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Antifungal Efficacy of *Warburgia ugandensis* Extracts Against Pathogenic Fungi and their application in Maize Grain Preservation

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Abstract
Maize is a staple food crop in Kenya which is often affected by the growth of fungi. These fungi produce mycotoxins which are hazardous to both human and animal health. The common mycotoxins present in maize and maize products are aflatoxins, Fumonisins, ochratoxins and penicillins. To address this concern, several methods have been put in place to counter the growth of fungi, however, no single method have been found effective. There has been an increased interest on plant based antifungal agents over synthetic fungicides due to environmental and health impacts. This study assessed antifungal activity of *Warburgia ugandensis* water and methanol extracts against *Aspergillus flavus* and *Fusarium verticillioides,* phytochemical composition and application in maize grain preservation. The antifungal activity of *Warburgia ugandensis* extracts were assessed using disc diffusion assay and the minimum inhibitory concentration (MIC) of the extracts also determined. Effectiveness of these extracts in preserving maize grains was also evaluated by treating the inoculating maize grains with the extracts. Chemical composition of the extracts was also determined using gas chromatography-mass spectrometry (GC-MS). *Warburgia ugandensis* extracts exhibited potent antifungal activity against *both F. verticillioides* and *A. flavus*. Methanol extract had higher antifungal against *F. verticillioides* (ZOI=30mm) while water extracts had a higher antifungal activity against *A. flavus* (ZOI=18mm). The MIC values of both extracts were 125mg/ml. Treatment with plant extracts effectively preserved maize grains from fungal contamination, with methanol extract being more effective. The GC-MS revealed the presence of a variety of chemical compounds in the extracts. Gas Chromatography-Mass Spectrometry analysis revealed 9-t-Butyl-4-iodo-2,2-dimethyladamantane (45.01%) and Cycloisolongifolene, 8,9-dehydro-9-formyl-(7.60%), while Ledene oxide-(II) (31.19%) as the most dominant compounds in water extracts. Notably, 9-t-Butyl-4-iodo-2,2-dimethyladamantane dominated the water extract, while while Ledene oxide-(II) (31.19%), Pregn-4-ene-1,20-dione, 12-hydroxy-16,17-d (21.51%) were dominating compounds in methanol extract. The most dominant compounds have been reported to possess antifungal activities hence underscoring the potential of *Warburgia ugandensis* extracts as natural antifungal bio preservatives therefore contributing to food safety and security.

Keywords: *Warburgia ugandensis,* antifungal activity, maize grain preservation, natural antifungal agents

1. Introduction

Maize (*Zea mays*), also known as corn, is the staple food and most commonly grown cereal in Kenya, accounting for nearly 80% of all cereal production produced from over 3 million hectares of land (Kang'e the *et al*., 2020). It is mostly used to produce hard maize flour porridge (ugali), providing over 50% of daily calorie intake in Kenya (Wanjiru *et al*., 2023). The major food safety issue in maize is the presence of pathogenic fungi, including *Aspergillus flavus, Aspergillus parasiticus, Aspergillus ochraceous, Fusarium verticillioides*, and *Fusarium proliferatum*. These molds thrive in warm and moist environments and secrete mycotoxins such as Aflatoxins and fumonisins, which have adverse health effects (Lewis *et al*., 2005) [9]. Generally, significant factors that affect fungal growth and toxin production include temperature, relative humidity, water activity, P^{H}, and fungal strain (Moghaddam & Farhadi, 2015) [14]. Fungal species belonging to the genera *Aspergillus flavus* and *Aspergillus parasiticus* are the most common strains secreting aflatoxins, while *Fusarium verticillioides* and *Fusarium proliferatum* produce fumonisins (Pierron *et al*., Tournas & Niazi). These toxins pose severe risks to human and animal health hence of major concern when it comes to food safety (H. Gourama & L. B. Bullerman, 1995) [5]. Their toxicity can cause either short- or long-term illness, or even death (Oliveira *et al*., 2014) [24].

Efforts to prevent the growth of pathogenic fungi and reduce mycotoxin contamination have been ongoing, yet no single method has proven effective (Maina *et al.*, 2016; A. W. Njoroge *et al*., 2019) [10, 16]. Postharvest strategies such as proper drying, sorting, and the implementation of food safety management systems like Hazard Analysis and Critical Control Point (HACCP) are commonly employed to manage mycotoxins (Jnr *et al*., A. W. Njoroge *et al*., 2019) [16]. Synthetic antifungal chemical preservatives are also widely used due to their perceived effectiveness, particularly among small and large-scale producers (Silva & Lidon, 2016) [26]. However, these chemical preservatives pose a significant risk to the environment, humans and animals health (i.e. lung cancer, allergy, headache, nausea, diarrhea etc.) (Kamala *et al*., 2019) [7]. Due to this, there has been an increasing interest in plant-based antifungal agents as potential alternative. However, there is lack of adequate information, and scientific findings regarding the efficacy of plant-based extracts in managing fungi and mycotoxin contamination (Kumari *et al*., 2019) [7].

Among the 1,562 medications approved by the FDA for the period between 1981-2014, 4% were purely natural goods, 9.1% were mixtures of different botanical drugs, 21% were derivatives of natural products, and 4% were synthetic drugs based on natural products (Ochola *et al*., 2015) [18]. This data underscores the importance of evaluating herbal extracts for their efficacy against mycotoxin-producing microorganisms. *Warburgia ugandensis* for instance commonly known as "Ugandan greenheart" or "pepper bark tree", is a member of the Conellaceae family and belonging to the genus *Warbugia* has broad spectrum of antibacterial and antifungal properties. Due to this, *Warburgia ugandensis* was used by herbalists for managing various ailments and disorders (Maobe *et al*., 2013; Njoroge *et al.*, 2010; Okello *et al.*, 2018; Rugutt *et al.*, 2006) [12, 17, 20, 23]. This study further sort to determine the efficacy of *Warburgia ugandensis* in inhibiting the growth of *Aspergillus flavus* and *Fusarium verticillioides*, determine the phytochemical composition and the potential application of in maize grain preservation against fungal contamination thereby enhancing food safety.

2. Materials and Methods

2.1 Study site

This research was conducted at the Food science Microbiology Laboratory department of Dairy and Food Science and Technology, Egerton University, with assistance from the Soils and Biotechnology Laboratory at Egerton University and the Biochemistry Laboratory at Jomo Kenyatta University of Science and Technology, Kenya.

2.2 Plant identification and sample collection

Samples of *Warburgia ugandensis* stem bark were collected from Nyeri County, Kenya, with the assistance of herbal medicine practitioner Dr. Jack Githae. The collected samples were stored in a cool box and transported to the Food Microbiology Laboratory at Egerton University, Kenya. Afterward, the samples were dried under shade for three days and then further dried in an oven at 40 °C until a moisture content of 14% was attained.

2.3 Extraction of crude methanol and aqueous extracts

Methanol extraction: The crude methanol extracts were obtained according to Adongo *et al*. (2012) [2]. Briefly, the dried and ground samples (50 g) of *Warburgia ugandensis* stem bark were mixed with 86% methanol in a ratio of 1:3 (m/v) in an Emerylene flask and allowed to stand for 72 hours with frequent shaking. The mixture was then filtered using a muslin cloth then with a filter paper (Whatman No.1) to remove coarse and fine particles, respectively. The filtrate was concentrated using a rotary evaporator (model ZJ-TFG-18, China) and dried to completion.

Water Extraction: Finely ground dried samples (50g) were mixed with boiling distilled water in a ratio of 1:3 and incubated for 72 hours. After incubation, the mixture was filtered to remove coarse particles, and the filtrate was centrifuged at 10,000rpm for five minutes. The supernatants were collected and dried under a laminar flow vacuum dryer (LDZH-100KBS) until a constant weight was achieved. The final aqueous crude extracts were standardized and transferred into well-labeled falcon tubes and stored under a refrigerator waiting further testing.

2.4 Determination of antifungal potency of *Warburgia ugandensis* water and methanol extracts

The antifungal potency of the crude methanol and water extracts of *Warburgia ugandensis* was assessed using the disk diffusion method (Belmekki *et al*., (2013) [3]. The dried extracts were redissolved in their respective solvents to obtain a high concentration of 1000 µg/mL. Pathogenic fungal strains of *A. flavus* and *F. verticillioides* (1 mL containing 10^6 CFU/mL), were spread on potato dextrose agar (PDA) in Petri dishes. Disks impregnated with the crude extracts under concentrations range (1000-125mg/ml) were placed on the surface of the inoculated agar. Negative control treatment was conducted using methanol, while Natamycin served as a positive reference standard. The plates were incubated at 33 °C and 24 °C for *A. flavus* and *F. verticillioides*, respectively, for seven days. The zone of inhibition was measured to determine antifungal potency and minimum inhibitory concentration of crude extract in inhibiting fungal growth. The experiments were conducted in triplicates and zones of inhibition were measured in millimeters.

2.5 Application in maize grain preservation

To determine the effectiveness of herbal extracts in preserving maize grain, *Warburgia ugandensis* methanol and water extracts at 125 µg/mL which was the established minimum inhibitory concentration were applied to the maize grain samples according to Abdelazm *et al*. The surface of the maize kernels was sterilized for one minute with a 5% sodium hypochlorite (NaOCl) and rinsed with sterile water. Twenty-five grams (25 g) of maize grains were sprayed with 2 mL of water and methanol extract separately, while one plate was left untreated to act as controls. One milliliter (1 mL) of a spore suspension of *Aspergillus flavus* and *Fusarium verticillioides* (1×10^8 spores/mL) was then added to the maize grains as an inoculant. The growth of *A. flavus* and *F. verticillioides* on the maize kernels was visually assessed after incubation at 33 °C and 24 °C, respectively, for seven days. Three replicates were conducted for each treatment.

2.6 Determination of phytochemical composition

The crude extracts were filtered and analyzed using SHIMADZU's GC-MS QP2010SE equipped with a BPX5 column (length (30m), thickness (0.25µm) and internal diameter (0.25mm)). The samples were injected in a split mode at a temperature of 200 °C, column oven temperature set at 60 °C and temperature program used to bring about the separation of compounds starting from 60 °C, and then ramped at 10^0C/minute held for 8 minutes. The interface temperature was 250 °C, the ion source temperature was set at 200 °C and the solvent cut times were 4.5. The mass spectrometer ran in scan mode for masses from 35Hz to 550Hz. The different identified compounds were characterized by matching their mass spectra with those of reference compounds recorded in the NIST 2014 mass spectral library.

2.7 Data analysis

The data obtained were tested for normality using Pearson's correlation in SAS 9.4. At the same time, the general linear model (GLM) technique was used to check the significant differences between the treatments. Where treatments were found significant, means separation for the individual effects of the type of extract, concentration, fungi, and their interactions were conducted using Tukey's Honestly Significant Difference at $p \leq 0.05$.

3. Results

3.1 Antifungal efficacy of *Warburgia ugandensis* water and methanol extracts

When generalized linear model (GLM) was conducted it was reported that the solvent used during extraction, the concentration, and the fungal strain significantly influenced the antifungal inhibitory activities of *Warburgia ugandensis* ($P<0.05$) (Table 1). It was reported that all the interactions had significant effects on the inhibitory activities of *Warburgia ugandensis* crude extracts except for the interaction between the type of solvent, concentration and fungal strain (Table 1). When mean separation was conducted Tukey's studentized range to determine the inhibitory effect between the types of solvents used, it was found that the type of solvent significantly influenced the antifungal activities of crude extracts with methanol producing a higher mean inhibitory effect. GLM analysis revealed a concentration dependent inhibitory effect with a higher concentration 1000µg/ml producing a higher mean zone of inhibition (Table 1). When mean separation was conducted it was found that there was a significant difference inhibitory effect between different concentration levels used (Table 2). GLM analysis furthermore revealed that the fungal strain significantly influenced the inhibitory effect *Warburgia ugandensis* crude extracts with *Fusarium verticillioides* being more susceptible (Figure 1). Mean separation conducted using Tukey's studentized range to compare the variation in the inhibitory effects of different extracts at different concentration revealed that Natamycin used as a positive control exhibited significantly highest inhibitory effect against both fungal stains yielding a zone of inhibition measuring 22.00±0.00mm and 28.50±0.50mm against *A.flavus* and *F. verticillioides* respectively as shown in table 3 bellow. There was a significant antifungal activity between methanol and water extracts of *Warburgia ugandensis*. Crude water extracts under high concentration 1000 µg/ml produced a mean zone of inhibition measuring 18.00±1.15mm and 26.00±0.00mm against *Aspergillus flavus* and *Fusarium verticillioides* respectively. This inhibitory effect was relatively high comparable to positive control however, there was a significant difference in their inhibitory effects ($P<0.05$). Concerning the inhibition effect of methanol extracts it was found that under high concentration, the extract exhibited higher inhibitory effect 16.67±0.67mm and 30.00±0.00mm against *A.flavus* and *F.verticillioides* respectively comparable to the positive control. Nevertheless, mean comparison to positive control revealed that there was a significant difference in inhibiting the growth of *A.flavus* as shown in Table 3 bellow. It was also observed that there was difference in inhibitory effects under different concentration levels.

Table 1: Inhibitory effect Warburgia ugandensis due to the type of solvent, concentration, fungal strain and their interaction effect

Source	DF	Mean Square	Pr > F
Extract	1	221.02	<.0001
Conc	3	95.18	<.0001
Extract*Conc	3	20.35	<.0001
Fungi	1	270.31	<.0001
Extract*Fungi	1	105.02	<.0001
Conc*Fungi	3	22.52	<.0001
Extract*Conc*Fungi	3	1.79	0.1897

Table 2: Comparison of inhibitory effect at different concentration levels $P<0.05$

Concentration Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
100 - 1000	9.0000	7.51	10.4876	***
100 - 500	11.9167	10.42	13.4043	***
100 - 250	14.0000	12.51	15.4876	***
100 - 125	15.5000	14.01	16.9876	***
1000 - 100	-9.0000	-10.48	-7.5124	***
1000 - 500	2.9167	1.70	4.1313	***
1000 - 250	5.0000	3.78	6.2146	***
1000 - 125	6.5000	5.28	7.7146	***
500 - 100	-11.9167	-13.40	-10.4291	***
500 - 1000	-2.9167	-4.13	-1.7021	***
500 - 250	2.0833	0.86	3.2979	***
500 - 125	3.5833	2.36	4.7979	***
250 - 100	-14.0000	-15.48	-12.5124	***
250 - 1000	-5.0000	-6.21	-3.7854	***
250 - 500	-2.0833	-3.29	-0.8687	***
250 - 125	1.5000	0.28	2.7146	***
125 - 100	-15.5000	-16.98	-14.0124	***
125 - 1000	-6.5000	-7.71	-5.2854	***
125 - 500	-3.5833	-4.79	-2.3687	***
125 - 250	-1.5000	-2.71	-0.2854	***

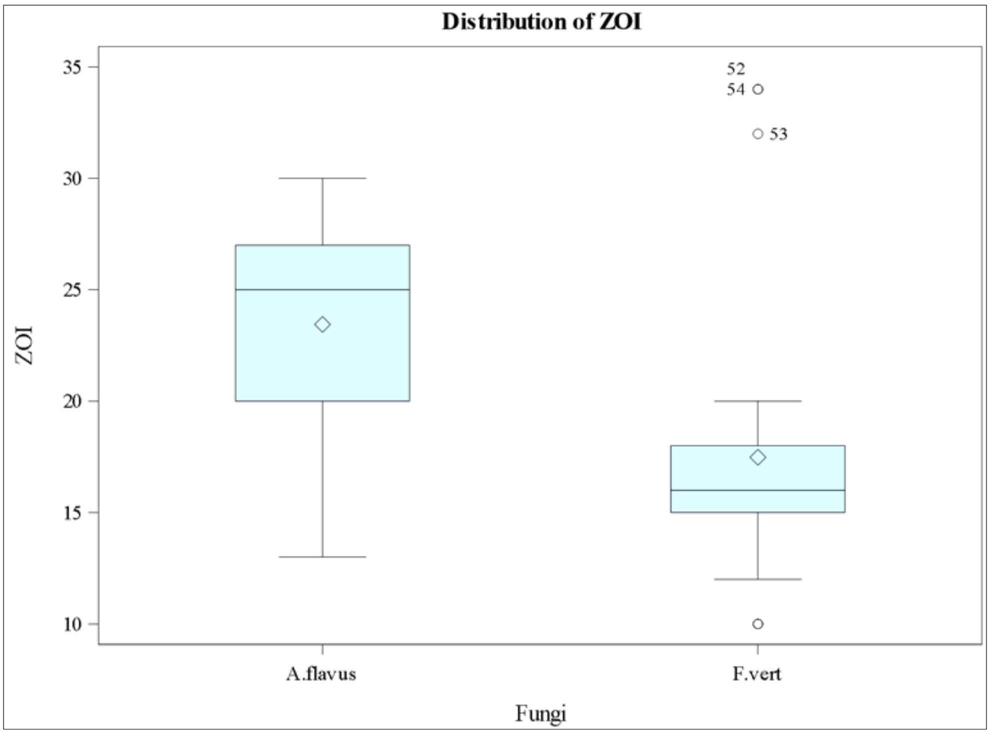


Fig 1: Mean susceptibility of fungal strains to crude plant extracts

Table 3: Mean comparison of fungal strain susceptibility to crude extracts at different concentration level

Plant	Extract type	Concentration (mg/ mL)	Zone of inhibition for *A. flavus* (mm)	Zone of inhibition for *F. verticillioides* (mm)
Positive control	Natamycin		22.00±0.00[c]	28.58±1.00[a]
Warburgia ugandensis	water	1000	18.00±1.15[cd]	26.00±0.00[b]
		500	15.67±0.88[de]	20.67±0.33[c]
		250	15.00±0.58[de]	15.00±0.00[de]
		125	10.67±0.67[ef]	8.33±0.67[f]
		0	0.00	0.00
	Methanol	1000	16.67±0.67[de]	30.00±0.00[a]
		500	14.67±0.67[de]	26.00±0.58[b]
		250	10.33±0.33[ef]	18.33±0.88cd
		125	8.33±0.58[f]	12.67±0.33[e]
		0	0.00	0.00

Values are means ± standard deviations of triplicate measurements. Values in the same column having the same superscript are not significantly different at p>0.05.

3.2 Minimum inhibitory concentration (MIC)

The findings from this study as shown in Table 2 were used to determine the Minimum Inhibitory Concentration (MIC) of the *Warburgia ugandensis* crude extracts. MIC is the lowest concentration of an antimicrobial or antifungal agent (in this case, plant extracts) that can inhibit the visible growth of a microorganism. The clear zones of inhibition were interpreted according to Odongo *et al*., (2022) [19] as low activity (1 mm-6 mm), moderate activity (7 mm-10 mm), high activity (11 mm-15 mm), and very high activity (>16 mm). From the findings in Table 3, it was identified that the lowest concentration with clear zones of inhibition for each combination of solvent extract and fungal pathogen was established at a concentration producing mean zone of inhibition measuring 11-15mm. It was observed that water and methanol extracts had high antifungal activity against Fusarium verticillioides at lower concentration (MIC = 125 mg/mL), producing a zone of inhibition measuring 8.33mm and 11.67mm respectively. For the case of *Aspergillus flavus*, the both water and methanol extracts had high activity at (MIC=125mg/ml) with a zone of inhibition measuring 10.67mm and 8.33mm respectively as shown in table 3 above.

3.3 Application of *W. ugandensis* extracts for maize grain preservation

Warburgia ugandensis water and methanol extracts were both applied in maize grain preservation at concentrations of 125 mg/mL and for both fungi Figure 1 and 2 respectively. The findings from this study revealed that the application of *Warburgia ugandesnis* water and methanol extract at a concentration of 125 mg/mL completely inhibited the growth of both Aspergillus flavus and *Fusarium verticillioides*. For the broken grains, A. flavus was not completely by water extracts while methanol extracts completely inhibited the growth of fungi in maize grains. Both water and methanol extracts of *Warburgia ugandesnis* completely inhibited the growth of Fusarium verticillioides. The petridish plate containing broken maize grains treated with water extracts (Z1) was found to contain signs of contamination.

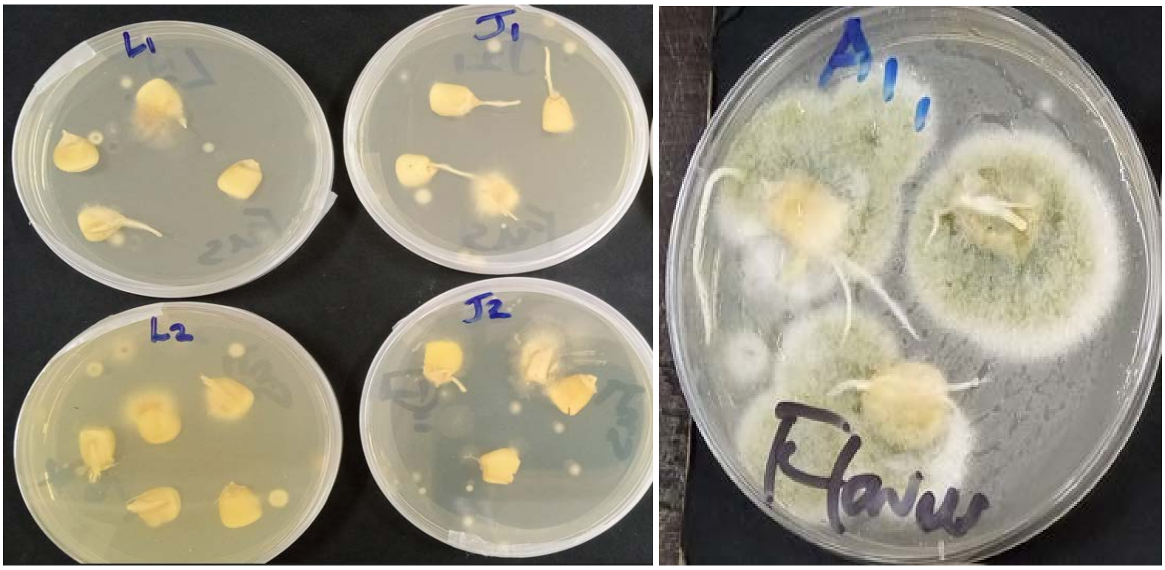


Fig 2: Maize grain preservation using W. ugandensis crude extracts (125mg/ml) against A. flavus.

J and L denotes methanol and water extracts respectively while 1 and 2 denotes healthy and broken maize grains.

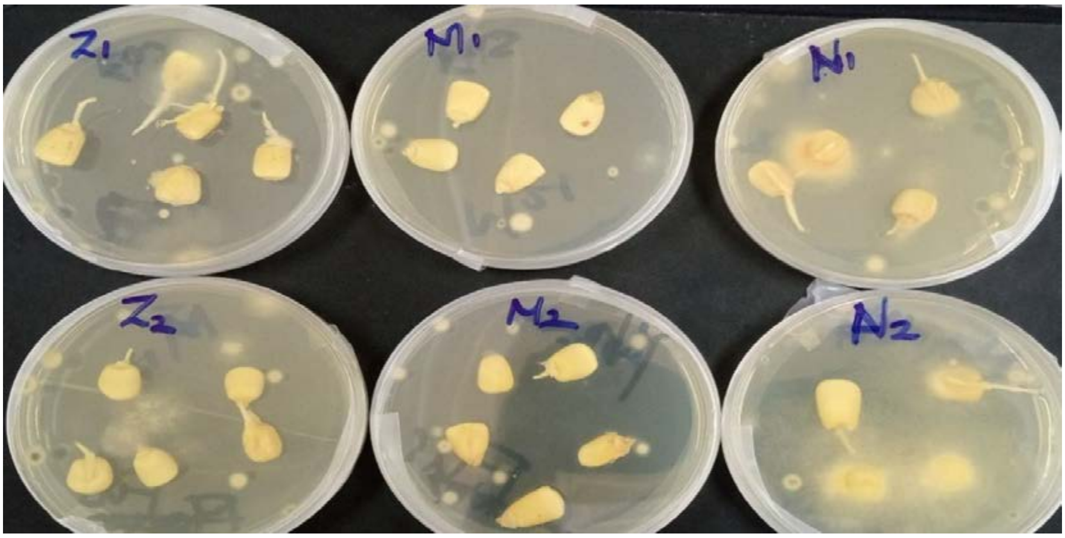


Fig 3: Maize grain preserved using W. ugandensis crude extracts (125mg/ml) against F. verticillioides.

Z-water extracts, M-methanol extracts, and N-negative control. The labels “1” and “2" denote healthy and broken maize grains, respectively.

3.4 Phytochemical composition of *Warburgia ugandensis* crude extracts

Gas chromatography mass spectroscopy was used to analyzed the major chemical components of *Warburgia ugandensis* crude extracts. In water extracts the most dominating compounds were 9-t-Butyl-4-iodo-2,2-dimethyladamantane (45.01%) and Cycloisolongifolene, 8,9-dehydro-9-formyl-(7.60%), while Ledene oxide-(II) (31.19%), Pregn-4-ene-1,20-dione, 12-hydroxy-16,17-d (21.51%), and 2,4-Cholestadien-1-one (12.46%), were the most dominating compounds in methanol extracts as shown in Table 2 and 3 respectively. Notably, there were several compounds were only identified in the methanol and water extract. Methanol extracts contained nine compounds that was not identified in water extract including; (4aS,7R)-7-(2-Hydroxypropan-2-yl)-1,4a-dim (2.24%), 2H-2a,7-Methanoazuleno[5,6-b]oxirene, octa (1.15%), 1,8,15,22-Tricosatetrayne (6.97%), 24-Noroleana-3,12-diene (1.81%), 1H-3a,7-Methanoazulene, octahydro-1,9,9-tri (2.22%), 2-tert-Butyl-4-hexylphenol(1.93%), Ledene oxide-(II)(31.19%), 6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a(2.81%), alpha.-Farnesene(0.86%), and 24-Noroleana-3,12-diene(0.72%). Conversely, nine compounds were also identified in water extract; Aristolene epoxide (0.63%), Neoisolongifolene, 8, 9-dihydro- (4.68%), and Neoclovene oxide (1.96%), among others. These findings highlight the diverse and distinct phytochemical profiles of the water and methanol extracts of *W. ugandensis*.

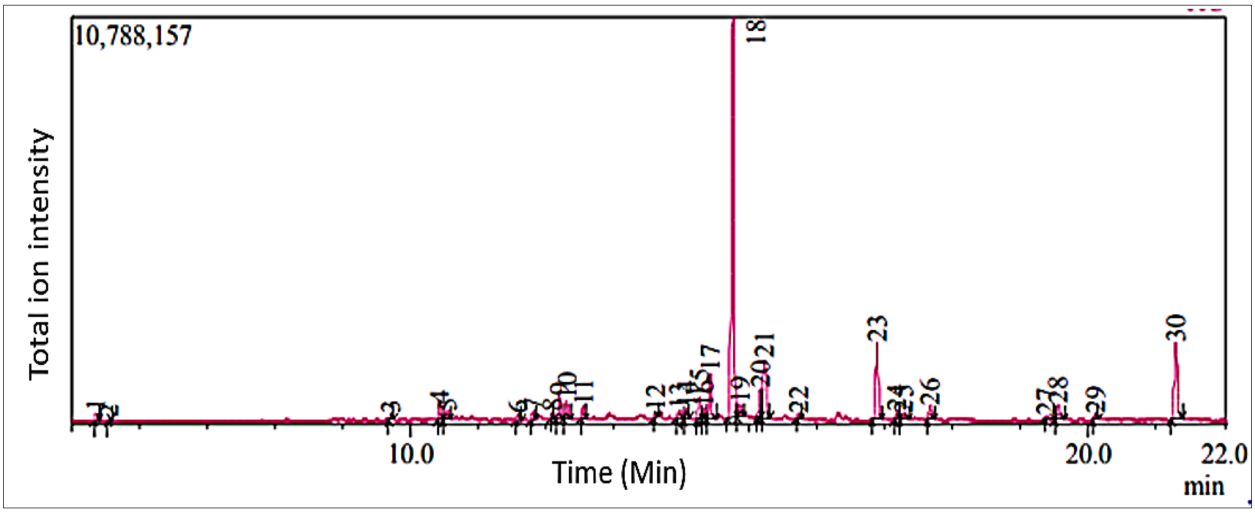
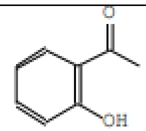
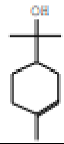
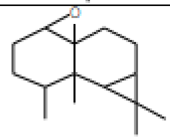
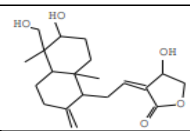
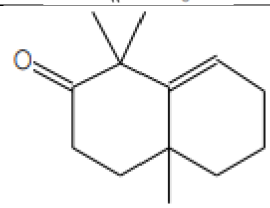
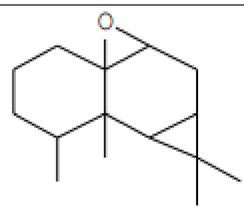
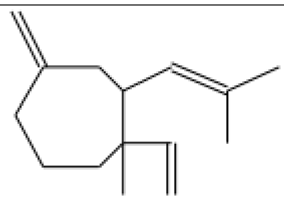
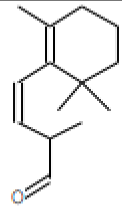
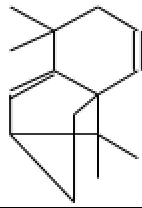
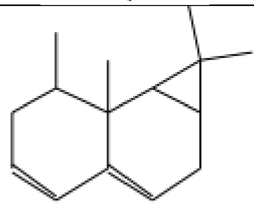
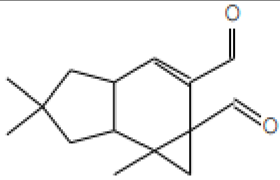
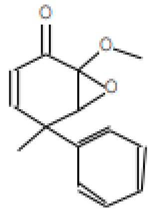
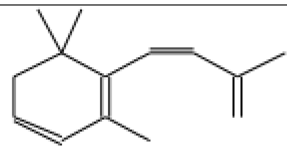
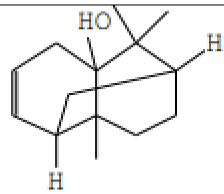
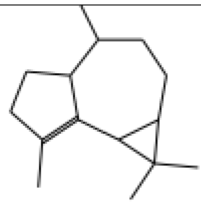
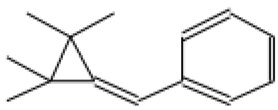
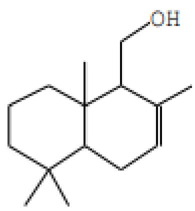
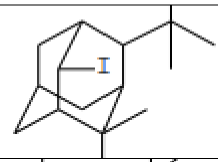
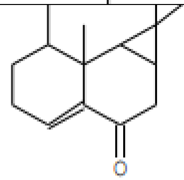
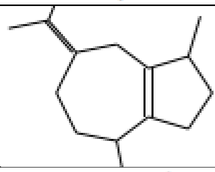
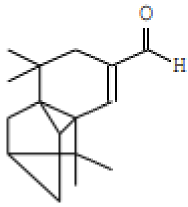
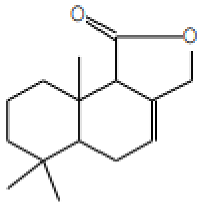
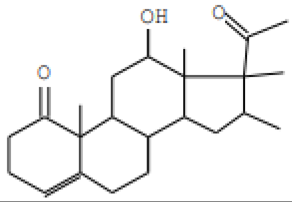
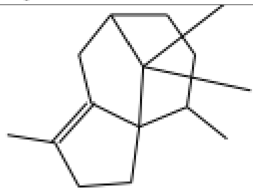
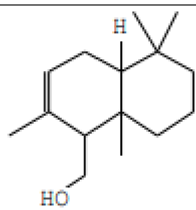
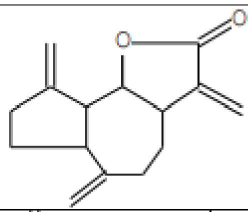
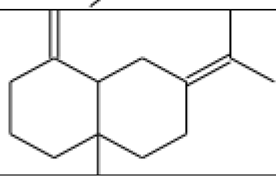


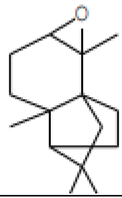
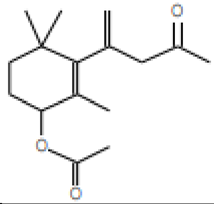
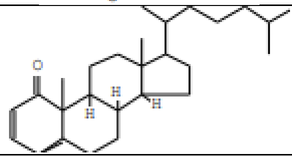
Fig 4: Chromatogram of Warburgia ugandensis water extract

Table 4: Chemical compounds identified in Warburgia ugandensis water extract

Peak	M.W.	Formula	Quantity (%)	Name	Chemical structure
1	136	$C_8H_8O_2$	0.50	Ethanone, 1-(2-hydroxyphenyl)-	
2	154	$C_{10}H_{18}O$	0.15	L-. alpha. -Terpineol	
3	220	$C_{15}H_{24}O$	0.42	Calarene epoxide	
4	350	$C_{20}H_{30}O_5$	1.25	Andrographolide	
5	192	$C_{13}H_{20}O$	0.68	2(1H)-Naphthalenone, 3,4,4a,5,6,7-hexahydro-1,1,4a-t	
6	220	$C_{15}H_{24}O$	0.63	Aristolene epoxide	
7	204	$C_{15}H_{24}$	0.70	Cycloheptane, 4-methylene-1-methyl-2-(2-methyl-1-pr	

8	206	$C_{14}H_{22}O$	0.47	But-3-enal, 2-methyl-4-(2,6,6-trimethyl-1-cyclohexeny	
9	202	$C_{15}H_{22}$	2.21	Neoisolongifolene, 8,9-dehydro-	
10	202	$C_{15}H_{22}$	1.63	1H-Cyclopropa[a]naphthalene, 1a,2,6,7,7a,7b-hexahyd	
11	232	$C_{15}H_{20}O_2$	1.23	Cycloprop[e]indene-1a,2(1H)-dicarboxaldehyde, 3a,4,	
12	230	$C_{14}H_{14}O_3$	0.43	1-Methoxy-5-methyl-5-phenyl-7-oxabicyclo [4.1.0]hept	
13	188	$C_{14}H_{20}$	0.92	1,3-Cyclohexadiene, 2,6,6-trimethyl-1-(3-methyl-1,3-b	
14	206	$C_{14}H_{22}O$	1.02	(1R,4aS,6R,8aS)-8a,9,9-Trimethyl-1,2,4a,5,6,7,8,8a-o	
15	204	$C_{15}H_{24}$	2.23	1H-Cycloprop[e]azulene, 1a,2,3,4,4a,5,6,7b-octahydro	
16	186	$C_{14}H_{24}$	0.94	Benzene, [(tetramethylcyclopropylidene)methyl]-	
17	222	$C_{15}H_{26}O_5$	4.68	1-Naphthalenemethanol, 1,4,4a,5,6,7,8,8a-octahydro-2	

18	346	$C_{16}H_{27}I$	45.01	9-t-Butyl-4-iodo-2,2-dimethyladamantane	
19	218	$C_{15}H_{22}O$	1.37	(1aR,7R,7aR,7bS) -(+)-1a,2,3,5,6,7,7a,7b-Octahydro-1	
20	204	$C_{15}H_{24}$	2.93	. beta. -Guaiene	
21	230	$C_{16}H_{22}O$	7.60	Cycloisolongifolene, 8,9-dehydro-9-formyl-	
22	234	$C_{15}H_{22}O_2$	0.39	(5a.alpha.,9a.beta.,9b.beta.)-5,5a,6,7,8,9,9a,9b-octahy	
23	358	$C_{23}H_{34}O_3$	7.83	Pregn-4-ene-1,20-dione, 12-hydroxy-16,17-dimethyl-	
24	204	$C_{15}H_4$	0.75	3H-3a,7-Methanoazulene, 2,4,5,6,7,8-hexahydro-1,4,9	
25	222	$C_{15}H_{26}O$	0.84	Drim-7-en-11-ol	
26	230	$C_{15}H_{18}O_2$	1.48	Azuleno[4,5-b] furan-2(3H)-one, decahydro-3,6,9-tris (m	
27	204	$C_{15}H_{24}$	0.08	Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-m	

28	220	$C_{15}H_{24}O$	1.96	Neoclovene oxide	
29	264	$C_{16}H_{24}O_3$	0.49	Acetate, 2-cyclohexenyl-3-[1-(2-oxopropyl) ethenyl]-2,	
30	382	$C_{27}H_{42}O$	9.17	2,4-Cholestadien-1-one	
			100.00		

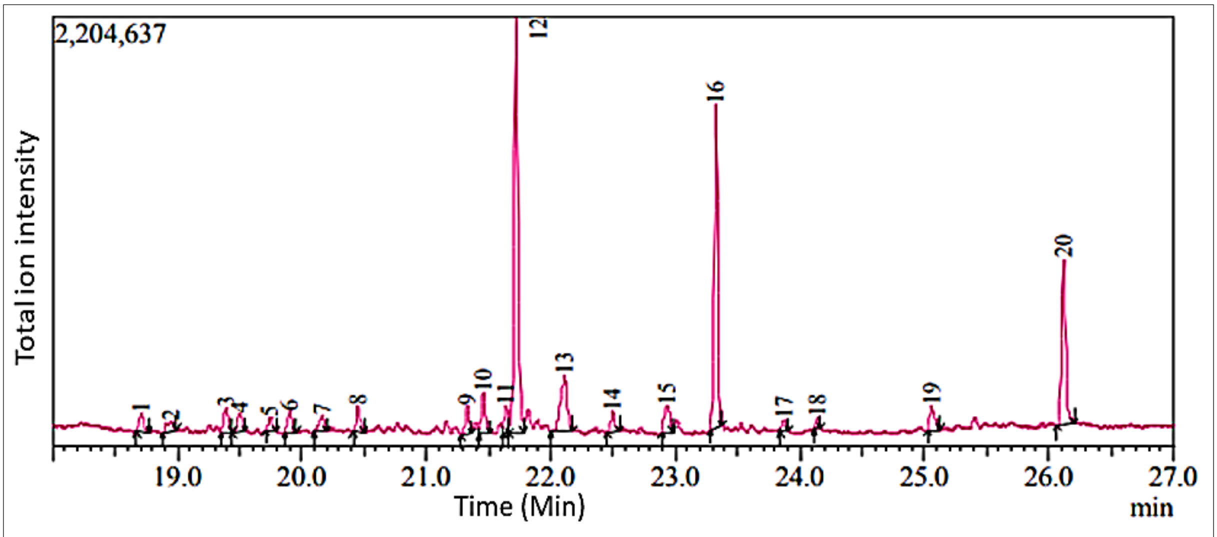
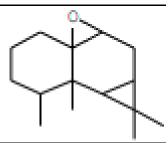
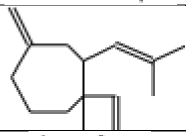
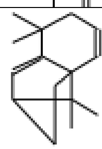
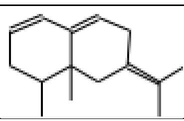
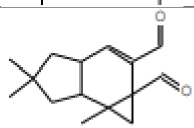
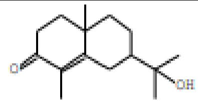
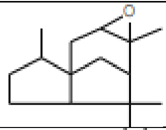
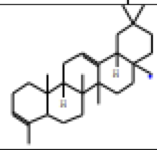
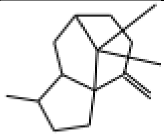
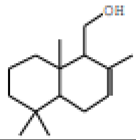
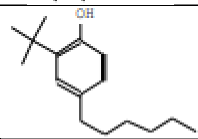
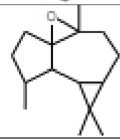
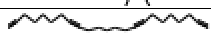
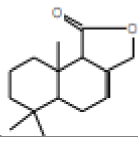
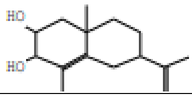
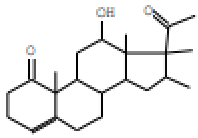
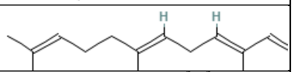
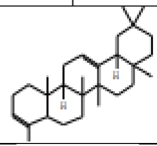
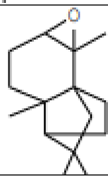
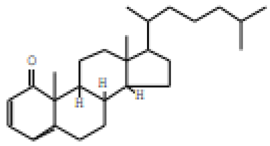


Fig 5: Chromatogram of Warburgia ugandensis methanol extract

Table 5: Chemical compounds identified in Warburgia ugandensis methanol extract

	M.W.	Formula	Quantity (%)	Name	Chemical stracture
1	220	$C_{15}H_{24}O$	1.58	Aristolene epoxide	
2	204	$C_{15}H_{24}$	1.12	Cycloheptane, 4-methylene-1-methyl-2-(2-me	
3	202	$C_{15}H_{24}$	2.05	Neoisolongifolene, 8,9-dehydro-	
4	202	$C_{15}H_{24}$	1.41	. beta. -Vatirenene	
5	232	$C_{15}H_{20}O_2$	1.03	Cycloprop[e]indene-1a,2(1H)-dicarboxaldehy	

6	236	$C_{15}H_{24}O_2$	1.57	(4aS,7R)-7-(2-Hydroxypropan-2-yl)-1,4a-dim	
7	220	$C_{15}H_{24}O$	1.51	2H-2a,7-Methanoazuleno[5,6-b]oxirene, octa	
8	394	$C_{29}H_{46}$	1.81	24-Noroleana-3,12-diene	
9	204	$C_{15}H_{24}$	2.22	1H-3a,7-Methanoazulene, octahydro-1,9,9-tri	
10	222	$C_{15}H_{26}O$	3.32	1-Naphthalenemethanol, 1,4,4a,5,6,7,8,8a-oc	
11	234	$C_{15}H_{22}O_2$	1.93	2-tert-Butyl-4-hexylphenol	
12	220	$C_{15}H_{24}O$	31.19	Ledene oxide-(II)	
13	308	$C_{23}H_{32}$	6.97	1,8,15,22-Tricosatetrayne	
14	234	$C_{15}H_{22}O_2$	1.60	(5a. alpha.,9a.beta.,9b.beta.)-5,5a,6,7,8,9,9a,9b-octahydro-6,6,9a-trimethylnaphtho[1,2-c]furan-1-(3H)-one (drimenin)	
15	236	$C_{15}H_{24}O_2$	2.81	6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a	
16	358	$C_{23}H_{34}O_3$	21.51	Pregn-4-ene-1,20-dione, 12-hydroxy-16,17-d	
17	204	$C_{15}H_{24}$	0.86	. alpha. -Farnesene	
18	394	$C_{29}H_{46}$	0.72	24-Noroleana-3,12-diene	
19	220	$C_{15}H_{24}O$	2.15	Neoclovene oxide	
20	382	$C_{27}H_{42}O$	12.64	2,4-Cholestadien-1-one	
			100.00		

Comparisons significant at the 0.05 level are indicated by ***.				
Extract Comparison	**Difference Between Means**	**Simultaneous 95% Confidence Limits**		
Water - Methanol	10.4583	9.3021	11.6146	***
Water - Water	14.7500	13.5938	15.9062	***
Methanol - WATER	-10.4583	-11.6146	-9.3021	***
Methanol - Water	4.2917	3.5604	5.0229	***
Water - WATER	-14.7500	-15.9062	-13.5938	***
Water - Methanol	-4.2917	-5.0229	-3.5604	***

Comparisons significant at the 0.05 level are indicated by ***.				
Conc Comparison	**Difference Between Means**	**Simultaneous 95% Confidence Limits**		
100 - 1000	9.0000	7.5124	10.4876	***
100 - 500	11.9167	10.4291	13.4043	***
100 - 250	14.0000	12.5124	15.4876	***
100 - 125	15.5000	14.0124	16.9876	***
1000 - 100	-9.0000	-10.4876	-7.5124	***
1000 - 500	2.9167	1.7021	4.1313	***
1000 - 250	5.0000	3.7854	6.2146	***
1000 - 125	6.5000	5.2854	7.7146	***
500 - 100	-11.9167	-13.4043	-10.4291	***
500 - 1000	-2.9167	-4.1313	-1.7021	***
500 - 250	2.0833	0.8687	3.2979	***
500 - 125	3.5833	2.3687	4.7979	***
250 - 100	-14.0000	-15.4876	-12.5124	***
250 - 1000	-5.0000	-6.2146	-3.7854	***
250 - 500	-2.0833	-3.2979	-0.8687	***
250 - 125	1.5000	0.2854	2.7146	***
125 - 100	-15.5000	-16.9876	-14.0124	***
125 - 1000	-6.5000	-7.7146	-5.2854	***
125 - 500	-3.5833	-4.7979	-2.3687	***
125 - 250	-1.5000	-2.7146	-0.2854	***

4.0 Discussion

Fungal infections and mycotoxin contamination are a major threat to human health and agriculture. Mycotoxins produced by fungi such as A. flavus and F. verticillioides can cause a variety of diseases in humans and animals, can also contaminate food and feed and can also lead to economic loss. There is no single technique that has been found effective in preventing fungal infection hence development of new and effective antifungal agents is urgently needed. Medicinal herbs contain variety of phytoconstituents, mostly secondary metabolites produced in response to various environmental. Medicinal plants have been used for centuries to treat fungal infections, however, limited scientific understanding of the antifungal properties of most medicinal plants limits their application (Chitopoa *et al*., 2019; Okello *et al*., 2018; Omwenga *et al*., 2015; Rugutt *et al*., 2006) [4, 20, 21, 23]. The significance of assessing the antifungal activities of the medicinal extracts cannot be understated, given its potential applications in agriculture and medicine.

Natamycin, employed as the positive control, exhibited significant inhibition zones (ZOI) against both tested fungi (*Fusarium verticillioides* and *Aspergillus flavus*) with the mean zone of inhibition 28.58mm and 22mm respectively. These substantial inhibition zones indicated very high fungal inhibition activity as per Odongo *et al*., (2022) [19] classification. These findings highlight the reliability of the experimental setup and validate the capacity of this study to discern significant antifungal effects of *Warburgia ugandensis* extracts. Significant differences in antifungal activities were observed between the two selected solvent extracts where methanol extracts displayed a greater inhibitory effect against both *Fusarium verticillioides* and *Aspergillus flavus* compared to water. These findings were similar to of Chitopoa *et al*., (2019) [4], who reported that ethyl acetate extracts of *Erythrina abyssinica* leaves exhibited the highest zone of inhibition (25 mm) when compared to dichloromethane extract (12mm) against C. albicans. This suggests that the solvent type significantly impacted the extraction of antifungal compounds from *Warburgia ugandensis* due to dispersibility of active compounds due to hydrophobicity and hydrophilicity of chemical compounds (Masoko & Makgapeetja, 2015) [13].

There was a clear concentration-dependent effect, where an increase in extract concentrations generally resulted in the formation of larger ZOIs. This implies that the inhibitory activity of medicinal herb extract against the tested fungal pathogens was enhanced by an increase in extract concentration. Therefore, selecting the appropriate concentration is critical for effective fungal pathogen control. Moreover, there was a significant fungal strain-dependent antifungal activity, with *Fusarium verticillioides* being more susceptible to the crude plant extracts compared to *Aspergillus flavus*. This could be attributed to the genetic potential of each pathogen such as energy metabolism, remodeling and difference in oxidative stress response which underscores the importance of considering the specific fungal strain when evaluating antifungal properties of medicinal herb (Shishodia *et al*., 2019) [25].

Determining the minimum inhibitory concentration (MIC) of an antifungal/antimicrobial agent is a key parameter in assessing their effectiveness against microbial pathogens (Mansouri *et al*., 2021; Ochola *et al*., 2015) [11, 18]. The interpretation of the zones of inhibition as low, moderate, high, or very high activity provides useful classification for the antimicrobial efficacy of the herbal extracts. Methanol extract had high activity while water extracts had moderate activity against *Fusarium verticillioides* at a relatively low concentration (MIC = 125 mg/mL), with a zone of inhibition measuring 12.67mm and 8.33mm respectively suggesting that *Warburgia ugandensis* water extract was effective at inhibiting the growth of *F. verticillioides* even at low concentrations. At a higher concentration (1000 mg/mL), it exhibited very high activity with a zone of inhibition measuring 26mm highlighting its potency as comparable to the positive control. In contrast, the MIC for *Warburgia ugandensis* water and methanol extract against *A. flavus* was 125mg/mL, producing a zone of inhibition measuring 10.67 mm and 8mm. This finding was an important indicator of the quantity of crude extracts applied in maize grain for preservation.

The application of both methanol and water extract at a concentration of 125 mg/mL (MIC) completely inhibited the growth of both *F. verticillioides* and *Aspergillus flavus* on healthy maize grains. It is possible that the methanol-soluble compounds in the methanol extracts are responsible for the higher antifungal activity (Okello *et al.*, 2018) [20]. Synergistic effect of active compounds may be another possible explanation for the higher antifungal activity of methanol extracts. Synergy occurs when more than one compound interacts to produce a greater effect than the sum of their individual effects (Scorzoni *et al.*, 2017) [24]. Therefore, it is possible that the active compounds in the methanol extracts work together to produce a synergistic antifungal effect. The results from this study moreover, revealed that methanol extracts were effective in inhibiting the growth of both *Fusarium verticillioides* and *Aspergillus flavus.* This finding suggests that *Warburgia ugandensis* crude extracts are effective in inhibiting the growth of fungi on maize grains.

The phytochemical analysis using gas chromatography mass spectroscopy technique revealed that 9-t-Butyl-4-iodo-2, 2-dimethyladamantane (45.01%) and (21.51%) Pregn-4-ene-1, 20-dione, 12-hydroxy-16, 17-d, were the most dominant compounds identified in *Warburgia ugandensis* water extract. Chemical compounds identified in this study were similar to those identified in earlier studies, however, there was variation in concentration which could be attributed to climatic conditions and method of extraction (Maobe *et al*., 2013; Okello *et al*., 2018; Otieno,) [12, 60]. The most dominant compounds were mostly phenolic compounds characterized by the presence of methyl, hydroxy and carbonyl groups which have been reported to possess antifungal activities. 9-t-Butyl-4-iodo-2,2-dimethyladamantane for example is made up of methyl (i.e., butyl, dimethyl), adamantane groups and electron donating hydroxy and methoxy which have been reported to contributes to increase the antibacterial and antifungal activities (Abubacker & Deepalakshmi, 2013; Nagashree *et al.*, 2013; Orzeszko *et al*., 2002) [1, 15, 22]. Moreover, the degree of substitution of hydroxy and methoxy groups in phenyl ring have been reported to increase the antimicrobial efficacy (Orzeszko *et al*., 2002) [22]. This therefore means that the presence of hydroxyl and methoxy in Ethanone, 1-(2-hydroxyphenyl)- and 1-Methoxy-5-methyl-5-phenyl-7-oxabicyclo [4.1.0] hept at different degree of substitution could have contributed to enhanced antifungal activity of *Warburgia ugandensis* extracts.

A study conducted by Kong *et al.*, (2019) [8] on the antifungal mechanisms of α-terpineol and terpene-4- alcohol against *Aspergillus ochraceus* in postharvest grapes found that, these compounds could exert antimycotic activities which leads to abnormal spore and negative downgrading metabolic pathways due to disruption of cell membrane and hence weakening the life of the fungi. It was further observed that α-terpineol exhibited the strongest inhibitory effect against *A. ochraceous*. The retarded growth of *Aspergillus flavus* and *Fusarium verticillioides* in the maize grains preserved using *Warburgia ugandensis* extracts could be attributed to the presence of α-terpineol. Some compounds that were found and identified in *Warburgia ugandensis* water extracts have such as Neoisolongifolene in Neoisolongifolene, 8, 9-dehydro-, and Neoisolongifolene, 8,9-dehydro- have not been reported to possess antifungal potency (Xiang *et al*., 2017) [27]. Furthermore, according to Lewis *et al.*, (2005) [9] the contribution of naphthalene derivatives in plant extracts has not been described hence the antifungal activities and a need for further analysis to determine the antifungal potency of naphthalene derivatives.

5. Conclusion

Both the methanol and water extracts of *Warburgia ugandensis* demonstrated high antifungal activity against *Aspergillus flavus* and *Fusarium verticillioides*. At a minimum inhibitory concentration (MIC) of 125 mg/mL, *Warburgia ugandensis* crude extracts effectively preserved maize grains. Methanol as extract had better inhibitory effect hence could be recommended as a suitable solvent in extracting *Warburgia ugandensis*. The excellent preservative effect of extracts against fungal growth suggests that *Warburgia ugandensis* could serve as a potential substitute for synthetic chemicals. Further research should focus on determining the sensory attributes of maize products preserved using these extracts. Additionally, exploring the upscaling application of these crude extracts in maize storage is a promising avenue for future studies.

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