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RESEARCH ARTICLE

EVALUATION OF THE CHEMICAL COMPOSITIONS AND ANTIFUNGAL ACTIVITIES OF LEAF EXTRACTS OFGARCINIA KOLA ON CUCUMBER FRUIT ROT FUNGAL PATHOGENS

***Jacinta, N. Akalazu**

Department of Plant Science and Biotechnology at Imo State University in Owerri, Nigeria

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Corresponding Author:
Jacinta, N. Akalazu.

ABSTRACT

Medicinal plants hold significant value for researchers engaged in the study of plant and animal disease management.The objective of the study was to evaluate the chemical composition and antifungal activities of *Garcinia kola* (bitter kola) leaf extracts on cucumber fruit rot fungal pathogens.*Garcinia kola* leaf methanol extract at 5, 10, 15, 20, and 25mg/ml concentration was employed to assess the fungal inhibition, while the extract diluted with 1 mL of potato dextrose agar broth at 100, 50, 25, 20, 20, 15, 15, 10, and 5 mg/Ml, was utilised to assess thein-vitro minimum inhibitory concentration (MIC). Gas Chromatography-Mass Spectrometry (GC-MS) was used to identify the compounds present in the *G. kola* leaves. The study found *Botryodiploidiatheobromae, Fusariumoxysporium,* and *Aspergillus flavus* in diseased cucumber fruits.As extract concentrations decreased, fungal mycelial growth inhibition decreased linearly. Synthetic fungicide, Mancozeb had the highest inhibition, having a specific inhibition value of23.0 mmon *Aspergillus flavus*, 26.23 on *Fusariumoxysporium*, and 26.70 on *Botryodiploidiatheobromae*, this was followed by 25mg/mlof the extract,having inhibition zone of 19.567 on *Aspergillus flavus*, 20.57 on *Fusariumoxysporium*, and19.01mmon *Botryodiploidiatheobromae*. *Garcinia kola* extract with lowest MIC was 100 mg/Ml, having a specific MIC value of 7.40 for *Aspergillus flavus*, 9.40 for *Fusariumoxysporium*, and 6.40 for *Botryodiploidiatheobromae*. However, 5 mg/mL of *Garcinia kola* extract had the highest MIC. The Gas Chromatography-Mass Spectrometry (GC-MS) analysis, revealed the presence of 30 bioactive compounds with a variety of pharmacological activities. The predominant compounds include 2,5-Methanofuro[3,2-b]pyridine, octahydro- (21.416%), 5-Amino-2-methoxy phenol (17.539%), 2,5-Methanofuro[3,2-b]pyridine, oc (17.465%), and 8-Azabicyclo[5.1.0]octane (22.035%). Hence, the inhibitory effects of bitter cola leaf on Cucumber fruit rot fungal pathogens may be attributed to the presence of these compounds, suggesting their potential as a viable substitute for synthetic fungicides.

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INTRODUCTION

Cucumber, *Cucumissativus* L., belongs to the family Cucurbitaceae. It is a flowering plant species under the genus Garcinia. It is a vine that produces elongated cylindrical fruits. It is cultivated in both temperate and tropical environments, within Nigeria, Cameroon, and many other nations located in sub-Saharan Africa (Mortimore, 2015). Cucumber is a significant vegetable, ranking as the fourth most important vegetable following tomato, cabbage, and onion. The cultivation of cucumbers is primarily driven by their significant nutritional and medicinal importance (Bates *et al*., 1990; Aboloma *et al*., 2009). Bitter kola (*Garcinia kola* Heckel) belongs to the family Clusiaceae, and is a flowering plant. It is distributed within the subtropical and tropical moist lowland forests of Nigeria, Cameroon, and various other nations located in sub-Saharan Africa. It has been extensively utilised in traditional medicine for a wide range of maladies since ancient times. Various studies have explored the medicinal properties of different parts of this tree (Adesuyi *et al*., 2012; Durand *et al*., 2015;Manourova *et al*., 2019, and Erukainure *et al*., 2021).

Plant extracts have been documented to possess medicinal properties and have been utilised in the treatment of diverse ailments, such as gastric and liver disorders, diarrhoea, bronchial diseases, throat infections, colds, fever and malaria (Erukainure *et al*., 2021; Tauchen *et al*., 2023). The plant contains several components that exhibit pronounced bitterness, resinous properties, and astringency, and possesses multiple bioactive chemicals, including tannins, alkaloids, and flavonoids, that are used to manageliver diseases, pulmonary and gastrointestinal conditions, as well as in the prevention of flea infestations (Burkil 2004; Adesuyi *et al*., 2012). *Garcinia kola* contains some compounds that appear to be very specific for G. kola,they include, garcinianin, kolanone, gakolanone, garcinoic acid, garcinal, garcifuran A and B, and garcipyran) (Terashima *et al*. 1995; Akoro *et al*., 2020). Phytopathogenic fungi are very detrimental plant diseases that inflict damage during all phases of crop development (Doehlemann *et al*., 2017; Hussain and Usman, 2019; Nazarov *et al*., 2020). Fruits and vegetables, such as cucumbers, are classified as extremely perishable commodities. The quality of these items can be significantly influenced by various factors, including post-harvest treatment, shipping, storage, and marketing (Agrios, 2005).

Post-harvest infections have a significant impact on cucumber fruits, resulting in potential losses of up to 30% of the overall crop output (Naureen *et al*., 2009). The cost of commonly used synthetic fungicides for managing fungal diseases has been documented to be high, and they have been found to have negative impacts on human and animal health, and the environment (Soanes and Richards, 2014; Scherbakova, 2019). In recent years, there has been a growing interest among researchers regarding the use of phytochemicals in plant disease control, because they possess antibacterial, insecticidal, and fungicidal effects. Phytochemicals have no detrimental effects, are environmentally acceptable, readily accessible, economically feasible, and demonstrate promising efficacy. The objectives of this study are to assess the active components in bitter kola leaf extracts and their ability to suppress the growth of isolated Cucumber fungal pathogens.

MATERIALS AND METHODS

Experimental site: The research was conducted in the humid tropical region of Nigeria, spanning from longitude 3° E to 12°E and latitude 4°N to 9°N. This area is situated within the Guinea Coast zone of West Africa, as referenced by Ogungbenro and Morakinyo (2014) and Akinsanola *et al*. (2016). The agroecological zones of Nigeria, namely the humid rainforest, derived savanna, and southern Guinea savanna, are recognised for their significant contribution to the country's agricultural output (Dania *et al*., 2019). The selection of the places is predicated on their status as primary producers of cucumbers and *Garcinia kola*.

Isolation and Identification of Cucumber fruit rot fungal pathogens: The fungal pathogens were obtained from Cucumber fruits that were affected and subsequently identified based on their culture characteristics and microscopic features (Marsh *et al*., 2013; Jidda 2017).

Pathogenicity Test: The fungi that were isolated were subjected to testing in order to assess their capacity to initiate decay in uninfected Cucumber fruits, following the procedure described by (Jidda 2017).
The determination of the pathogen city of each fungus on the inoculated samples was conducted by measuring the dimensions (length and width) of decayed portions at two-day intervals over a period of ten days, as described by (Jidda 2017).

Preparation of plant material: The fresh bitter kola leaves were subjected to identification and authentication by Prof Mbagwu, a distinguished taxonomist in the Department of Plant Science and Biotechnology at Imo State University, Owerri. The bitter kola leaves methanol solution (volume/volume) stock solution, and stored in dry containers at a temperature of (25 ± 1) ºC, as documented by (Al-Mizraqch *et al*. 2010).

Antifungal sensitivity: Following the procedures of (Al-Mizraqch *et al*., 2010), the agar diffusion technique (well diffusion method), was employed to evaluate the antifungal activity of the extracts. After the 24-hour incubation period, the colonies were resuspended in tubes containing 5 mL of Potato Dextrose Broth. Each well with a diameter of 6mm was then filled with 50 µl of various plant extracts, as well as mancozeb at a concentration of 1000 µg/ml as a positive control, and distilled water was used as a negative control. A series of concentrations (5, 10, 15, 20, and 25mg/ml) were created for each plant extract. All trials were conducted in triplicate and the results were provided as averages.

Minimum inhibitory concentration (MIC): The MIC refers to the lowest concentration of a substance required to impede the growth of a microorganism. This was determined using the broth dilution method, as described by (Ismael and Abdul Latif, 2019)), with some modifications. Each plant extract was diluted in 1 mL of broth at concentrations of 100 mg/mL, 50 mg/mL, 25 mg/mL, 20 mg/mL, 15 mg/mL, 10 mg/mL, and 5 mg/mL. Subsequently, 1 mL of each fungal pathogen suspension was added to the respective dilutions. The positive control, Mancozeb, was employed at a concentration of 1000 µg/ml, while the negative control consisted of DMSO at a purity of 99.9%. The tubes were subjected to incubation at a temperature of 37ºC for a duration of 24 hours. The experiments were conducted in duplicate and replicated three times to ensure reproducibility.

Gas chromatography-mass spectrometry (GC-MS) analysis of the bioactive compounds in bitter kola leaf: The GC-MS analysis was conducted using a 7890A gas chromatograph system (Agilent 19091-433HP, USA) coupled with a mass spectrophotometer. The system was equipped with an HP-5 MS fused silica column (5% phenyl methyl siloxane, dimensions: 30.0 m × 250 µm, film thickness: 0.25 µm) and interfaced with a 5675C Inert MSD with Triple-Axis detector. The carrier gas employed in this study was helium gas, which was carefully calibrated to achieve a column velocity flow rate of 1.0 ml/min. Additional GC-MS parameters include an ion-source temperature of 250 °C, an interface temperature of 300 °C, a pressure of 16.2 psi, an out time of 1.8 mm, and a 1 µl injector operating in split mode with a split ratio of 1:50. The injection temperature is set at 300 °C.

The initial temperature of the column was 36 °C and remained constant for a duration of 5 minutes. Subsequently, the temperature was increased to 150 °C with a rate of change of 4 °C per minute. The temperature was increased to 250 °C at a pace of 20 °C per minute and maintained at that level for a duration of 5 minutes. The overall elution time was 47.5 minutes. The relative percentage of each component was determined by comparing its average peak area to the total areas. The system was controlled and data was acquired using the MS solution software provided by the supplier.

Identification of compounds: The identification of components was accomplished by utilising their retention indices, while the interpretation of the mass spectrum was completed by referencing the database provided by the National Institute of Standards and Technology (NIST). The database encompasses a collection of around 62,000 patterns representing known chemicals. The acquired spectra of the unidentified constituents within the palm kernel bunch fraction were compared to the standard mass spectra of recognised constituents contained in the NIST library (NISTII).

Statistical analysis: The means and standard deviations of the mean were computed for the zones of inhibition and MIC that were measured in both sets of experiments for each respective case. The means were subjected to statistical comparison using the one-way analysis of variance (ANOVA) to assess whether they exhibited significant differences at a significance level of ($P < 0.05$).

RESULTS

Fungal mycelium growth inhibiton: A comparison analysis was conducted using a one-way ANOVA to assess the effectiveness of *Garcinia kola* leaf extract, distilled water, and mancozeb in preventing fungal mycelial growth. The results revealed extremely significant differences ($P<0.05$) when compared to the positive control (Mancozeb). The results of the experiment indicated that Mancozeb had the greatest inhibitory effect on the growth of *Aspergillus flavus* (23.0), *Fusariumoxysporium* (26.23), and Botryodiploidiatheobromae (26.70). Subsequently, the leaf extract of *Garcinia kola* at a concentration of 25mg/ml demonstrated a somewhat lower inhibitory effect.

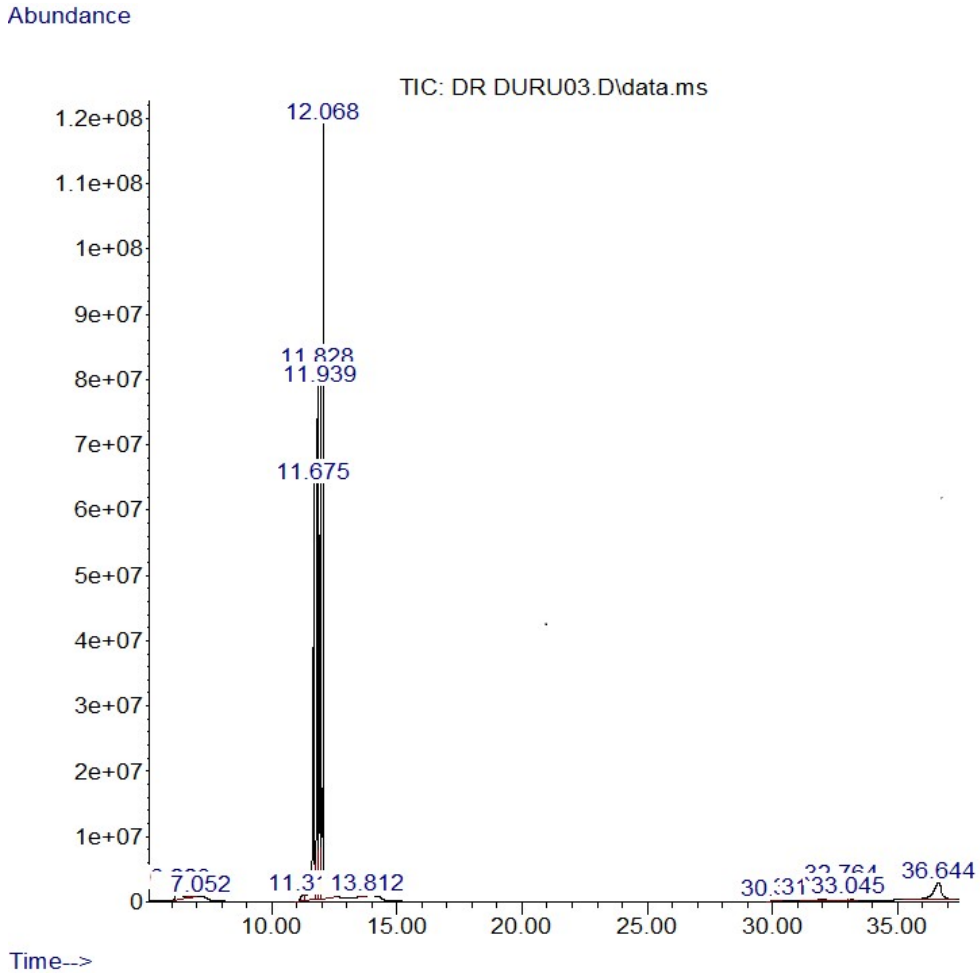
No statistically significant changes were seen in the susceptibility of all the fungal pathogens to the plant extracts. The findings of the study demonstrated that the utilisation of methanol extract derived from Garcinia kola leaves, at different concentrations ranging from 5.0 to 25mg/ml, exhibited a notable suppression of fungal mycelial growth. This observation is supported by the data presented in (Tables 1 and 2). A linear drop in the suppression of fungal mycelial growth was seen as the concentration of the extract decreased. Means that do not share a letter are significantly different ($p\leq0.05$). Values in brackets are standard deviation.

Table 1. Diameter of crude extracts' inhibition zones (mm) against different fungal species

	Fungal pathogens		
	Aspergillus flavus	Fusariumoxysporium	Botryodiploidia theobromae
Garcinia cola leaf extract concentrations.			
Distilled water(I ml)	0.2333 (0.15)g	0.10(0.10)g	0.28(0.096)g
5%	5.767(0.25)f	6.17(0.35)f	4.73(0.3)f
10%	9.467(0.31)e	8.87 (0.50)e	7.70(0.48)e
15%	13.300(0.27)d	12.67 (0.31)d	11.8 (0.192d)
20%	17.533 (0.61)c	16.30 (0.36)c	15.3 (0.47)c
25	19.567 (0.67)b	20.57 (0.74)b	19.01(0.38)b
Mancozeb (1000 µg/ml)	23.000 (0.20)a	26.23(0.25)a	26.70(0.44)a
F- value	1236.30	1362.31	2452.07
P- value	0.000	0.000	0.000

Table 2. Minimum inhibitory concentration (Percentage) of crude algal extracts against test fungal strains

	Fungalpathogens		
	Aspergillus flavus	Fusariumoxysporium	Botryodiploidia theobromae
Garcinia cola leaf extract concentrations.			
5%	82.30(3.03)b	68.03(0.896)b	72.70(0.265)b
10%	74.533(0.61)c	52.20(0.200)c	55.63(0.32)c
15%	56.01(0.25)d	41.43(0.252)d	49.4(49.50)d
20%	32.50 (0.27)e	34.60(0.31)e	34.60(0.53)e
25%	29.73 (0.67)f	34.60 (0.36)f	28.60(0.36)f
50%	16.33 (0.32)g	21.30(0.17)g	20.1(0.56)g
100%	7.40 (0.10)h	9.40(0.10)h	6.40(0.153)h
DMSO (99.9%)	85.20 (0.20)a	92.30(0.27)a	88.50(0.50)a
Mancozeb (1000 µg/ml)	0.33 (0.15)i	0.37(0.16)i	0.43(0.21)i
F-value	2803.15	18118.15	19321.29
P- value	0.000	0.000	0.000



[contents] count=2 Name=D:\MassHunter\GCMS\1\5977\Abdul\PGR\DUR\DR DURU03.D
1=INT TIC: DR DURU03.D\data.ms 2=PBM Apex [INT TIC: DR DURU03.D\data.ms]
Time=Thu Jun 15 19:05:54 2023

Fig.1.Thechromatograms

Table 3. Identified compounds

[PBM Apex]							
Time=	Thu Jun 15 19:06:00 2023						
Header=	PK	RT	Area Pct	Library/ID	Ref	CAS	Qual
1=	1	6.2228	2.8309	(Dimethylamino)ethyl methacrylate	30434	002867-47-2	83
2=	2	6.6053	0.039	Ethanamine, 2-chloro-N,N-dimethyl-	5225	000107-99-3	72
3=	3	6.6254	0.0487	(Dimethylamino)ethyl methacrylate	30436	002867-47-2	64
4=	4	6.689	0.0554	(Dimethylamino)ethyl methacrylate	30436	002867-47-2	64
5=	5	6.7087	0.0327	Acetic acid, 2-(dimethylamino)ethyl ester	14227	001421-89-2	59
6=	6	6.8254	0.0951	Betaine	8629	000107-43-7	72
7=	7	6.8492	0.0236	Betaine	8629	000107-43-7	80
8=	8	6.9745	0.0777	Betaine	8629	000107-43-7	64
9=	9	7.0063	0.014	Betaine	8629	000107-43-7	64
10=	10	7.0516	0.0055	Ethanamine, N,N-dimethyl-	775	000598-56-1	72
11=	11	11.3159	1.0366	6,7-Dihydro-4H-tetrazolo[1,5-a]pyrimidin-5-one	18093	1000304-07-9	27
12=	12	11.6747	21.4158	2,5-Methanofuro[3,2-b]pyridine, octahydro-	18314	049656-63-5	38
13=	13	11.828	17.5389	5-Amino-2-methoxyphenol	18231	001687-53-2	35
14=	14	11.9388	17.4653	2,5-Methanofuro[3,2-b]pyridine, octahydro-	18314	049656-63-5	53
15=	15	12.0679	22.0347	8-Azabicyclo[5.1.0]octane	6263	000286-44-2	53
16=	16	13.5334	0.0049	Cyclohexanecarbonitrile, 1-hydroxy-	11073	000931-97-5	46
17=	17	13.5681	0.0031	Cyclobutane, 1,2-diethyl-, trans-	6932	019341-98-1	42
18=	18	13.5946	0.0048	Cyclobutane, 1,2-diethyl-, trans-	6932	019341-98-1	42
19=	19	13.6385	0.0047	Cyclobutane, 1,2-diethyl-, trans-	6932	019341-98-1	42
20=	20	13.6749	0.0068	Cyclobutane, 1,2-diethyl-, trans-	6932	019341-98-1	46
21=	21	13.746	0.0063	Cyclohexanecarbonitrile, 1-hydroxy-	11078	000931-97-5	50
22=	22	13.8123	0.003	Cyclobutane, 1,2-diethyl-, trans-	6932	019341-98-1	42
23=	23	30.1127	0.1447	Dodecanoic acid, 2,3-dihydroxypropyl ester	134497	000142-18-7	72
24=	24	30.1955	0.0169	Dodecanoic acid, 2,3-dihydroxypropyl ester	134497	000142-18-7	64
25=	25	30.2366	0.0957	Dodecanoic acid, 2,3-dihydroxypropyl ester	134497	000142-18-7	58
26=	26	31.518	0.3934	3-Eicosene, (E)-	140277	074685-33-9	53
27=	27	31.8768	0.1718	Octadec-9-enoic acid	142076	1000190-13-7	83
28=	28	32.7637	8.1888	Oxacyclotetradecane-2,11-dione, 13-methyl-	102128	074685-36-2	86
29=	29	33.0448	0.5387	Valeramide, N-hexyl-	52063	010264-25-2	30
30=	30	36.644	7.7023	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	210570	003443-84-3	96

Minimum inhibitory concentration: The results of the analysis of variance (ANOVA) revealed a statistically significant difference in minimal inhibitory concentrations among all treatment groups. The mancozeb treatment demonstrated the lowest minimum inhibitory concentration (MIC) values observed, namely 0.33 mg/ml for *Aspergillus flavus*, 0.37 for *Fusariumoxysporium*, and 0.43 for *Botryodiploidiatheobromae*. Conversely, the DMSO treatment presented the highest recorded MIC value. An increase in the*G.kola* extract concentration led to a decrease in MIC values (Tables 1 and 2). The lowest MIC value was observed at a concentration of 100 mg/ml; *Aspergillus flavus (7.40),Fusariumoxysporium (9.40), and Botryodiploidiatheobromae*(6.40). However, the highest MIC value was observed at a concentration of 5 mg/ml, for *Aspergillus flavus* (82.30), *Fusariumoxysporium* (68.03), and *Botryodiploidiatheobromae* (72.70) (Tables 1 and 2).

GCMS of the identification of compounds: GCMS result revealed that the primary compounds detected in the methanolic leaf extract of *G.kola* include; 2,5-Methanofuro[3,2-b]pyridine, octahydro (21.416%), 5-Amino-2-methoxy phenol (17.539%), 2,5-Methanofuro[3,2-b] pyridine, oc (17.465%).(Table 3and figure 1) Hence, the potential inhibitory effects of bitter cola leaves on fungal infections causing Cucumber fruit rot may be attributed to the presence of phytochemicals, suggesting their potential as an alternative to synthetic antifungal drugs.

DISCUSSION

Mycelial growth inhibition: The reduction of fungal mycelial growth observed in this study can be attributed to the presence of antifungal chemicals inside the *G.kola* leaf extract. The lack of major variations in the sensitivity of fungal pathogens to plant extracts may be attributed to their comparable cell wall composition and plasma membrane fluidity(Aranda-Martinez *et al*.,2016). The result on isolated fungal pathogens are consistent with (Naureen *et al*., 2009) who identified*Rhizopusstolonifer, Fusariumoxysporium, Aspergillus flavus* as post-harvest fungi pathogens associated with cucumber rot.

Several authors have reported that leaves of *Garcinia kola* possess a wide range of antifungal properties against *Fusariumverticillioides, Penicilliumcitrinium,* and *Aspergillus tamari*, as well as antibacterial activities against *Enterococcus faecalis, Escherichia coli, Staphylococcus lentus, Staphylococcus simulans, and S. sciuri*after a 48-hour incubation period (Berche *et al*., 1991; Durand *et al*., 2015). Inhibitory activity of *G.kola* leaf extract on the fungal pathogens was consistent with Hioki *et al*.,2020, and Tauchen *et al*., 2023, who reported that *G.kola* contains garcinoic acid (GA), and garcinol that exhibited antimicrobial, anti-yeast and antiprotozoal activities.

The minimum inhibitory concentration (MIC): The current research findings on minimum inhibition concentration support the previously mentioned results, indicating that the *Garcinia kola* leaf ethanol extract exhibited the lowest minimum inhibitory concentration (0.156 mg/ml) against *Pseudomonas aeruginosa*, and 0.625 mg/ml against *Staphylococcus saprophyticcus* (Durand *et al*., 2015). Inaddition, report showed that the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of *Garcinia kola* leaf extract on*Fusariumverticillioides, Penicilliumcitrinium,* and *Aspergillus* were between 12.5 and 25 mg/ml, respectively (Igbojionu,2023).

Gas chromatography-mass spectrometry (GC-MS) for the identification and characterization of substances: The GCMS analysis align with the aforementioned research, indicating that the leaf extract of *Garcinia kola* exhibited a greater concentration of alkaloids (10.05%), phenols (9.54%), and flavonoids (4.80%). These chemicals are known to possess antifungal properties (Igbojionu, 2023). However, antifungal compounds in the leaf extract of *Garcinia kola* has been reported by (Eleazu *et al*., 2012;Durand *et al*., 2015).

CONCLUSION

Phytochemicals hold significant value due to their potent antibacterial properties and their ability to increase both plant and animal productivity.

The study's results suggest that *Garcinia kola* leaf extract possesses significant chemical constituents and demonstrates antifungal properties against Cucumber fruit rot fungal pathogens. Moreover, the effectiveness of the extract in inhibiting the growth of these pathogenic fungi varied considerably across different concentrations. Therefore, it is recommended to employ the *Garcinia kola*leaves for managing fungal infections causing Cucumber fruit rot and improving the yield of Cucumber plants. However, the use of Garcinia cola leaf extract alone does not provide a definitive solution for effectively managing these fungal diseases. Therefore, the adoption of integrated biocontrol techniques is crucial in order to achieve sustainable control of plant fungal diseases in Nigeria

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