DOI: http://dx.doi.org/10.32571/ijct.1278596 **E-ISSN: 2602-277X**

International Journal of Chemistry and Technology

http://dergipark.org.tr/ijct

Research Article

Chemical Compositions, Phytochemical Screenings and Antifungal Activities of Cashew Nut Shell Liquid Extracts on Phytopathogenic Fungi of Yam (Dioscorea rotundata)

Nurudeen Olanrewaju Sanyaolu[1]*, Olufemi Omotunde Agbola[1], Ahmed Adeola Ibikunle[1], Segun Ajibola Ogundare[1], Rasaq Adejare[2]

[1]*Department of Chemical Sciences, Faculty of Science, Olabisi Onabanjo University, 120107, Ago-Iwoye, Ogun State, Nigeria* ,

[2]*Department of Microbiology, Federal University of Agriculture, 110211, Abeokuta, Ogun State, Nigeria*

Received: 6 April 2023; Revised: 11 September 2023; Accepted: 25 September 2023

*****Corresponding author e-mail: nurusanya@yahoo.com

Citation: Sanyaolu, N.O.; Agbola, O.O.; Ibikunle, A.A.; Ogundare, S.A.; Adejare, R. *Int. J. Chem. Technol.* **2024**, 8(1), 10-18.

ABSTRACT

This study was carried out to evaluate the antifungal activity of Cashew (Anacardium occidentale L.) nutshell liquid on fungal pathogens of dry rot diseases of yam (Dioscorea cayennensis subsp. rotundata). The Soxhlet extraction method was used and constituents were identified and quantified using Gas chromatography. Isolation and identification of fungi complexes associated with dry rot disease of yam were made using Potato dextrose agar (PDA) as a medium. The antifungal activity of CNSL was evaluated using the food poisoning technique on PDA at 25, 50, 75 and 100% concentrations of CNSL dissolved in dimethyl sulphoxide. Azulene (57.65%) was the major chemical component of CNSL with phenolic compounds; anacardol, cresols and anacardic acid, among other compounds. CNSL phytochemical screening showed the presence of flavonoids, alkaloids, saponins, tannins, phenols, terpenoids, volatile oil and steroids. Infected yam tubers were isolated, four fungal strains (Fusarium solani, Lasiodiplodia theobromae, Aspergillus fumigatus, and A. niger) were identified, and pathogenicity test confirmed the association of the fungi species with the dry rot disease of white yam. CNSL was active against the identified fungi and could potentially compete with conventional standard as a suitable fungicide against dry rot disease of white yam.

Keywords: Cashew nutshell liquid, Phytochemical compositions, Antifungal activities, Dioscorea rotundata.

1. INTRODUCTION

The cashew tree belongs to the class of arboreal native plants, which originated from Northeastern Brazil and could also be found in some of the countries in the tropics like Tanzania, Indonesia, Mozambique, Thailand and India.[1,2] Cashew (*A. occidentale*) is an important export commodity not just for its juicy fruit, which is rich in vitamins A and C, but also for its nut, a source of protein and essential minerals. The by-product of nut production is the shell which constitutes the pericarp that encases the nut. The shell provides the cashew nut shell liquid (CNSL), which has varying compositions depending on the method and extraction condition.[3,4] Moreover, CNSL is rich in phenolic compounds, majorly anacardic acid, and its decarboxylate derivative, cardanol.[5-7] The potential of CNSL has been explored not only in producing environmentally friendly biofuel but also in synthesizing other phenolic compound derivatives and for its biological activities.[7,8-10] The presence of phenolic compounds has been established to be responsible for these biological activities, including larvicidal, antioxidant, antigenotoxic and antimicrobial activities.[9-11] The antimicrobial potency of CNSL has been investigated using methicillin-resistant *Staphylococcus aureus* (MRSA) clinical isolates. The results of the study showed that the minimum inhibition concentration of CNSL against this pathogen was

DOI: http://dx.doi.org/10.32571/ijct.1278596 **E-ISSN: 2602-277X**

0.00024 µg mL^{-1}.[11] Similarly, the antifungal property of CNSL was studied using five different human pathogenic fungi; *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *Fusarium sp*. and *Curvalaria sp*[12]; the study revealed that CNSL showed different degree of inhibition against tested fungi with percentage inhibition in the range 95-41%. However, CNSL showed no significant effect against *Curvalaria sp.*[12]

CNSL is a plant-based product that farmers can readily access. Hence, it can be used as a pesticide, insecticide and antimicrobial agent in minimizing the devastating effect of pests, insects and fungi that attack farm produce. This will reduce the cost of procuring expensive conventional synthetic pesticides, insecticides and antimicrobial agents by farmers and improve the availability of farm products all year round at relatively reduced cost with good returns to the farmers.[7] One such farm product on which CNSL can be applied is yam (*Dioscorea* spp.), a common staple food rich in carbohydrates for millions of households globally.[13] Farmers experience poor quality yam products stored over a period due to the harmful effect of fungi, which cause yam to rot in storage facilities.[14] This has reduced the quality of yam and made it less attractive in the international market.[13] Being readily accessible, less toxic, biodegradable and environmentally friendly, CNSL potential can be explored in curbing the menace of fungi in yam tubers. More recently, the demand for environmentally benign products has increased to replace the conventional synthetic products used in crop management to mitigate the threat imposed on global food security caused by plant pathogens, which reduce farm products' economic and nutritional values.[15] The control and management of plant pathogens can also be achieved using plant-derived products rich in bioactive chemicals that can provide potential alternatives to the currently used antimicrobial control agents.[16]

To a great extent, fungi diseases have been a great threat to crop production compared to other plant parasites like bacteria, nematodes and viruses.[16] Significant scope exists for the exploration of huge plant biodiversity to develop new plant-based crop protectants. CNSL is well known due to its diverse applications. However, its potential as a fungicide in crop management has not been investigated despite reports on its insecticidal and antimicrobial potential without any adverse effect on the environment, plants and human health due to its biodegradability.[17,18-19] It will therefore be worthwhile to study the influence of CNSL in improving the quality of yam tubers by minimizing the effect of rots which result in the largest post-harvest losses in yams.

CNSL is a caustic, dark reddish-brown, viscous liquid constituting nearly 25% of the cashew and approximately 30-35% of the nutshell by weight. CNSL can be categorized as natural or technical based on the adopted extraction method. CNSL extracted at low temperature (< 50 ºC) with the aid of solvent or supercritical CO_2 is considered natural because its composition will not be modified extensively, and it is rich in anacardic acid, as most reported. However, the composition varies. There are other aromatic and aliphatic compounds present in natural CNSL. The technical CNSL is extracted at high temperatures (150-200 ºC). This will lead to the decarboxylation of anacardic acid with an increase in cardanol.[16] This phenolic compounds contain hydrophobic side chains (-$C_{15}H_{31-2n}$), which differ in the degree of unsaturation, approximately 48 - 49% monoene, 16 - 17% diene and 29 - 30% triene.[18-20] Hamad and Mubofu[7] reported the different components of CNSL based on the extraction method used. However, CNSL, whether cold solvent extracted or heat extracted, is known to contain anacardic acid, cardol, cardanol and other polymeric materials.[21] Similarly, the presence of azulene has been previously reported in CNSL.[22] It is important to note that azulene has diverse biological activities, including antimicrobial, antiviral and antifungal activities.[23]

Yam belongs to the genus *Dioscorea* in the family *Dioscoreacea*, and it is one of the most important staple foods in the world, especially in some parts of the tropics and subtropics.[24] Nigeria is known to be the largest producer of yam in the world. Annual yam production in the country is estimated at 38 million metric tonnes accounting for approximately 65% of the world's production.[12] Yam attracts pests that plague it both before and after harvest. A major factor limiting the post-harvest life of yam is rot, and the loss incurred is often huge and devastating. Losses due to post-harvest rot significantly affect the incomes of both farmers and traders, food security and seed yams stored for planting. The quality of yam tubers is negatively impacted by rots, making them less attractive to consumers.[25,26] Most rots of yam tubers are caused by pathogenic fungi such as *Aspergillus flavus*, *A. niger* (Tiegh), *Fusarium oxysporum, F. solani, Botryodiplodia theobromae, Penicilllium chrysogenum, Rhizoctonia spp, P. oxalicum, Trichoderma viride* and *Rhizopus nodosus.*[14,27] Microbial deterioration of yam starts in the soil, which reduces its capacity to germinate and its survival in the field and progresses in storage, infecting the tubers without sign of external symptoms. Physical damage to the tuber during harvesting and lesions from insect attack predisposes the yam tuber to this disease.[28]

The most recent global trend in controlling agricultural pests is green pesticides based on their moderate rate of efficiency.[29] Sources of green pesticides, which include Neem and pyrethrum, are rich in bioactive chemicals that provide potential alternatives to the conventional agents used by farmers for pest control. The long-term environmental hazard associated with using chemical agents to control plant pathogens has called for research to provide better alternatives that are more potent against pathogens but non-toxic and less harmful to the

DOI: http://dx.doi.org/10.32571/ijct.1278596 E-ISSN: 2602-277X

environment. Hence, this study was designed to identify the pathogenic fungi in rotting yam (*Discorea rotundata*) samples and investigate the antifungal potential of CNSL against the fungi species identified in anticipation of establishing a readily available alternative to the conventional agents.

2. EXPERIMENTAL

2.1. Materials

All reagents used in this study were of analytical grade, including n-hexane, dimethyl sulphoxide, sulphuric acid, hydrochloric acid, ferric chloride, picric acid, and acetic anhydride procured from Sigma-Aldrich. Deionized water was used to prepare and dilute solutions throughout the experiment. Fifteen kilograms (15.0 kg) of cashew nuts samples were collected from the Cocoa Research Institute of Nigeria, Ibadan, Oyo State, South-Western Nigeria. Samples of infected white yam (*Dioscorea cayennensis* subsp. *rotundata*) were randomly purchased from the Oja-Oba market in Ibadan, Oyo State, South-Western Nigeria.

2.2. Methods

2.2.1. Extraction of CNSL

The nuts were washed with a detergent solution and rinsed with deionized water to remove extraneous materials. This was followed by drying under ambient conditions. The dried nuts were shelled manually with the aid of a knife cutter. Finally, the dried shells were properly crushed to small sizes with the aid of a mortar and pestle to increase the surface area for easy extraction of the CNSL. The extraction of CNSL was carried out using a Soxhlet extractor and n-hexane as solvent (10 mL^{-1} g). The hexane extract was concentrated by evaporation under reduced pressure to yield a dark viscous liquid (CNSL). [30]

The percentage yields of CNSL were obtained using equation[29,31];

$$Percentage\ Yield = \frac{Mass\ of\ Oil\ Extract}{Mass\ of\ Sample} x\ 100 \qquad (1)$$

2.2.2. GC-MS Analysis of CNSL

Qualitative and quantitative analyses of the constituent of the CNSL were conducted using Gas Chromatography-Mass Spectrometry (GCMS-QP2010SE SHIMADZU, JAPAN) and GC-FID (5890-11 SHIMADZU, JAPAN) at Shimadzu Training Centre, Lagos. An HP-5 MS GC Column (30 m x 0.25 u x 0.25 mm id) was used. The conditions adopted were: temperature programming from 70-270 ºC held at 70 ºC for 2 min, and at 170 ºC for 2 min (ramped at 10 ºC/min), at 220 ºC for 2 min (ramped at 10 ºC/min) and finally at 270 ºC for another 2 min (ramped at 10 ºC min^{-1}). The injection temperature was 220 ºC. Helium was used as a mobile phase with a flow rate of 15 mL min^{-1}. The identification of the compounds based on their corresponding mass spectra was done by comparison with data of compounds available on NIST 1. MS data Library on the GC-MS.

2.2.3. Phytochemical Analysis

In the establishment of the presence of phytochemicals of interest, qualitative tests were conducted using established standard methods[32,33] to identify the constituents.

2.2.4. Isolation and Characterization of Fungal Pathogens

Isolation of fungi associated with dry rot disease of yam was done using standard microbiological techniques. The yams were peeled to expose the rot tissues, cut, minced and surface-sterilized with 70% ethanol for 1 min and 5% (v v^{-1}) sodium hypochlorite solution for 3 min. The pieces were rinsed twice with sterile distilled water and air-dried. The cut surfaces of the yam samples were directly inoculated on acidified potato dextrose agar (PDA) plates, and the plates were incubated at 28±2 ºC for 7 days.[34] The fungal isolates were subcultured on the PDA plates, and the pure cultures were maintained on the PDA slants at 4 ºC. The fungal isolates were identified based on their morphological and microscopic characteristic.

The method described by Khadijat[35] was adopted for the pathogenicity test with few modifications. Briefly described, ten healthy yam tubers were washed with clean distilled water for 10 minutes, surface sterilized with 10 %(v v^{-1}) sodium hypochlorite solution, rinsed in three changes of sterile distilled water and air-dried. The tuber lengths were measured and divided into three segments for fungal inoculation. A sterile 5 mm cork borer created a hole 3 mm deep. A 5 mm agar disc of the mycelia mat of each fungal isolate was inoculated on each cut hole. The holes were covered by replacing the tuber peel and sealing with masking tape, while the control tubers were inoculated with sterile distilled water. The tubers were incubated at room temperature (28±2 ºC) for nine days. Then, tubers were transversely cut in each inoculated segment, observed, recorded and re-isolated to obtain the pure cultures of the inoculated fungi.[35]

2.2.5. In-vitro Antifungal Activities of CNSL on Phytopathogenic Fungal Isolates

Oxidation was carried out on the extracted 2.5 g of CNSL by dissolving it in 7.5 % aqueous sulphuric acid solution (100 ml), and 2.5 g of potassium dichromate was added. The mixture was stirred at 29 ºC for 24 h. Dimethyl sulphoxide (DMSO) solvent was dissolved in the same mixture to obtain four serial dilutions of 25, 50, 75 and 100 % concentrations of CNSL. The resultant dilutions were used to evaluate the antifungal activity of CNSL using the food poisoning technique on PDA.[35] 1.0 ml of 25, 50, 75 and 100 % concentrations of CNSL was separately pipetted into a petri dish, and

DOI: http://dx.doi.org/10.32571/ijct.1278596 **E-ISSN: 2602-277X**

15 ml of sterilized molten PDA was poured and swirled to homogenize. The mixture was allowed to solidify, and a 5 mm agar disc of 7-day old culture mycelia mat of each pathogen was inoculated at the centre of the plate. The plates were incubated at (28±2 ºC) for 48 hours, and the mycelia extension of the pathogen was measured in millimetres (mm) as the zone of inhibition. This experiment was performed in duplicates along with mancozeb 80% WP as standard, DMSO as the negative control and antifungal agents as the positive control. The percentage inhibition was calculated by the formula[36];

$$Percentage\ Inhibition = \frac{Growth\ in\ control - Growth\ in\ test \ \ x \ \ 100}{Growth\ in\ control} \quad (2)$$

2.2.6. Statistical Analysis

Data collected were subjected to analysis of variance (ANOVA) using Statistical Analysis Software (SAS, 2002). Significant means were separated with Duncan's Multiple Range Test (DMRT) at $p \leq 0.05$.

3. RESULTS and DISCUSSION

The extraction of CNSL from cashew nutshell using n-hexane as solvent gave a yield of 30.23%, higher than the 25.85% reported by Idah and co workers[37] and within the 30-35% established in the literature.[10,38,39] The CNSL was dark brown with a choking odour. These physical features are characteristics of CNSL, as previously reported.[15,18,37] The high yield of CNSL indicated sustainable availability if considered suitable as a potential antifungal agent against yam rots.[40]

3 .1. GC-MS Analysis of CNSL

Major constituents are shown in bold based on the Kovalt retention indexes in the HP-5MS column. From the results presented in **Table 1**, 18 constituents were identified in the extracted CNSL, representing 83.02 % of the total composition. The chromatogram of the CNSL is shown in the supplementary information. This analysis revealed the presence of phenolic compounds, including derivatives of anacardic acid, anacardol and cresol, with varying percentage compositions.[41] Among other components, azulene predominated (~57%). As established in the literature, the chemical composition of CNSL depends on the extraction method.[42] The chemical constituents of the extracted CNSL reflected the method adopted for the extraction. The presence of terpenes and aromatics, among other bioactive compounds, could potentially synergize to provide the anticipated antifungal against yam rots.

.

Table 1. Chemical composition of CNSL.

No	Compounds	Retention Time	Retention Index	Composition (%
1	Ethanone,1-Cyclopropyl-2-(4-pyridinyl)-	13.725	1323	0.50
2	Cyclopropene, 3-Methyl-3-vinyl-	15.014	566	0.91
3	2-Methylphenyl-N-methylcarbamate (benzylamine derivatives of anacardic acid)	15.133	1372	2.08
4	16-Heptadecenal	16.400	1890	1.11
5	Phthalic acid, Cyclobutyl tridecyl ester	16.609	2954	1.55
6	Cyclohexane ethanol	16.723	1123	2.07
7	3-Hexanone, 2,2-dimethyl-	16.840	868	0.74
8	Pentanoic acid, 2-methyl-	17.018		0.89
9	Butane, 2,2-dimethyl-	17.517		0.92
10	M-cresol acetate	18.123	1430	1.49
11	Decane, 1-Iodo	18.158		1.57
12	Sulfurous acid, 2-ethylhexyl hexyl ester	18.768	1972	1.36
13	P-cresol	19.264	1014	1.26
14	Phenol, 3-pentadecyl- (Anacardol)	19.440		3.37
15	Oxalic acid, Dodecyl-2-methylphenyl ester	19.925	2534	2.58
16	Azulene,1,2,3,5,6,7,8,8a-octahydro-1,4-dim	21.628	1490	57.65
17	Apha-bulnesene	21.669	1490	3.22
18	Rotundene	21.835	1450	1.36
	TOTAL			84.63
1	Sesquiterpenes			57.65
2	Aromatic Compounds			9.49
3	Monoterpenes			6.18
4	Aliphatic esters			2.60
5	Alkanols			2.07
6	Aliphatic hydrocarbons			1.83
7	Aliphatic ketones			1.24
8	Alkyl halides			1.57
9	Aliphatic aldehydes			1.11
10	Aliphatic acid			0.89

***** Compounds arranged according to elution from an HP-5 column.

DOI: http://dx.doi.org/10.32571/ijct.1278596 E-ISSN: 2602-277X

3.2. Phytochemical Screening

The Phytochemical constituents of the CNSL are presented in Table 2. This revealed the presence of flavonoids, alkaloids, saponins, tannins, terpenoids, phenols, volatile oils and steroids, while glycosides and reducing sugar were absent in the extract. As established by phytochemical screening, the constituents corresponded to the compounds identified in the extract *via* GC-MS. The presence of these phytochemicals in CNSL could contribute to its antifungal activities. Researchers have reported similar observations where such phytochemical contents and antimicrobial activity of cashew nut shell oil confirmed its activity as an antifungal agent.[43,44]

Table 2. Phytochemical constituents of CNSL.

Phytochemical	Results
Flavonoids	+
Alkaloids	+
Saponins	+
Tannins	+
Terpenoids	+
Glycosides	-
Volatile oils	+
Steroids	+
Reducing sugar	-
Phenol	+

* +/-: present/absent

3.3. Pathogenicity Test

Four fungal isolates were observed to induce visible rot symptoms on the healthy yam tubers, implying that these isolates are phytopathogens. These phytopathogenic fungi were re-isolated from infected yam tubers, and based on their colonial and microscopic characteristics, they were identified as *F. solani, A. fumigatus, A. niger and Lasiodiplodia theobromae*. The appearances of the phytopathogenic fungal isolates on potato dextrose agar (PDA) plates are shown in Figure 1, while Table 3 shows colonial and microscopic characteristics of the phytopathogenic fungal isolates. Some of the identified fungi are potentially harmful to the crop, animals, and man if consumed. *F. solani* is usually found in the soil and manifests as a causal agent of crop diseases, including root and fruit rots. *A. fumigatus* uses the soil and decaying organic matter as natural habitats and can be potentially harmful to the individual with a compromised immune system. Similarly, *Aspergillus niger*, which causes black mold, is a common food contaminant capable of secreting harmful mycotoxins to man and animals. *L. theobromae* usually attacks a wide range of post-harvest farm products, causing rot. The results obtained closely agreed with the earlier results reported by Okigbo and Emeka[45] on the biological control of rot-inducing fungi of water yam (*Dioscorea alata*) with *Trichoderma harzianum*, *Pseudomonas syringae*, and *P. chlororaphis*; Ajayi and Olorundare[46] on bacteria and fungi species associated with yam (*Dioscorea rotundata*) rots and Ray and co workers[47] on microorganisms associated with post-harvest spoilage of yams.

Table 3. Morphological characteristics of fungal isolates.

No	Colony Morphology	Microscopic Observation	Identities
1	Fluffy white colour	Colourless white creamy with dark brown zonation pigment	Fusarium solani
2	Greenish-ash colour	Pigmentless green echinate conidial with green phialides	*Aspergillus fumigatus*
3	Fluffy dark grey colour	Aseptate paraphyses, pycnidia. Reverse greyish blue	*Lasiodiplodia theobromae*
4	Black loose colour	Conidia are globose to subglobose and tend to split to lose columns with age	*Aspergillus niger*

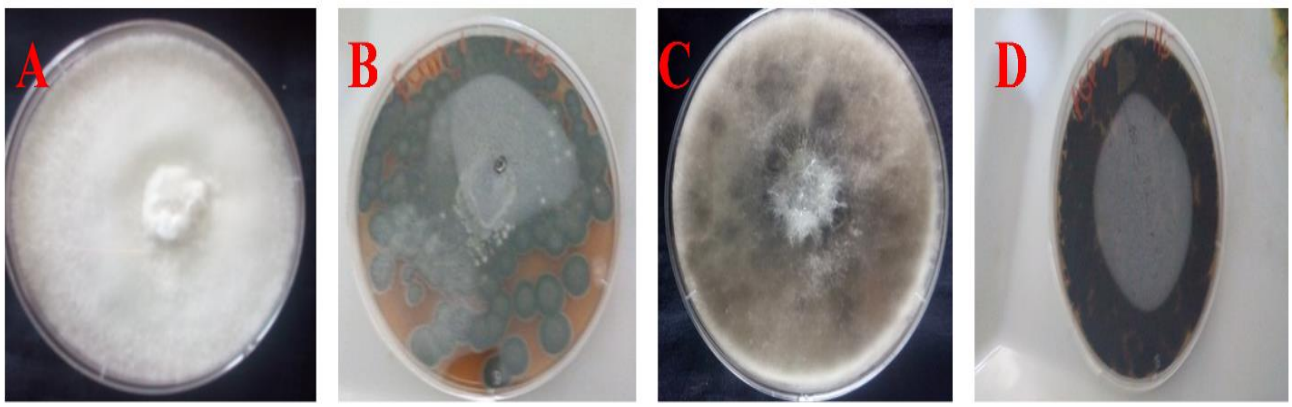

Figure 1. Appearances of phytopathogenic fungal isolates on PDA plates: (A) *Fusarium solani*, (B) *Aspergillus fumigatus*, (C) *Lasiodiplodia theobromae* and (D) *Aspergillus niger*.

DOI: http://dx.doi.org/10.32571/ijct.1278596 **E-ISSN: 2602-277X**

3.4. Antifungal Activity

The results of *in vitro* antifungal activity conducted on the isolated fungi using different concentrations of CNSL extract revealed its antifungal potential by inhibiting the growth of *A. fumigatus*, *F. solani*, *L. theobroma and A.* niger at a varied degree of inhibition. The mean mycelia diameter of fungal isolates using CNSL against fungal growth at different concentrations are given in Table 4. Based on the mycelia diameters, it was observed that out of four fungal isolates evaluated, two fungi (*L. theobromae* and *F. solani*) were significantly inhibited by CNSL at 50, 75 and 100% concentration when compared with the standard. *A. fumigatus* had no significant difference at 50, 75 and 100% concentration of CNSL but was significantly different from the control. *A. niger* at 25 and 75% concentration of CNSL showed no difference from the standard. While at 25% concentration of CNSL, all fungi isolates showed significant growth of mycelia diameter when compared to the standard. Among the different concentrations, 100% CNSL with the least growth of mycelia diameter had the highest zone of inhibition compared to the standard. At 50, 75 and 100% concentrations of CNSL, the results obtained for *L. theobromae* and *F. solani* showed no significant difference to the standard but were significantly different from the control. For *A. niger*, there was no significant difference between the concentrations at 50% of CNSL with the standard, but 75 and 100% concentrations were significantly different from the standard. While for *A. fumigatus*, there was no significant difference between 25 and 50% concentrations and between 75 and 100% concentrations, but they were significantly different from the standard and more so from the control. At 100% concentration of CNSL, the mycelia growths of all tested fungi were inhibited at a varying degree in comparison to the standard. The results of the study clearly showed that the CNSL extract was effective with an increase in concentration.

The level of inhibition observed in *A. fumigatus* and *A. niger* in this study was also reported by Salgueiro and co workers[48] in their study on the chemical composition and antifungal activity of the essential oil, and Pinto and co workers[49] made a similar observation on antifungal activity of the essential oil of thymus pulegioides on *candida*, *Aspergillus* and dermatophyte species. The antifungal activity of CNSL extract observed in this study could be attributed to the presence of phenolic compounds and azulene, which constituted the major chemical component, as revealed by the GC-MS result. Azulene had been reported to have possessed the antibacterial ability and had been used for skin care treatment which explained the fungicidal ability in CNSL. This activity could also be attributed to the presence of oxygenated mono and sesquiterpene hydrocarbons, which is in agreement with the previous work reported.[50]

Table 4. Mean Mycelia diameter of fungi isolates at different concentrations of CNSL.

	Mean mycelia Diameter (mm)						
Organisms	25%	50%	75%	100%	DMSO	CTRL	STD
A. fumigates	28.00^{b}	28.50^{b}	21.00^{c}	17.50^{c}	31.00^{b}	47.50^{a}	12.00^{d}
L.theobromae	20.75^{c}	6.50^{d}	7.00^{d}	6.25^{d}	80.00^{b}	85.25^{a}	7.00^{d}
F. solani	12.50^{c}	6.50^{d}	6.25^{d}	6.50^{d}	16.50^{b}	84.00^{a}	6.00^{d}
A. niger	73.25^{b}	61.75^{cd}	65.50^{c}	61.00^{d}	71.00^{b}	80.50^{a}	62.25^{cd}

* DMSO- Dimethylsulphoxide, CTRL- control, STD-Standard fungicide used; a, b, c, d, cd, significant differences (figures with same superscript are not different)

The percentage zone of inhibition of CNSL against fungal growth at different concentrations is summarized in Table 5. Considering the percentage zone of inhibition at different concentrations of CNSL against each fungal isolate, CNSL showed a relatively similar trend with the standard on inhibition of *L. theobromae* and *F. solani*.[40]

However, CNSL showed slightly lower activity in comparison with the standard against *A. fumigatus*, but a higher performance was noted for CNSL against *A. niger*. The result revealed the suitability of the extracted CNSL as a fungicide, and this could be associated with the synergic interaction between the chemical constituents of CNSL in the inhibition of the identified fungal isolates tested. Representative pictorial illustrations of the inhibition activities of CNSL on the phytopathogens are presented in Figure 2. A related study by Buxton and co workers[15] extracted CNSL using different solvents (ethanol, ethyl acetate and acetone) and tested it against five human pathogenic fungi. The result revealed that CNSL was active against *A. niger*, *A. flavus*, *A. fumigatus* and *Fusarium sp.* but showed no resistance against *Curvalaria sp.* The observed inhibition was attributed to phytochemicals, including flavonoids, triterpenoids, phenolic compounds and volatile oils in the extracted CNSL. This result supported our findings that CNSL showed activities against phytopathogenic fungi associated with rots in yam.

DOI: http://dx.doi.org/10.32571/ijct.1278596 E-ISSN: 2602-277X

Table 5. Percentage zone of inhibition of antifungal activity at different concentrations of CNSL.

	Percentage zone of inhibition (%)					
Organisms	25%	50%	75%	100%	DMSO	STD
A. fumigates	41.05	40.00	55.79	63.16	34.74	74.4
L. theobromae	75.66	92.38	91.79	92.67	6.16	92.96
F. solani	85.12	92.26	92.56	92.26	80.36	92.86
A. niger	9.01	23.29	18.63	24.22	11.80	22.67

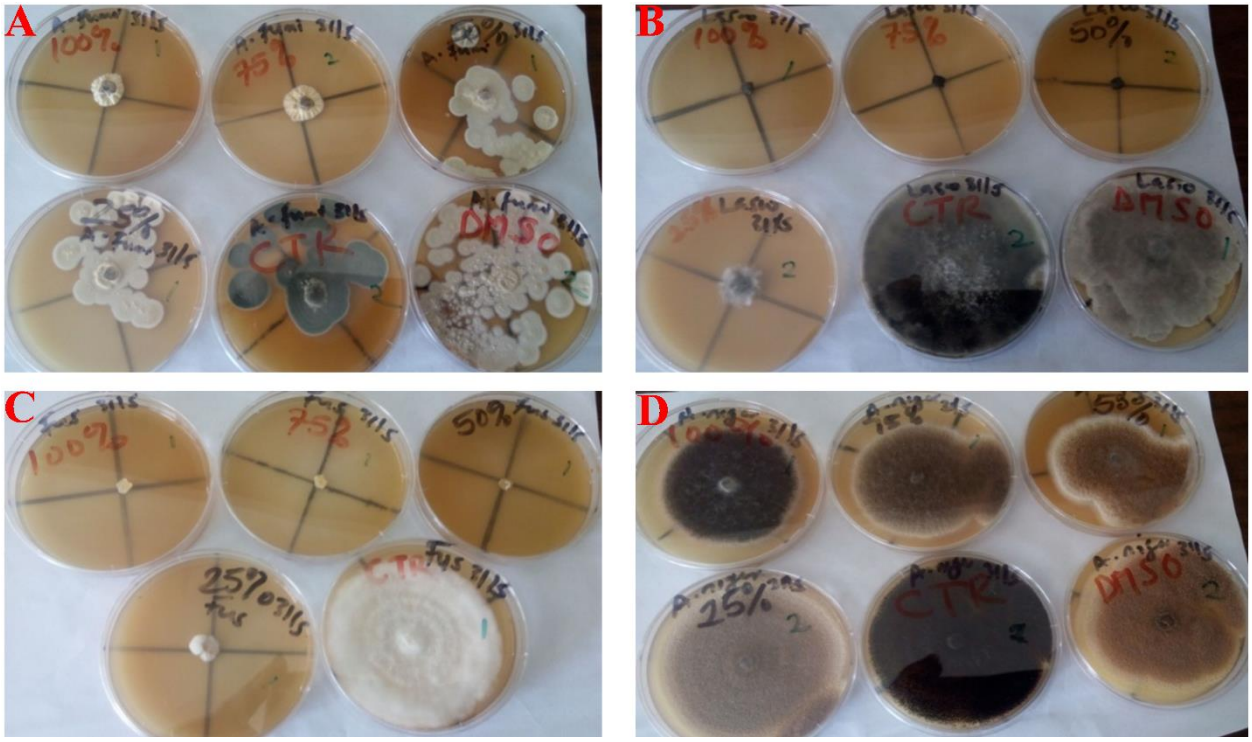

Figure 2. Representative images showing the inhibitions at different concentrations of CNSL against (A) A. fumigatus, (B) L. theobromae, (C) F. Solani and (D) A. niger.

4.CONCLUSION

This study investigated the antifungal activities of CNSL against causal agents of yam rots. The soxhlet extraction process adopted gave a percentage yield of 30.23 % w/w. The CNSL extract showed significant inhibition against four fungi isolated and identified from the yam rot samples used in this study. The chemical and phytochemical screening of the extracted CNSL revealed azulene, phenolic compounds, oxalic acid, pentanoic and phthalic acid, flavonoids, alkaloids, saponins, phenols, among others, as the chemical constituents responsible for the antifungal activities. The percent zone of inhibitions for all the isolates at 25, 50, 75 and 100 % concentrations ranged between 9.01 and 92.6%. This study showed that CNSL could completely serve as a fungicide compared to conventional standard fungicides.

Conflict of Interest

Authors declare that there is no a conflict of interest with any person, institute, company, etc.

REFERENCES

1. Da Silveira, N.F.; Gomes-Rochette, M.L.M.; de Oliveira, D.C.S.; Nunes-Pinheiro, A.R.; Tomé, F.Y.; Maia de Sousa, D.F.; de Melo, N.O. *Exp. Biol. Med.* **2015**, 240, 12, 1648-1655.

2. Sahin, C.; Magomedova, L.; Ferreira, T.A.; Liu, J.; Tiefenbach, J.; Alves, P.S.; Queiroz, F.G.; de Oliveira, A.S.; Bhattacharyya, M.; Grouleff, J.; Nogueira, P.C.; Silveira, E. R.; Moreira, D.C.; de Almeida Leite, J. S.; Brand, G. D.; Uehling, D.; Poda, G.; Krause, H.; Cummins, C.L.; Luiz A. S., *J. Med. Chem.* **2022**, 65, 1961–1978.

3. Rwahwire, S.; Tomkova, B.; Periyasamy, A.P.; Kale, B.M. *Elsevier*. **2019**, 7, 61-80.

4. Lomonaco, G.; Mele, S.E.; Mazzetto, M.T. *Springer, Cham*. **2017**, 11, 19-38.

DOI: http://dx.doi.org/10.32571/ijct.1278596 **E-ISSN: 2602-277X**

5. Leite, Ad.S.; Dantas, A.F.; Oliveira, G.S.; Gomes, Junior A.L.; de Lima, S.G.; Citó, G.L.; Freitas, R. M.; Amélia, de C.A.; Melo-Cavalcante, A.M.; José Arimateia, J.A.; Lopes, J.A.; *Biomed Res. Int.* **2015**, 10, 20-39.

6. Ike, D.C.; Ibezim-Ezeani, M.U. *Green Chem. Lett. Rev*. **2021**, 14, 620-633.

7. Hamad, F.B.; Mubofu, E.B. *Int. J. Mol. Sci*. **2015**, 16, 8569-8590.

8. Kumar, S.; Rosen, M.A. *Energy & Fuels*. **2018**, 32,7237-7244.

9. Morais, S.M.; Silva, K.A.; Araujo, H.; Vieira, I.G.; Alves, D.R.; Fontenelle, R.O.; Silva, A.M., *Pharm*. **2017**, 10, 31-39.

10. Taiwo, E.A. *Adv. in Petchem*. **2015**, 10,1-13.

11. Parasa, L.S.; Tumati, S.; Kumar, C.A., *Int. J. Pharm. Sci*. **2011**, 3, 436-40.

12. Kannan, V.R.; Sumathi, C.; Balasubramanian, V.; Ramesh, *N. Bot. Res. Int*. **2009**, 2, 253-257.

13. Verter, N.; Bečvářová, V.; Acta Univ. Agric. Silvic. Mendel. Brun. **2015**, 63, 659-665.

14. Raphael, O.N.; Ebere, E.C.; Kingsley, A.C.; Chidiebere, I.R.; Chidi, O.B.; Nsikak, A.S.; Anaukwu, C. G.; Okafor, I. O.; Ezenwa, C. U.; Iloanusi, A. C. *Adv. Appl. Sci. Res*. **2015**, 6, 7-13.

15. Buxton, T.; Takahashi, S.; Niwata, I.; Owusu, E.O.; Kim, C.S. *J. Entomol. Zool. Stud*. **2017**, 5, 1241-1246.

16. Sanyaolu, N.; Agboyinu, E.; Yussuf, S.; Sonde, O.; Avoseh, O.; Ibikunle, A., *IJS*. **2010**, 21, 129-37.

17. Dos Santos, T.A.; Araújo, B.Q.; Citó, G.L.; da Silva, J.; Saffi, J.; Richter, M.F.; Ferarz, B. F. *Food Chem*. **2011**, 126, 1044-1048.

18. Raja, K., *JBiopest*. **2015**, 8, 147-152.

19. Mahapatro, G. *Indian J. Entomol*. **2011**, 73, 121-124.

20. Mazzetto, S.E.; Lomonaco, D.; Mele, G. Quim. Nova. **2009**, 32, 732-741.

21. Ileke, K.; Olotuah, O., *Int. J. Biol*. **2012**, 4, 96-105.

22. Patela, R.; Bandyopadhyayb, S.; Ganesh, A., *CHEMCON*. **2005**, 20-35.

23. Bakun, P.; Czarczynska-Goslinska, B.; Goslinski, T.; Lijewski, S., *Med. Chem. Res*. **2021**, 30, 834 46.

24. Okigbo, R.; Ogbonnaya, U. *Afr. J. Biotechnol*. **2006**, 5, 14-28.

25. Agu, K.; Awah, N.; Nnadozie, A.; Okeke, B.; Orji, M.; Iloanusi, C.; Anaukwu, C.; Eneite, H.; Ifediegwu, M.; Umeoduagu, N; Udoh, E. *Food Sci. & Technol*. **2016**, 4, 103-106.

26. DN, A.; Umeh, O.; Onyenobi, F. Food Chem. **2016**, 15-36.

27. Frank, C.O.; Kingsley, C.A., *Br. Microbiol. Res*. J. **2014**, 4, 343-350.

28. Bassey, E.E., *Eur. J. Phy. Agric. Sci*. **2017**, 5, 14-25.

29. Pretty, J.; Bharucha, Z.P., *Insects*. **2015**, 6, 152-182.

30. Deepanraj, B.; Senthilkumar, N.; Mala, D.; Sathiamourthy, A. *Biomass Convers Bior*. **2021**, 5, 23-47.

31. Ikya, J.K.; Umenger, L.N.; Iorbee, A. J. *Food Resour. Sci*. **2013**, 2, 1-12.

32. Evans, W. Trease and Evans Pharma., 15th edition, Edinburgh, Saunders. **2002**, 249-275.

33. Talukdar, A.D.; Choudhury, M.D.; Chakraborty, M.; Dutta, B. Assam Uni. *J. of Sci. and Tech.* **2010**, 5, 70-74.

34. Pitt, J.; Hocking, Samson, R.A. & King, A. D.; *Elsevier*. **1992**; pp 112-134.

35. Pritha, D.S.; Karpagam, J.F. IOSR *J Pharm Biol Sci*. **2017**, 12, 61-64.

36. Rajani, P.; Rajasekaran, C.; Vasanthakumari, M.M.; Shannon B. O.; Ravikanth, G.; Uma Shaanker, R. *Microbiol. Res*. **2021**, 242, 126-195.

37. Idah, P.; Simeon, M.I.; Mohammed, M., *Food Chem*. **2014**, 5, 20-44.

38. Kyei, S. K., Akaranta, O., Darko, G., & Chukwu, U. J., Chem. Sci. Int. J. **2019**, 28(3), 1-10.

39. Zombe, K.; Nyirenda, J.; Lumai, A.; Phiri, H. Sustain. *Chem*. **2022**, 3, 334-344.

40. Souza, N. O.; Cunha, D. A.; Rodrigues, N. S.; Pereira, A. L.; Medeiros, E. J.; Pinheiro, A. A.; de Vasconcelos, M.A.; Neto, L. G.; Bezerra, T. T.; Mazzetto, S.E.; Lomonaco, D.; Teixeira, E. H.; de Paulo A. S. *Arch. of Oral Bio*. **2022**, 133, 105299.

DOI: http://dx.doi.org/10.32571/ijct.1278596 **E-ISSN: 2602-277X**

41. Makwana, K.; Ichake, A.B.; Valodkar, V.; Padmanaban, G.; Badiger, M. V.; Wadganaonkar, P. P. *Eur. Polym. J.* **2022**, 166, 111029.

42. Liu, Z.; Liu, H.; Wang, Y.; Yu, H.; Wang, J. Polym. *Eng. Sci*. **2022**, 6,18-32.

43. Santos, F.O.; Angélico, E.C.; da Costa, J.G.M.; Rodrigues, F.F.; Rodrigues, O.G.; de Medeiros, R.S. *Afr. J. Biotechnol*. **2013**, 12, 112-124.

44. Dorathy, S.; Jebapritha, S.; Karpagam, I. Journal of *Pharm. and Bio*. Sciences. **2017**, 12, 61-64.

45. Okigbo, R.; Emeka, A. J. Stored Prod. *Postharvest Res*. **2010**, 1, 18-23.

46. Ajayi, A.O.; Olorundare, S.D. *Appl. Sci. Res*. **2014**, 2, 12-28.

47. Ray, R.; Nedunzhiyan, M.; Balagopalan, C. Ann. *Trop. Pathol*. **2000**, 22, 31-40.

48. Salgueiro, L.; Pinto, E.; Goncalves, M.; Pina-Vaz, C.; Cavaleiro, C.; Rodrigues A.; Palmeira, A.; Tavares, C.; Costa-de-Oliveira, S.; Martinez-de-Oliveira, M. *J. Planta Medica*. **2004**, 70, 572-575.

49. Pinto, E.; Pina-Vaz, C.; Salgueiro, L.; Gonçalves, M.J.; Costa-de-Oliveira, S.; Cavaleiro, C.; Palmeira, A.; Rodrigues, A.; Oliveira, J.M. *J. Med. Microbiol*. **2006**, 55, 1367-1373.

50. Raja, K.; Sivasubramaniam, K.; Geetha, R. *Entomol. Gen.* **2015**, 34, 197-206.

51. Khadijat, A. Proceedings of the 27th Annual Conference of the Nigerian Institute of Food Science and Technology (NIFST), held in Kano 13th; 20-33, **2003**.