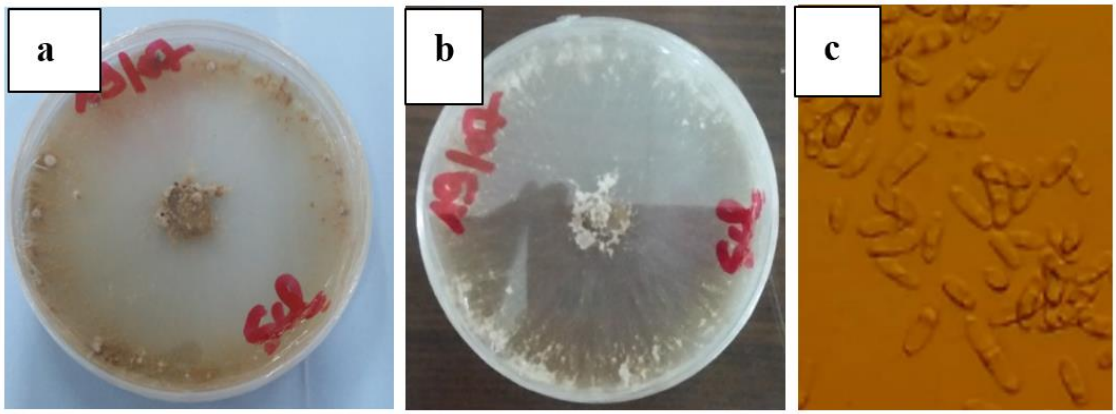


Fig. 1. Pure isolate of *S. sclerotiorum* a and b) macroscopic observation c) microscopic observation

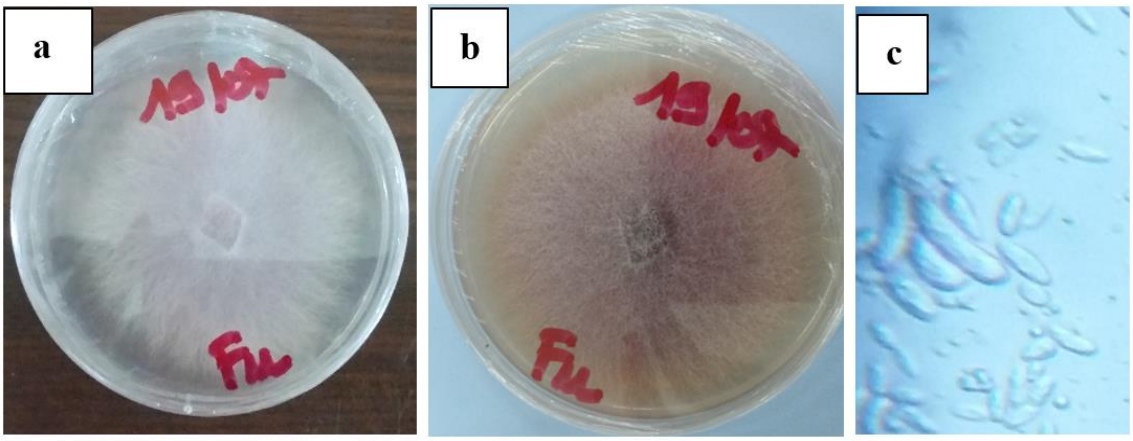


Fig. 2. Pure isolate of *F. oxysporum* a and b) macroscopic observation c) microscopic observation

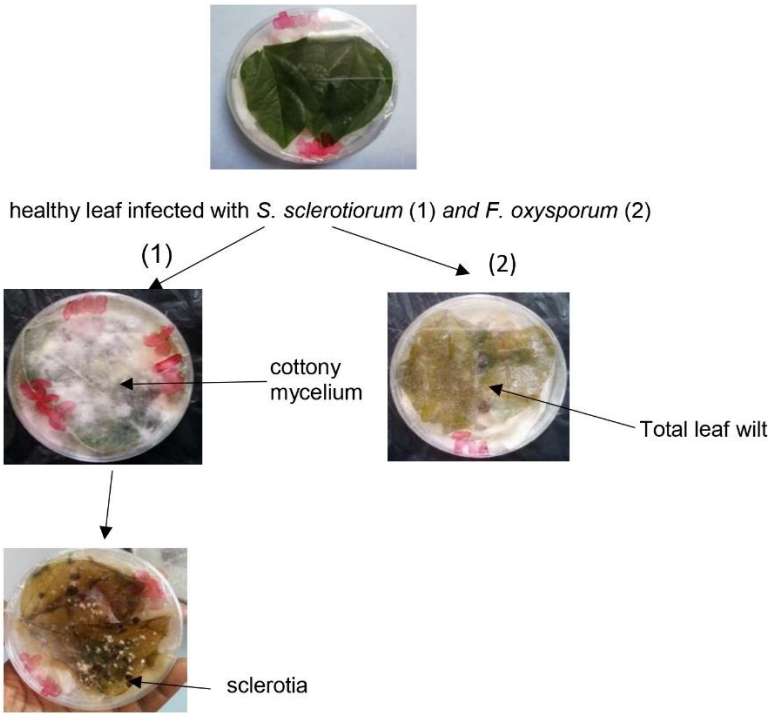
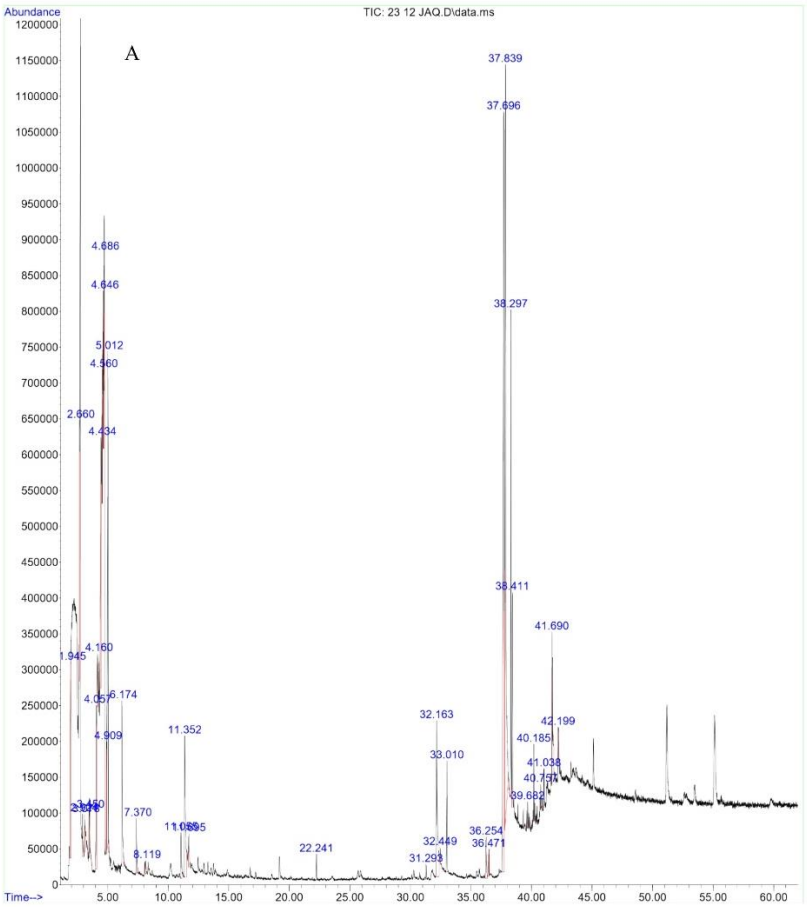


Fig. 3. Pathogenicity test on cowpea leaf in petri dish



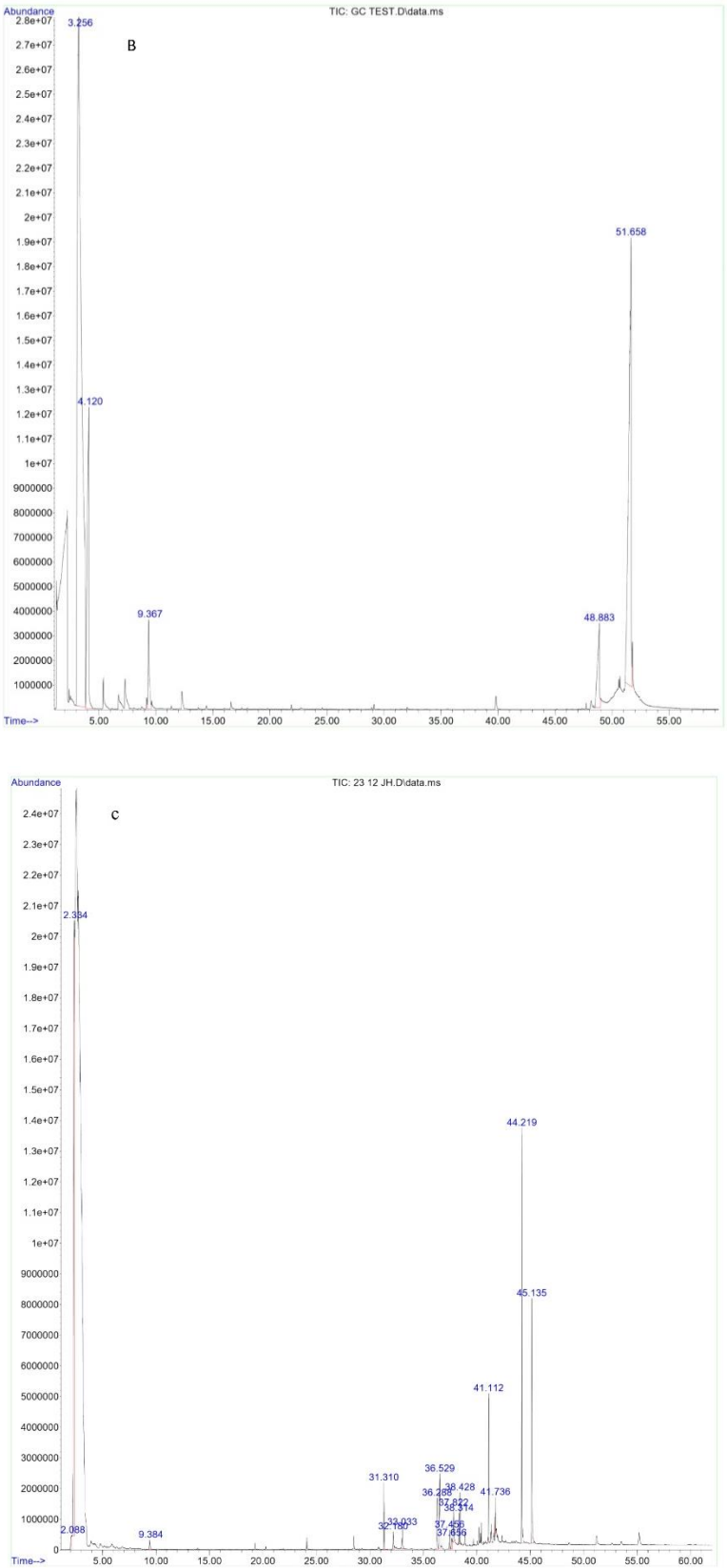


Fig. 4. Chromatographic profile of aqueous (A), acetone (B) and hexane (C) extracts of *Jatropha curcas* seeds

Table 1. Bioactive phytochemical compound from aqueous extract of *J. curcas* seeds by GC-MS F: fungicide; I: insecticide

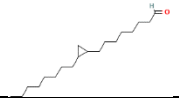
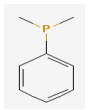
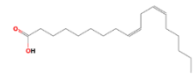
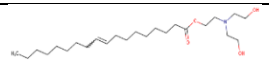
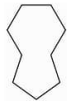
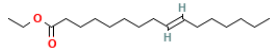
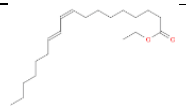
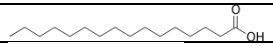
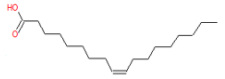
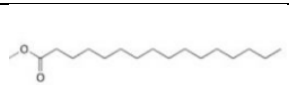
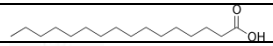
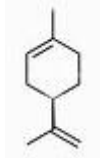
Compound names	Formular	RT (mm)	Activities	Chemical structure	Molecular weight (g/mol)
Cyclopropaneoctanal, 2-octyl-	C19H36O	39.682	F et I		280.5
Dimethyl-cyano-phosphine	C3H6NP	3.450	F		62.05
9,12-Octadecadienoic acid (Z,Z)-.	C18H32O2	36.254	F et I		280.4
9-Octadecenoic acid (Z)-, 2-hydr...	C18H34O2	39.682	F et I		282.5
Cyclononanone	C_9H_{18}	40.185	F et I		126,239
Heptasiloxane, 1,1,3,3,5,5,7,7,9...	$C_{14}H_{44}O_6Si_7$	40.757	I		505.09
Ethyl 9-hexadecenoate	$C_{18}H_{34}O_2$	38.411	F et I		282.5
Ethyl 9.cis.11.trans.-octadecad...	C20H36O2	38.297	F et I		308.498
Hexadecanoic acid, methyl ester	C17H34O2	31.293	I		270.45
n-Hexadecanoic acid	C16H32O2	32.163	F et I		256.42
Oleic acid	C18H32O2	37.839	I		282

Table 2. Bioactive phytochemical compounds of acetone *Jatropha curcas* seed extract by GC-MS F: fungicide; I: insecticide

Compound names	Formular	RT (mm)	Activities	Chemical structure	Molecular weight (g/mol)
Pyridine	C_5H_5N	3.582	F et I		79,09
Pyridine	C_5H_5N	4.029	F et I		79,09
Pyridine	C_5H_5N	11.209	F et I		79,09
1 N-(Methylsulfonyl)-O-methylhydro.	C3H9NO5S2	4.519	F et I		203.2
1 N-(Methylsulfonyl)-O-methylhydro.	C3H9NO5S2	6.542	F et I		203.2

Table 3. Bioactive phytochemical compounds of hexane *Jatropha curcas* seed extract by GC-MS F: fungicide; I: insecticide

Compound names	Formular	RT (mm)	Activities	Chemical structure	Molecular weight (g/mol)
Oleic Acid	C18H32O2	37.822	I		282
Hexadecanoic acid, methyl ester	C17H34O2	31.310	I		270.45
n-Hexadecanoic acid	C16H32O2	48.694	F et I		256.42
D-Limonene	C10H16	9.384	F et I		136,234

3.4 *In vitro* Antifungal Activity of *Jatropha curcas* Seed Extracts against *Sclerotinia sclerotiorum* and *Fusarium oxysporum*

The results showed that acetone and aqueous extracts significantly reduced mycelial growth of the different fungal isolates, the hexane extract, however, had a weak inhibitory effect. Control dishes were fully colonized by *S. sclerotiorum* and *F. oxysporum* five and eight days after inoculation, respectively, while dishes containing the synthetic fungicide inhibited mycelial growth by 100% (Fig. 5A and 5B). It should be noted that the acetone and aqueous extracts inhibited the mycelial growth of the isolates in the same way as the synthetic fungicide, from a concentration of C3 = 60 µL/mL for the aqueous extract and C4= 120 µL/mL for the acetone extract for the two fungi tested (Figs. 6,7). The percentage inhibition of fungal mycelial growth also depended on the type of extract used and its concentration. The higher the concentration, the greater the inhibition.

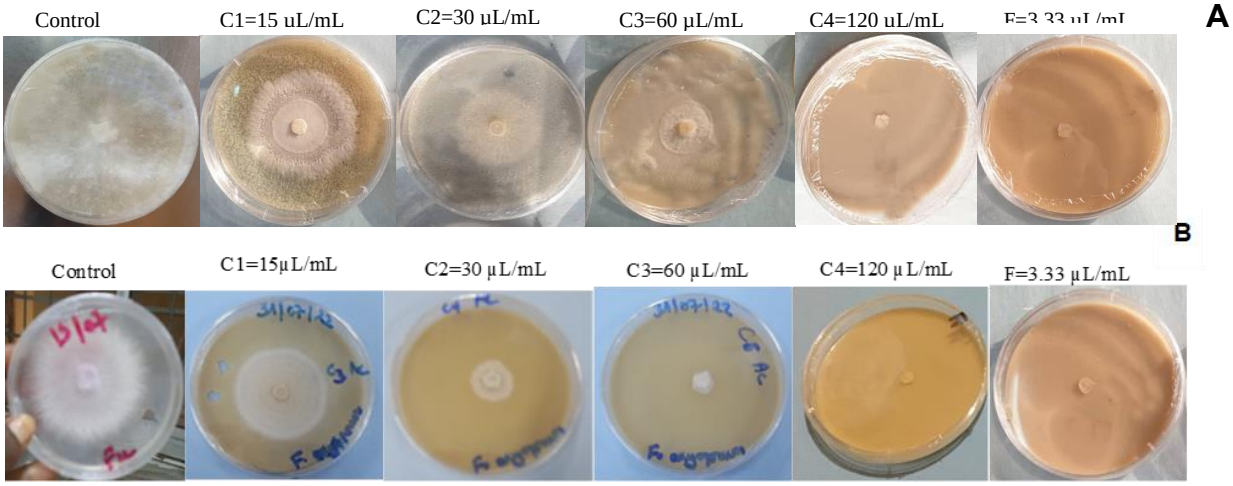


Fig. 5. Mycelial growth inhibition of *Sclerotinia sclerotiorum* isolates (A) and *Fusarium oxysporum* isolates (B) in acetone extract

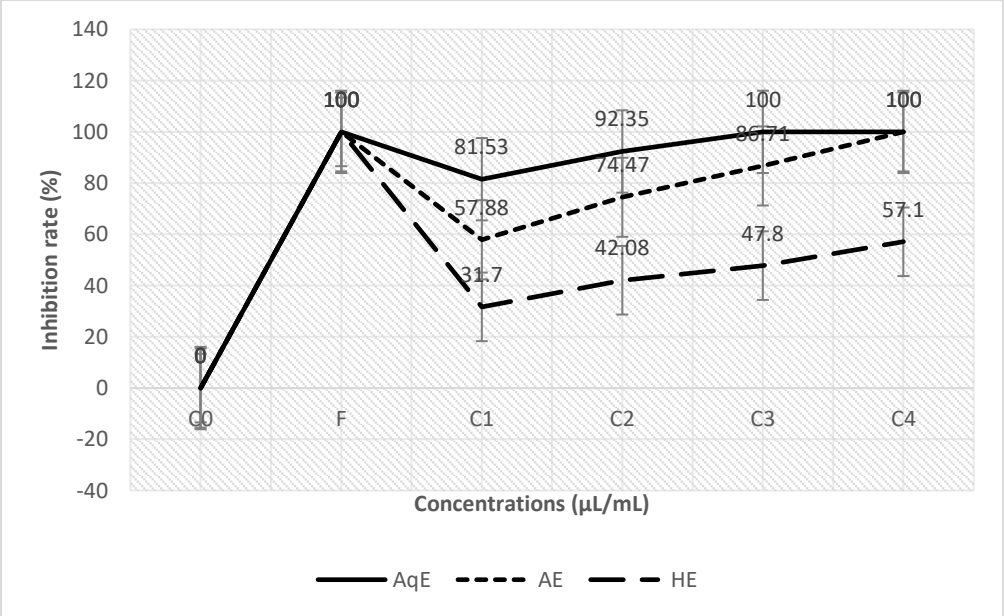


Fig. 6. *In vitro* inhibition of *Sclerotinia sclerotiorum* mycelium growth by *Jatropha curcas* extracts

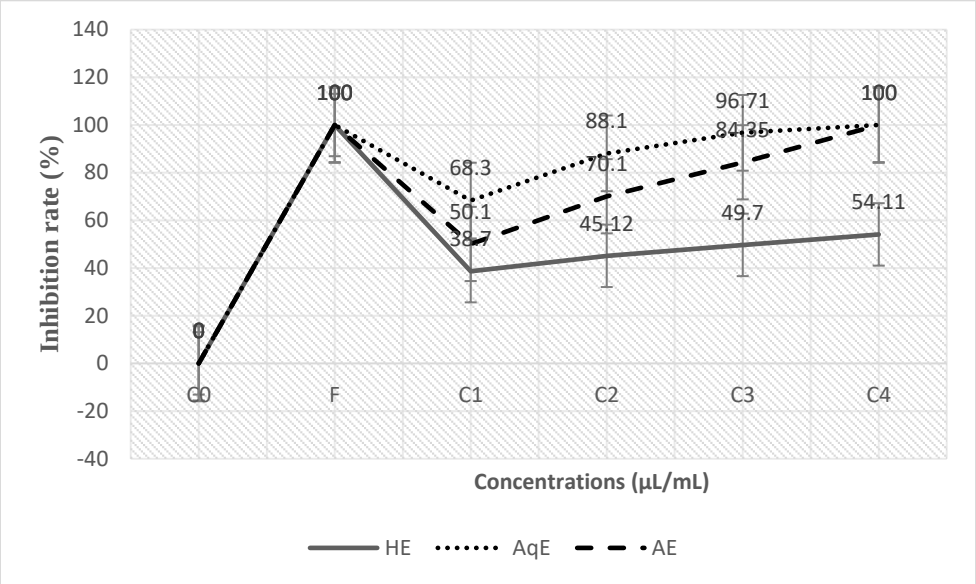


Fig. 7. *In vitro* inhibition of *Fusarium oxysporum* mycelium growth by *Jatropha curcas* extracts

Table 4. Minimum inhibitory concentrations MIC 50 and MIC 90 in µL/mL

Phytopathogenic fungi	Extracts	CMI 50	CMI 90
Fusarium oxysporum	Aqueous extract	9.96	17.93
	Acetone extract	14.97	26.95
	Hexane extract	19.38	34.88
Sclerotinia sclerotiorum	Aqueous extract	9.2	16.55
	Acetone extract	12.96	23.32
	Hexane extract	23.66	42.59

3.5 Minimum Inhibitory Concentrations of the Various Extracts

The lowest minimum inhibitory concentrations (MIC50) obtained with isolates of *Sclerotinia sclerotiorum* and *Fusarium oxysporum*, were 9.2 µL/mL and 9.96 µL/mL with the aqueous extract followed by the acetone extract (12.96 µL/mL and 14.97 µL/mL), respectively. The lowest minimum inhibitory concentrations (MIC90) were also with the aqueous extract (16.55 and 17.93 µL/mL) followed by the acetone extract (23.32 and 26.95 µL/mL) of *S. sclerotiorum* and *F. oxysporum* isolates, respectively.

3.6 Fungicidal and Fungistatic test

All *Jatropha curcas* lethal extracts tested showed fungistatic activity, however the synthetic fungicide showed fungicidal activity.

4. DISCUSSION

Sclerotinia sclerotiorum and *Fusarium oxysporum* are among the most causal pathogens destroying cowpea crops. They are identified on the basis of morphological characteristics. White mould caused by *Sclerotinia sclerotiorum* is a disease that is widespread throughout the world. Temperatures between 18 and 23°C and high humidity favour the appearance of the pathogen [36]. Understanding the morphology and pathogenicity of the fungus is fundamental for controlling the disease. Morphological characteristics after culturing the fungus on PDA showed the uniform and rapid growth of two types of *S. sclerotiorum* colonies, one off-white and the other brown, and the presence of sclerotia. Black, oval, irregular sclerotia were formed with approximately 15 to 25 sclerotia per Petri dish. These sclerotia were arranged in the centre and at the edge of the Petri dishes. Under the microscope, the hyphae were hyaline and branched. Ascospores were oval, unicellular and hyaline. The cultural and morphological characteristics of the pathogens in this study are in accordance with the findings of other researchers [50,51]. The mycelial colours of *Fusarium oxysporum Schl. f.sp.tracheiphilum* observed after growth on PDA medium varied between the two isolates. Some isolates had slow growing, white, cottony colonies, irregular in

shape with pink filaments. On the other hand, other isolates showed purple colonies and filaments. Microscopic observation showed the presence of macroconidia and microconidia. Some are septate, while others are not. This result is in agreement with the observations reported by Metsena et al. [4].

The pathogenicity test revealed the appearance of specific symptoms similar to those of white rot and fusariosis. Healthy young cowpea leaves infected with inocula of the two fungi *S. sclerotiorum* and *F. oxysporum* showed symptoms identical to those observed on infected organs in the field, characterised by cottony mycelial growth, formation of sclerotia as well as presence of yellowing and wilting symptoms on the leaves, among others, similar to those confirmed by Rodrigues et al., [16] ; Sharma et al. [52] and Rather et al. [37].

Jatropha curcas seed oil obtained has a yellowish colour and an oily texture. The aqueous extract has a whitish colour and a milky texture. The best storage conditions for these oils would be 4°C to maintain the quality of the oil. According to Tapanes et al. [53] this oil is potentially more valuable because of the great properties it possesses (low viscosity compared to castor oil, better oxidation stability compared to soybean oil, low acidity, less processing compared to corn ethanol and good cold properties compared to palm oil).

Natural products derived from plants are an effective option against chemical products. GC-MS analysis of *Jatropha curcas* seed extracts in acetone, aqueous and hexane shows a chromatogram profile with majority, minority and ultra-minority peaks. The results reveal four bioactive compounds with fungicidal activity in acetone, and more than five in aqueous extract and hexane extract. *Jatropha curcas* contains more steroids, terpenoids, flavonoids and alkaloids (mainly phorbol esters), flavonoids and alkaloids (mainly jatrophin) in the leaves and seeds. One of the most common biological properties of alkaloids is their toxicity against the cells of foreign organisms. Alkaloids constitute one of the largest groups of phytochemicals in plants, with amazing effects that could explain the efficacy of *J. curcas* against phytopathogenic fungi [54]. Rampadarath et al. [54], reported also that Jatropha extracts showed microbicidal activity. This activity of Jatropha extracts is probably due to their ability to form complexes with extracellular and soluble proteins, and bacterial cell wall through non-specific forces such as hydrogen bonding, hydrophobic effects, and the formation of covalent bonds.Thus, their antimicrobial mode of action may be related to their ability to inactivate microbial adhesins, enzymes, cell envelope transport proteins, etc. Toka et al. [26] revealed that methanol extract of *Azadiracta indica* and *Balanites aegyptiaca seeds* contributes to lytic membrane and inhibition of the protein of *F. oxysporum*. Saetae and Suntornsuk [55] showed that, the ethanolic extract of *J. curcas* seed cake has been shown to have antifungal activities against important fungal phytopathogens. Other several studies have reported that, the bioactive compounds present in *Jatropha curcas* seed extracts have antibacterial, anti-inflammatory, antifungal, insecticidal activity [56,57,58,52]. Some studies show that n-hexadecanoic acid, 9,12-octadecadienoic acid (Z, Z), 1-penten-3-ol, 4-methyl-pentane, 2,2,4-trimethyl-, cis-13-octadecenoic acid and octadecanoic acid possess antifungal properties [59,60,61]. Oleic, hexadecanoic and octadecanoic acids are fatty acids with hydrocarbon chains of twelve to eighteen carbons with a terminal carboxyl group that are absorbed, digested and transported in animals and humans [33].

The results of this study indicated that the tested *Jatropha curcas* extracts inhibited the mycelial growth of *F. oxysporum* and *S. sclerotiorum*. This inhibition may be due to the bioactive compounds present in these extracts (acetone, aqueous and hexane). The antifungal activities of *J. curcas* have been tested against other pathogens. Cordova-Albores et al. [62], previously explained that all seeds of the genus Jatropha contain substances such as curcine and curcusone B, which have antifungal activity against phytopathogenic fungi, namely *Botrytis cinerea*, *F. oxysporum*, *F. moniliforme* and *Aspergillus flavus*.

In the present study, aqueous extracts, significantly, reduced the growth of *F. oxysporum* and *S. sclerotiorum in vitro*, followed by acetone extracts and finally hexane extracts, which showed weak inhibition. This could be explained by the presence of polar substances of the different phytoconstituents present in the aqueous and acetone extracts of *J.curcas*. On the other hand, the effect of hexane extracts could be due to the non-polar substances [63]. Solvent polarity may play a role in the extraction of many compounds [64]. The aqueous and acetone extracts inhibited mycelial growth by

100% at concentrations C3 = 60 µL/mL and C4 =120 µL/mL, respectively. Koné et al. [44] showed that the aqueous and acetone extracts of *J. curcas* seeds inhibited the mycelial growth of *Cercospora malayensis*, the causal agent of cercosporiosis on okra leaves, to the same extent as the synthetic fungicide at concentration C5. Seweta et al. [65] concluded in their work that *Jatropha* oil has a promising antifungal effect on *Aspergillus niger*, *Penicillium sp*., *Rhizopus sp*., *Pestaliotiopsis funerea* and *Rhizoctonia*, *Penicillium glabrum* and *Aspergillus niger*. According to Doughari et al. [66], *Jatropha curcas* Linn extracts have antifungal activity against *Candida albicans*. *Jatropha curcas* and *Azadirachta indica* oils have antibiosis-type antifungal activity reported by [67]. Cordova-Albores et al. [62] reported that the phorbol ester content of *J. curcas* seed oil of the Mexican species used in their study can be considered non-toxic and, therefore, potentially used as a biofungicide.Sharma et al. [52] showed that *J. curcas* seed extracts contain terpenes, flavonoids, phenols and alkaloids. Flavonoids and terpenes are molecules known for their antifungal activity [68].

The study found that the aqueous and acetone extracts of *Jatropha curcas* seeds had the lowest minimum inhibitory concentrations (MIC50), indicating their effectiveness in inhibiting fungal growth. These extracts were fungistatic at concentrations that completely inhibited fungal growth. This is consistent with a previous study by Koné et al. [44], which also demonstrated the fungistatic effect of *J. curcas* seed extracts. Furthermore, the low MIC values of these extracts suggest their potential as fungicides against *Fusarium oxysporum* and *Sclerotinia sclerotiorum*. This finding is supported by a study conducted by Doumbouya et al. [69], who found that low MIC values indicated the strong inhibition of phytopathogenic fungi by *Ocimum gratissimum* extracts. Taken together, the results highlight the antifungal properties of *J. curcas* seed extracts and their potential use as natural fungicides.

5. CONCLUSION

The objective of this work was to test the *in vitro* antifungal effect of *Jatropha curcas* (*Euphorbiaceae*) seed extracts using the GC-MS analytical method on the development of *Fusarium oxysporum* and *Sclerotinia sclerotiorum* phytopathogenic fungi of cowpea. Our study revealed that, *Jatropha curcas* seed extracts contain several volatile chemical compounds as identified by GC-MS. These chemical compounds exhibited antifungal activity; the main ones being: 9,12-octadecadienoic acid (Z,Z) ; n-Hexadecanoic acid ; Squalene ; D-Limonene ; 9,15-octadecadienoic acid, methy and 1,3-Dioxane, 5-ethyl-2,2-dimethyl.These chemical compounds are present in the extracts (aqueous, acetone and hexane). Aqueous and acetone extracts significantly inhibited the mycelial growth of *Fusarium oxysporum* and *Sclerotinia sclerotiorum* at all concentrations tested. Total inhibition (100%) of mycelial growth of *F. oxysporum* and *S. sclerotiorum* was obtained in Petri dishes contaminated with the aqueous and acetone extracts at concentrations C3 = 60 µL/mL and C4 = 120 µL/mL, as well as with the synthetic fungicide. The antifungal activity of *J. curcas* seed extracts should be considered when controlling fungal diseases in crops, therefore, a bio-formulation is required.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that generative AI technologies such as Large Language Models, etc have been used during writing or editing of manuscripts. This explanation will include the name, version, model, and source of the generative AI technology and as well as all input prompts provided to the generative AI technology.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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