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GC-MS analysis of the Phytocomponents in the plant extracts of Bitter Apple (*Citrullus Colocynthis*) and their Antibacterial activities against selected pathogenic and food spoilage microorganisms

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Abstract:
The antibacterial potency of the ethanolic and aqueous extracts of the seeds and fruit epicarp of *Citrullus colocynthis* was evaluated against *Bacillus cereus, Pseudomonas aeruginosa* and *Staphylococcus aureus* using agar-well diffusion method. GC-MS analysis and phytochemical screening of the ethanolic and aqueous extracts of the seeds and fruit epicarp were carried out. The ethanolic and aqueous extracts of the fruits of the bitter apple showed inhibitory activities against the test organisms with zones of inhibition ranging between 2 mm and 16 mm, However, the range of inhibition with the seed extract was 5 mm and 18 mm. The GC-MS analysis revealed a total of forty-four and forty-nine phyto-compounds in the extracts of fruits and seeds of *C. colocynthis* respectively. This study showed that ethanolic extracts of the seeds of bitter apple could be employed in the pharmaceutical industry as a potent antimicrobial agent in the formulation of drugs

***Keywords: GC-MS, Citrullus colocynthis* Phytocomponents, Antibacterial, Plant extracts**

1. INTRODUCTION

PLANT components and extracts have been used medicinally in the treatment of infections and diseases since antiquity [1]. The utilization of plant materials and extracts arose from the search for bioactive compounds that could cure infectious diseases. Scientists discovered that some active compounds in these plants can prevent or eradicate infectious agents. The secondary metabolism of medicinal plants produces organic molecules such as tannins, alkaloids, carbohydrates, triterpenoids, steroids, and flavonoids that have physiological effect on the human body and antimicrobial activities against diverse groups of microorganisms [2].

The bioactive compounds and extracts from the roots, seeds, or flowers of medicinal plants can be used to treat infectious diseases [3]. In recent times, there is an increasing level of acceptability and preference of plant extracts and phyto-products over the orthodox medicine due to their low cost, biodegradability, low toxicity, and minimal residual toxicity in the ecosystem [4]. There is a rising trend of antibiotics abuse, which is responsible for antibiotic-resistance by some pathogenic microorganisms, rendering them ineffective most especially with the genetic potential to acquire and transmit drug resistance, new multi-resistant strains continue to emerge [5].

The plant, *Citrullus colocynthis* is commonly known as colocynth, bitter apple and bitter cucumber. It was originally known *as Colocynthis citrullus* but is now classified as *Citrullus colocynthis.* It is from the family *Cucurbitaceae* and other commonly utilized plants in this family include *Cucurbita pepo* (pumpkin), *Cucumis melo* (melon) and *Cucumis sativus* (cucumber) [6]. This plant, bitter apple has been utilized since ancient times as a traditional medicine for the treatment of diabetes, infections, ulcer, inflammation, jaundice and urinary disease in Asia and African countries [7].

It also possesses anticancer [8], antioxidant [9] insecticidal [10], antimicrobial [11] and anti-inflammatory properties [12]. It has been reported that this plant produces several secondary metabolites including cucurbitacins, flavonoids, caffeic acid derivatives and terpenoids which might be responsible for its medicinal properties [13].

Due to the increase in the development of drug resistant microorganisms which is a global challenge, there is need for intensified effort towards searching for plants with active ingredients that exhibit antimicrobial properties. These active ingredients can then be developed into drugs at specific concentrations that can be used by humans and animals to combat new strains of microorganisms causing infection. This study therefore seeks to evaluate the phytochemical and antimicrobial properties of *Citrullus colocynthis* against selected pathogenic and food-spoilage microorganisms.

2.0 Materials and methods

2.1 Source of bacteria

Clinical isolates of *Staphylococcus aureus* and *Pseudomonas aeruginosa* were obtained from Microbiology laboratory, Crawford University, Faith City, Igbesa, Ogun State. Spoilage microorganisms were isolated from samples of cooked rice purchased from the University Cafeteria. All samples were cultured and sub-cultured on nutrient agar plates till pure cultures were obtained.

2.2 Collection and processing of plant materials

The fruits of *Citrullus colocynthis* were purchased from Lusada market, Igbesa, Ogun State. The fruit epicarp of *Citrullus colocynthis* was peeled and air dried for two weeks at room temperature. The seeds were removed from the fruits, washed with water were also air dried for two weeks. The dried plant materials were milled into powdery form using a waring blender. This was sieved through 1.0 mm sieve to obtain a fine powder which was kept in an airtight container until further use.

2.3 Preparation of plant extracts.

2.3.1 Preparation of aqueous extracts

The aqueous extract was prepared by mixing 10 g of plant materials with 100 ml (10 % w/v) of previously sterilised distilled water [14]. The conical flasks were labelled as WES (water extract of seeds) and WEB (water extract of the fruit epicarp). The samples were then placed on the vibrator for 48 h.

2.3.2 Preparation of ethanolic extracts

The ethanolic extract was prepared by mixing 10 g of the plant extracts with 100 ml (10 % w/v) ethanol inside a conical flask and corked tightly [14]. Each of the conical flasks was labelled as EES (ethanolic extract of seeds) and EEB (ethanolic extract of epicarp). The conical flasks were placed on a vibrator for 48 h to allow for proper extraction of the plant materials. The extracts were stored in sterile airtight container at 4 ^{o}C in the refrigerator and used for the assay of antimicrobial activity.

2.4 Phytochemical analysis of the Ethanolic and Aqueous extracts of *C. colocynthis*.

2.4.1 Qualitative phytochemical analysis of the Ethanolic and Aqueous extracts of *C. colocynthis*.

The Ethanolic and Aqueous extracts of *C. colocynthis* was analyzed for the presence of secondary metabolites such as tannins, saponins, flavonoids, steroids, alkaloids and phenols as described by Tiwari et.al. [15]

2.4.2 Quantitative phytochemical analysis of the Ethanolic and Aqueous extracts of *C. colocynthis*.

2.4.2.1 Quantification of Alkaloids

Estimation of the quantity of Alkaloids present in the extracts was carried out according to the method of Longbap et al. [16]. To 5 g of the sample, 200 ml of 10 % acetic acid in ethanol was added and covered for 4 h after which it was filtered. Concentrated NH_4OH was added drop wise to the filtrate until precipitation was totally formed. The precipitate was collected and washed with dilute NH_4OH and then filtered. The residue collected (alkaloid) was dried and weighed.

2.4.2.2 Quantification of Flavonoids

This was carried put with the extraction of 10 g of the plant sample with 100 ml of 80 % aqueous methanol at room temperature. The solution was filtered into a pre weighed 250 ml beaker. The filtrate was transferred into a water bath and allowed to evaporate to dryness and the weight was recorded [17].

2.4.2.3 Quantification of Total Saponins

The method used was described by Obdoni and Ochuko [18]. Twenty grams of the plant sample mixed with 100 ml of 20 % aqueous ethanol was heated over a hot water bath for 4 h with continuous stirring at about 55 ^{o}C. The mixture was filtered and the residue re-extracted with another 200 ml 20 % ethanol. The concentrate was transferred into a 250 ml separation funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5 % aqueous sodium chloride. The remaining solution was heated in a water bath and was further dried in the oven to a constant weight after which the Saponin content was calculated.

2.4.2.4 Quantification of total tannins

Estimation of the total tannins in the plant sample was done by mixing 500 mg of the sample with 50 ml of distilled water. This was filtered into a 50 ml volumetric flask and made up to the mark with distilled water. Then 5 ml of the filtrate was pipette into a test tube and mixed with 2 ml of 0.1M $FeCl_3$ in 0.1 N HCl and 0.008M potassium ferrocyanide. Absorbance was measured at 120 nm within 10 mins [19].

2.4.2.5 Quantification of Total phenolic content

Total phenolic content of the extracts was quantified using the Folin-Ciocalteu method [20] with some modifications. Each

extract (1.0 mg/ml) was added to 2 ml distilled water in 10 ml volumetric flask followed by the addition of Folin-Ciocalteu reagent (1.0 N, 1 ml). After 5 min, Na_2CO_3 (0.2 N, 3 ml) solution was added and the volume was made up to 10 ml with distilled water. The solution was mixed thoroughly and incubated for 2 h at room temperature. After incubation, the absorbance was measured at 760 nm with a spectrophotometer.

2.4 GC-MS (Gas Chromatography-Mass Spectrometry) analysis of the ethanolic extracts of the seeds and fruit epicarp of Citrullus colocynthis

The Gas Chromatography-Mass Spectrometry was carried out at Federal Institute for Industrial Research Oshodi (FIIRO) on the plant extracts according to the methods of Rukshana et al. [21]. The first step of GCMS was done by injecting the sample into the injection port of the Gas chromatogram (GC). The GC instrument vaporizes the sample and then separates and analyses the various components. Each component produces a specific spectral peak that may be recorded on a paper chart electronically. The time elapsed between elution and injection is called the "retention time". Differences between some compounds were identified using the Retention time. The peak is measured from the base to the tip of the peak.

2.5 ISOLATION AND IDENTIFICATION OF SPOILAGE MICROORGANISM

2.5.1 Isolation of spoilage bacteria

Spoilage bacteria were isolated from the rice samples after 72 h of deterioration. Using standard microbiological technique (serial dilution), 1 g of the deteriorated rice sample was pipetted and mixed in another 9 ml of sterile distilled water in a test-tube. The test-tube was shaken vigorously to homogenize. The exponential dilution continued to the fourth factor (10^{-4}). One millilitre (1 ml) of the fourth factor was aseptically transferred and plated in duplicate sets using sterile molten lukewarm nutrient agar. The poured plates were allowed to set and were incubated at 37 °C for 24 h. Sub culturing of distinct colonies were carried out to obtain pure cultures for further identification.

2.5.2 Preparation of media

Culture media used for this evaluation were Nutrient agar and potato dextrose agar (BDH Chemicals, UK) for bacteria and fungi respectively. The powdered nutrient agar of 28 g was dissolved in 1 litre of deionized water, allowed to soak for 10 minutes and then sterilized by autoclaving for 15 minutes at 121 ^{o}C. Potato dextrose agar of 39 g was also dissolved in 1 litre of distilled water and boiled to dissolve the medium completely before sterilizing with autoclave at 121 ^{o}C for 15 minutes. The pH of the sample was adjusted to 3.5, after adding 10 ml of lactic acid. The medium was thereafter cooled to 55 ^{o}C.

2.5.3 Identification of spoilage bacteria

Preliminary identification of the bacteria isolates was based on microscopy and colonial morphological characteristics.

2.5.3.1 Colonial morphology

The bacteria isolate was examined for its colonial appearance and the colonial characteristic was identified by the size, shape, consistency, colour, elevation and opacity.

2.5.3.2 Cellular morphology

Each pure bacteria isolates was stained by Gram staining techniques. A smear of the isolates was made on a clean grease free glass slide and was fixed by passing through the flame three times. It was then stained with Crystal violet for 60 s. Thereafter, the slide was rinsed with water and then stained with Lugol's iodine for another 60 s. The slide was then rinsed with water and decolourised with acetone for 30 s. The slide was stained with Saffranin for 60 s, rinsed with water and allowed to air dry. The slide was then examined under the microscope with oil immersion using ×100 objective lens of the microscope. The shape, colour and arrangement of the bacteria cell was examined and recorded.

2.5.3.3 Biochemical identification

The following biochemical tests were performed to further characterise the bacteria isolate according to the method of Tewari and Singh [22].

2.5.3.3.1 Catalase test

A loopful of pure inoculum was dipped into 3 % hydrogen peroxide; bubble production indicates positive test while no bubble indicates negative result.

2.5.3.3.2 Sugar fermentation test

Sugar containing medium was inoculated with the pure test isolates and incubated at 37 °C for 18 h to 24 h. The production of acid and gas as a result of fermentation was shown by changes in the colouration of the medium and gas production with the following sugar solution containing 1 % Andrade indicator; glucose, lactose, maltose, mannitol, sucrose and xylose.

2.5.3.3.1 Citrate utilisation test

This test was done to demonstrate the ability of the organism to utilise citrate as its only source of carbon. Pure colony of the isolate was inoculated into Simmon-citrate agar medium which contain sodium citrate, an ammonium salt and an indicator bromothymol blue, then incubated at 37 °C for 24 h. Blue colouration indicates positive test while original green indicates negative test.

2.6 **Antimicrobial susceptibility testing**

2.6.1 Agar well diffusion technique

The antimicrobial activity of this plant was evaluated using the agar well diffusion method [23]. A loopful of one colony of the organism was picked with a wire loop and introduced into the broth culture and was placed in the incubator to allow uniform growth of the bacteria after which serial dilution was performed.

Mueller Hinton agar plates were seeded with 18-24 h old cultures of microbial inoculum (a standardized inoculum of $1\text{-}2 \times 10^{7}$ CFU ml^{-1} 0.5 McFarland Standard). Four wells (8

mm in diameter) were bored into the agar media with a sterilized cork borer. Three drops of the plant extracts with concentrations 4, 5 and 6 mg/ml were introduced into the wells. The control experiments were set up with ethanol and water used to fill up separate agar wells already seeded with test organisms; they served as control against the agar wells filled with the various extracts. Inoculated plates were then incubated at 37ºC for 24 h and zones of inhibition were measured in mm. Three replicates were prepared for each isolate.

3.0 RESULTS

3.1 ISOLATION OF SPOILAGE BACTERIA

The microorganism isolated from the biodeteriorated rice was identified as *Bacillus cereus* as presented in Table 1.

Table 1: Identification of Spoilage bacteria from biodeteriorated rice

S/N	Microscopy	CAT	TSIA	CIT	Bacteria
PLATE 10^{-4}	Purple rod shaped, gram positive	+	+	+	*Bacillus cereus*

Key: CAT- Catalase Test; TSIA- Triple Sugar Agar Test; CTI- Citrate Test

3.2 Phytochemical screening

The qualitative and quantitative phytochemical screening of *C. colocynthis* seed and fruit is presented in Table 2. The result of qualitative phytochemical screening indicated the presence of Alkaloids, flavonoids, saponins, tannins and phenols.

Table 2: Phytochemical Screening of Ethanolic and aqueous extracts of *C. colocynthis.*

Bioactive compound	Qualitative analysis (%)		Quantitative analysis (%)	
	Ethanol	Water		Ethanol Water
Tannins	+		+	
	0.06		0.04	
Flavonoids	+		+	
	0.62	0.54		
Alkaloids	+	+		
0.08	0.04			
Saponins	+		+	
0.04	0.02			
Phenols	+	+	0.43	0.22

Key: **+:** Presence of bioactive compounds;
-: Absence of bioactive compounds

3.3 Antibacterial activity of the ethanolic and aqueous extracts of *Citrullus colocynthis*

3.3.1 Antibacterial activity of the ethanolic and aqueous extracts of the fruit epicarp of *Citrullus colocynthis*

The average antibacterial activity of ethanolic extract of the fruit epicarp of *Citrullus colocynthis* is presented in Table 3. The results showed that zones of inhibition recorded by the isolates depend on the type of bacterial isolates and concentration of the extracts. Highest zone of inhibition of 16 mm was demonstrated by *S. aureus* at 200mg/ ml while the lowest zone of inhibition of 2 mm was recorded for *S. aureus* at 100mg/ ml.

Table 3: Antibacterial Susceptibility test of the ethanolic and aqueous extracts of the fruit epicarp of *Citrullus colocynthis*

Concentrations (mg/ml)	200		100	
Isolates	Et.F	AqF	Et.F	AqF
	Zones of Inhibition (mm)			
S. aureus	16	6	12	2
P. aeruginosa	13	11	12	10
B.cereus	15	14	12	10

Key: Et.F: Ethanolic extract of the epicarp of fruit
AqF: Aqueous extract of the fruit epicarp Control (Pure solvent)

3.3.2 Antibacterial activity of the ethanolic and aqueous extracts of the seeds of *Citrullus colocynthis*

The average antibacterial activity of ethanolic extract of the seeds of *Citrullus colocynthis* is presented in Table 4. The results showed that zones of inhibition recorded by the isolates depend on the type of bacterial isolates and concentration of the extracts. Highest zone of inhibition of 18 mm was demonstrated by *B. cereus* at 200mg/ ml while the lowest zone of inhibition of 5 mm was recorded for *P. aeruginosa* at 100mg/ ml.

Table 4: Antibacterial Susceptibility test of the ethanolic and aqueous extracts of the seeds of *Citrullus colocynthis*

Concentrations (mg/ml)	200		100	
Isolates	Et.S	AqS	Et.S	AqS
	Zones of Inhibition (mm)			
S. aureus	17	10	13	10
P. aeruginosa	12	11	6	5
B. cereus	18	14	15	12

Key: EtS: Ethanolic extract of the Seed
AqS: Aqueous extract of the Seed
Control: (Pure solvent)

3.4 Gas chromatography-Mass spectrometry analysis of the Ethanolic extracts of the fruit epicarp and seeds of *Citrullus colocynthis*

3.4.1 Gas chromatography Mass spectrometry analysis of Ethanolic extract of the fruit epicarp of *Citrullus colocynthis*

The GC-MS Chromatogram of the ethanolic extract of the fruit epicarp of *Citrullus colocynthis* presented as Figure 1 revealed forty-four peaks. This shows that forty-four different phytocompounds were present in the extracts. The names and molecular weight of the compounds in the ethanolic extract of the fruit of *C. colocynthis* is shown in Table 5

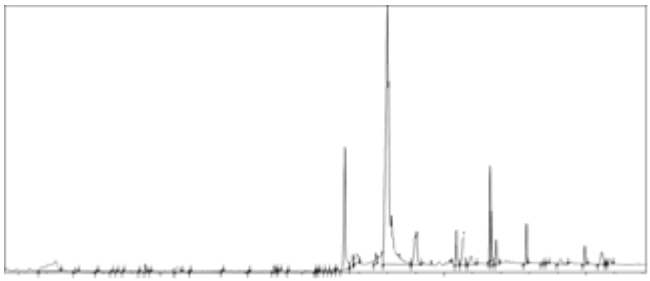

Figure 1. GC-MS chromatogram of the ethanolic extract of the fruit epicarp of *Citrullus colocynthis*

Table 5: GC-MS Analysis of ethanolic extract of the fruit epicarp of *Citrullus colocynthis*

Peak#	R.Time	A/H	Name
1	6.318	23.22	Glycerin
2	7.000	3.36	4,5-Diamino-6-hydroxypyrimidine
3	7.747	2.43	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-
4	8.270	2.58	Catechol
5	8.455	1.65	Benzofuran, 2,3-dihydro-
6	8.677	2.09	1,7-Heptanediol
7	9.245	1.53	Pyrrole-2-carboxaldehyde, 1-[1-(1-adamantyl)e
8	9.468	1.91	4-Hydroxy-2-methylacetophenone
9	9.630	2.20	3-Nonyn-2-ol
10	10.622	10.84	1,3-Propanediol, 2-ethyl-2-(hydroxymethyl)-
11	11.042	1.96	Spiro[androst-5-ene-17,1'-cyclobutan]-2'-one, 3
12	12.155	3.13	3-Deoxy-d-mannoic lactone
13	13.105	1.57	2(3H)-Benzothiazolone
14	14.013	2.40	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphen
15	14.086	1.98	1-Octadecanesulphonyl chloride
16	14.201	2.81	Hexadecane, 1-chloro-
17	14.474	1.50	Octadecanoic acid
18	15.443	1.43	Phthalic acid, butyl undecyl ester
19	15.501	2.02	Tetradecanal
20	15.599	2.28	2-Trifluoroacetoxydodecane
21	15.753	1.50	1-Hexacosanol
22	15.908	1.54	Tridecanal
23	16.095	3.84	Maltose
24	16.170	1.85	Heptadecanoic acid, 10-methyl-, methyl ester
25	16.506	3.71	n-Hexadecanoic acid
26	16.787	5.05	Hexadecanoic acid, ethyl ester
27	16.922	7.64	Ethyl iso-allocholate
28	17.601	3.18	Z,Z-8,10-Hexadecadien-1-ol
29	18.001	9.03	9,12-Octadecadienoic acid (Z,Z)-
30	19.031	7.21	.gamma.-Sitosterol
31	20.426	2.22	Hexadecanoic acid,2-hydroxy-1 (hydroxymeth)
32	20.694	5.27	.alpha.-Amyrin
33	20.944	7.18	.alpha.-Tocopheryl acetate
34	21.614	2.24	9,12-Octadecadienoicacid(Z,Z)-,2,3-dihydrox
35	21.647	2.37	E,E,Z-1,3,12-Nonadecatriene-5,14-diol
36	21.828	3.35	Octadecanoic acid,2,3-dihydroxypropyl ester
37	22.896	2.27	Squalene
38	23.438	3.24	Cyclohexane,1,1'-(2-tridecyl-1,3-propanediyl)
39	23.583	2.64	Di-n-decylsulfone
40	24.088	7.13	Olean-12-ene-3,28-diol, (3.beta.)-
41	24.949	2.89	.gamma.-Tocopherol
42	25.547	6.41	Urs-12-en-28-oicacid,3-hydroxy-,methyl este
43	25.687	3.11	Di-n-decylsulfone
44	25.820	6.72	Olean-12-ene-3,28-diol, (3.beta.)-

3.4.2 Gas chromatography-Mass spectrometry analysis of Ethanolic extract of seeds of *Citrullus colocynthis*

The GC-MS Chromatogram of the ethanolic extract of the seeds of *Citrullus colocynthis* presented as Figure 2 revealed forty-nine peaks. This shows that forty-nine different phytocompounds were present in the extracts. The names and molecular weight of the compounds in the ethanolic extract of the seeds of *C. colocynthis* is shown in Table 6.

Figure 2. GC-MS chromatogram of the ethanolic extract of the seeds of *Citrullus colocynthis*

Table 6: GC-MS Analysis of ethanolic extract of the seeds of *Citrullus colocynthis*

Peak#	R.Time	A/H	Name
1	4.523	1.07	Butanenitrile, 2,3-dioxo-, dioxime, O,O'-diacet
2	4.641	1.44	Butanal, 3-hydroxy-
3	4.697	1.22	2-Hexanol, 3-methyl-
4	4.744	1.00	Cyclohexanone, 3-hydroxy-
5	4.864	1.91	Nonanal
6	4.949	4.10	Cyclohexanone, 3-hydroxy-
7	5.357	2.85	N-Ethyl-N'-nitroguanidine
8	5.421	1.91	Butyrolactone
9	5.592	2.11	Urea, 1-methylcyclopropyl-
10	5.656	1.53	2-Cyclopenten-1-one, 2-hydroxy-
11	5.866	2.08	Butanal, 3-hydroxy-
12	6.060	3.48	2-Hydroxy-gamma-butyrolactone
13	6.268	2.57	1,5-Diazocine, octahydro-1,5-dinitroso-
14	6.538	2.40	Tetrahydro-4H-pyran-4-ol
15	6.615	1.88	3-Trifluoroacetoxydodecane
16	6.677	2.94	2,3-Pentanedione, 4-methyl-
17	6.838	2.04	2-Pyrrolidinone
18	6.889	1.89	2,5-Dimethyl-4-hydroxy-3(2H)-furanone
19	7.012	2.48	Cyclopropanecarboxylic acid, 1-amino-
20	7.078	2.43	Glutaraldehyde
21	7.164	2.90	Heptanal
22	7.282	4.79	4-Pyridinol
23	7.463	2.70	6-Acetyl-.beta.-d-mannose
24	7.515	4.54	1H-Indole, 2,3-dihydro-
25	7.817	2.72	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-
26	7.919	2.00	1-Pentanol, 2,2-dimethyl-
27	8.074	2.33	Ether, 3-butenyl propyl
28	8.297	3.35	Catechol
29	8.481	2.20	Benzofuran, 2,3-dihydro-
30	9.137	3.47	5-cis-Methyl-1R,3-cis-cyclohexanediol
31	9.186	3.34	Dodecanal
32	9.479	3.94	2-Methoxy-4-vinylphenol
33	9.631	4.88	Benzenemethanol, 4-hydroxy-
34	9.768	2.30	3,7-Diazabicyclo[3.3.1]nonane, 9,9-dimethyl-
35	9.818	3.44	E-2-Hydroxymethylcyclopentanol, bistrifluoroa
36	9.904	3.04	Cyclopentanecarbonitrile, 5-hydroxy-1-methyl-
37	10.471	2.82	Bicyclo[2.2.1]heptan-2-one, 4,7,7-trimethyl-, s
38	10.677	4.77	[3,3'-Bi-1H-1,2,4-triazole]-5,5'-diamine
39	11.075	5.90	1,3-Cyclohexanediol
40	11.731	2.29	2,4-Pentadien-1-ol, 3-ethyl-, (2Z)-
41	12.152	2.32	1,2,4-Cyclopentanetrione, 3-(2-pentenyl)-
42	12.473	2.55	Phenol, 2-amino-4-(1H-1,2,3,4-tetrazol-1-yl)-
43	12.692	2.06	exo-Tricyclo[5.3.1.0(2.6)]undecane
44	12.771	7.62	.alpha.-D-Galactopyranoside, methyl
45	14.022	2.55	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphen
46	14.162	3.57	10-Oxocyclodec-2-enecarboxylic acid, methyl
47	15.352	3.97	3-Azabicyclo[3.2.2]nonane
48	16.099	3.98	2-t-Butyl-4-methyl-5-oxo-[1,3]dioxolane-4-car
49	16.418	3.41	6,9,12-Octadecatrienoic acid, phenylmethyl est

4.0 DISCUSSION

The Gas Chromatography-Mass Spectrometry analysis of the ethanolic and aqueous extracts of the seed and fruit epicarp of Citrullus colocynthis revealed the presence of more phyto-compounds in the ethanolic extracts of the seeds than that of the fruit epicarp of *C. citrullus*. This may be responsible for

the higher antimicrobial activity of 18 mm exhibited by the ethanolic extract of the seeds compared to that of the fruit epicarp (15 mm) against *B. cereus*. The possibility of higher phytochemicals present in the fruit epicarp compared to that of the seed was reported by Gupta et al.[24] in which the GC-MS and HPLC profiling of the extracts established this fact. This explains the higher antimicrobial activity exhibited by the seed extract compared to the fruit extract since the bioactive compounds and their antimicrobial activity varies in plant parts [25].

The phytochemical screening of the ethanolic extract of the seeds and fruit epicarp of Citrullus colocynthis revealed the presence of certain phytochemicals. These bioactive compounds are classified as Alkaloids, flavonoids, saponins, cucurbitacins, tannins and cardiac glycosides. This correlates with the findings of Uma and Sekar [26] who reported the same groups of phytochemicals as being present in bitter apple. These phytochemicals exhibit various antimicrobial, pharmacological and biochemical actions and found to be beneficial to human health [27].

The phytocompounds present in the extracts of *Citrullus colocynthis* is responsible for the antimicrobial activity they exhibited against the test organisms. Alkaloids have been reported to form complexes with the DNA of the microorganism thereby altering their activities and ability to multiply [28]. The reaction of flavonoids with the amino acids in the cell wall of microorganisms is solely responsible for their antimicrobial activity which results in the inactivation of bacterial enzymes [29]. Tannins make microorganisms inaccessible to substrates necessary for their growth, resulting in the disruption of the respiratory process and also inhibiting the enzyme activity of the microorganisms [30].

The antibacterial sensitivity revealed that the highest zone of inhibition against the test organism was exhibited by ethanolic extract and the least zone of inhibition was exhibited by the aqueous extract of the *Citrullus colocynthis*. The test organisms were all inhibited at different rates by the ethanolic extracts of either the fruit epicarp or the seeds with *Bacillus cereus* recording the highest inhibition and *P. aeruginosa*, the least. This correlates with the findings of Bryan et al. [31] which showed that the ethanolic extract of *C. citrullus* showed inhibitory activity against *Escherichia coli, Proteus mirabilis, Staphylococcus aureus,* while the water extract exhibited less or no activity. It is also in conformity with a similar work in which the ethanolic extracts of bitter leaf was found to be more effective against *S. aureus* and *Shigella* [32] This may be due to the higher volatility of the ethanol which tends to extract more active compounds from the samples than water.

Conclusion

The search for plants with potent antimicrobial properties is necessitated by the occurrence of antibiotic resistance. This study investigated and compared the antibacterial potency of ethanolic and aqueous extracts of C. colocynthis whose fruits in homes against pathogenic and food spoilage bacteria. The GC-MS analysis of the ethanolic and aqueous extracts of the seed and fruit epicarp of Citrullus colocynthis revealed the presence of more phyto-compounds in the ethanolic extracts of the seeds than that of the fruit epicarp of C. citrullus. This resulted in the ethanolic extract of the seed exhibiting a higher zone of inhibition compared to that of the fruit epicarp. This shows that the ethanolic extract of the seed is more potent than that of the fruit epicarp. This efficacy of the seed of the bitter apple could be explored in the formulation of drugs and preservatives in the pharmaceutical and food industry respectively.

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