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Original Article

Natural Compounds of Ethanolic Fruit Extract of *Citrullus Colocynthis* Screening and Assessment of Its Antifungal Activity

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Abstract

Citrullus colocynthis is also known as bitter cucumber, bitter apple and colocynth. It is now classified as *Citrullus colocynthis*, where it used to be called *Colocynthis citrullus*. Medicinal plants have secondary metabolism to produce organic compounds, including tannins, alkaloids, carbohydrates, triterpenoids, steroids, flavonoid compounds, etc. These compounds possess physiological influences on human beings and antimicrobial action on a range of microbes. Based on the peak (%), the prevalent bioactive compounds were 1H-Pyrrole-2-carboxaldehyde, 2'-Hydroxy-5'-methylacetophenone, 3-Nonyn-2-ol, spiro[3H-chromene-2,1'-cyclobutane]-4-one, 4-propanoylphenyl 2,2-dimethylpropanoate, 6-Hydroxyhexanoic acid lactone, Octadecanoic acid, tetradecyl ester, 4-(3-Hydroxy-1-propenyl)-2-methoxyphenol, 4-Phthalimidobutyric acid, n-Tetradecanal, 3-Trifluoroacetoxydodecane, octahydropyrrolo[1,2-a]azocin-5(1H)-one, (R)-alpha-Hydroxy-gamma-butyrolactone, N-(4-Methoxyphenyl)-acetylacetamide, 1-Pentanol, 2,3-dimethyl, Glutaraldehyde, 3-methyl, 3,3-Diphenyldihydrofuran-2(3H)-one, Catechol diethyl ether, 1H-Indole, 2,3-dihydro-2-methyl and 9,12-Octadecadienoic acid, ethyl ester. Bioactivity of Hollyhock (Citrullus colocynthis) methanolic crude extract and ethanol fraction in inhibiting three fungi in comparison to traditional antibiotics. besides the popular antibiotics Posaconazole (PCZ) and Itraconazole (ICZ). Observed on Aspergillus flavus (18.08±0.39, 21.00±0.43, 31.09±0.52 and 25.00±0.46). Monitored on F. oxyporum (16.96 20.08, 12.00 22.08 and 19.45). Repeated versus Cladosporium herbarum concomitantly (24.58±0.45, 19.00±0.40, 34.00±0.57, and 27.95±0.50). The activity of citrullus colocynthis metabolites was very high (24.58±0.45) towards Cladosporium herbarum.

Keywords: Antifungal, Compounds, *Citrullus Colocynthis*, GC-MS.

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Introduction

Plant parts and extracts have been in use since ancient times to treat illnesses and infection. The search process of bioactive materials that are capable of treating infectious diseases resulted to the use of plant materials and extracts. Scientists discovered that these plants do have active chemicals, which may prevent or kill infectious organisms. The bioactive materials and extracts of medicinal plants in their roots, seeds, or flowers can be used in the treatment of disorders[1,2]. Plant extracts and phyto-products have gained increased acceptability and preference over traditional medication in recent years due to their low cost, minimum toxicity in the ecosystem and biodegradability. There is also an increase in antibiotic abuse thus rendering certain pathogenic germs to be resistant to antibiotics, making them useless. This is more so because new multi-resistant strains of bacteria are continuously being developed through their genetic ability to develop and transmit drug resistance[3,4,5,6]. *Citrullus colocynthis* is another common plant in the family. Bitter apples have been used to treat diseases such as diabetes, infections, ulcers, inflammation, jaundice, and urinary tract illnesses since ancient times and are used as traditional medicine in Asia[7]. *C. colocynthis* is a source of many bioactive substances that include proteins, tannins, phenolics, and terpenoids. Of its numerous pharmacological attributes, only a few can be mentioned: analgesic, gastrointestinal, reproductive, protective, antibacterial, and anti-inflammatory. The chemical composition of a plant and its biological activity might be influenced by a wide range of environmental and developmental factors[8,9,10]. No studies investigate the connection between changing season and the phenolic contents and antioxidant activity of *C. colocynthis* fruits. The weather patterns which prevail at various seasons of the year may affect the antioxidant activity, which is usually related to the phytomolecules like total phenolics. Polyphenols are naturally found substances that are antioxidants and eliminate free radicals. Phytochemicals like polyphenols are credited with the free radical-scavenging properties of Haldral extracts. Polyphenol constituents and antioxidant capacity of each portable part of fruit have been examined. Polyphenols, tannins, phenolic acids and flavonoids have been established to possess antibacterial properties. Moreover, those most commonly occurring bioactive compounds include terpenoids, glycosides, alkaloids, essential oils, and steroids[11,12]. To determine the biological qualities accurate, it is necessary to apply appropriate analytical methods to detect all the biological substances, both volatile and non-volatile. Every procedure should also have a variety of extraction solvents to be used in order to identify both polar and nonpolar compounds. The aerial part of *C. colocynthis* as crude extracts were tested in their bactericidal and inhibitory activity against 16 *Mycobacterium tuberculosis* drug-resistant strains, 2 Mycobacterium strains that were not tuberculosis (MOTT), and the drug-sensitive *Mycobacterium tuberculosis* H37Rv standard strain. However, against test bacterial, petroleum ether extract did not do as well[13]. It also contains antibacterial, anti-inflammatory, antioxidant, insecticidal and anticancer properties[14,15]. There is the need to put more effort in identifying plants with active compounds that possess antibacterial properties due to the increased prevalence of drug-resistant microbes, which is a problem that affects the whole world. It is then possible to develop drugs with these active chemicals in specific doses to assist people and animals in overcoming strains of microbes that cause shocks during infection. This research attempted to investigate the bioactive components of *Citrullus colocynthis* fruit ethanol extracts and their antibacterial effects under specific conditions.

Materials and Methods

Plant Material Collection, Extraction and Preparation of Ethanolic Extraction

Citrullus colocynthis fruits were purchased in Nader market of Hillah City. The *citrullus colocynthis* fruit epicarp was peeled and allowed to dry at the room temperature over a period of 2 weeks. Seeds of the fruits were removed, washed using water and left to dry in the air after two weeks. The dry plant materials were ground in a

Waring blender into powder. This was sieved using a 1.0 mm sieve to make a fine powder and stored in an airtight container until required. The ethanolic extract was prepared by mixing ten grams of plant extracts and one hundred milliliters (10 percent w/v) ethanol in a conical flask and tightly corked. The conical flasks were incubated on a vibrator during 48 hours to make the plant materials fully extracted. In order to conduct the antimicrobial activity test, 5 g of harvested and powdered plant samples were stored in a sterile, airtight container, on the refrigerator at 4 ^{o}C, using a variety of organic solvents (100 mL) such as ethanol and crude methanolic extract. Extraction was done at 60 ^{o}C temperature in a water bath and the entire procedure was repeated thrice. The solvents were extracted and allowed to evaporate in a rotary evaporator. Moreover, polytetrafluoroethylene (PTFE) 0.22-m filter was used to filter the extracts. The materials were weighed and stored in dark air tight and sterilized vessels to perform additional assays.

GC-MS Analysis of the *Citrullus colocynthis* fruit extract

Examples of volatile and semi-volatile bioactive chemicals that can be identified and measured based on GC-MS analysis of plant extracts, which is a potent and high-precision technique, are terpenoids, fatty acids, and alkaloids. It divides complex mixtures and it often takes 10-50mg of extract to generate a chemical fingerprint that can be used in industry, pharmaceutical, and medical applications. Fruits of citrullus colocynthis were analyzed by means of the GC-MS analysis with Thermo GCTrace Ultra Version: 5.0, Thermo MS DSQ II. The instrument has a non-polar column of the DB 35-MS Capillary Standard which has a 30 mm x 0.25 mm ID x 0.25 mm film. The carrier gas is helium and the minimum flow rate of 1.0 ml/min. To use the injector, the oven temperature was adjusted to 60 o C at 15 minutes, then gradually increased to 280 o C at 3 minutes to 250 o C to use the injector. The retention indices were compared to identify the components, and the Willey and NIST libraries were used. The constituents were identified after comparing them with the ones present in the computer library (NIST and Willey) linked to the GC-MS apparatus and tabulating the results.

An Antifungal Test in the form of a Well-In-Agar

The antifungal test was carried out using the well-in-agar technique. After pouring the medium into Petri dishes and letting it harden, a 5 mm well was made in the medium with a sterile cork punch. After that, 85 milliliters of the aqueous extract were transferred to different wells. The dishes were incubated in 48 at a temperature of 28-31 C$^{\circ}$ and at the expiry of the incubation period, the inhibitory zone was established. The well was duplicated three times to obtain three extracts.

Analysis of Statistics

All the data sets were statistically assessed with the help of the Statistical Package of the Social Sciences (SPSS) software, the analysis of variance (ANOVA) was conducted to define the efficiency of plant extract fractions. Each of the analyses was repeated (n = 3). The identification of significant differences and mean comparisons (alpha = 0.05) was conducted with the help of Tukey test with STATISTICA 13. Finally, the results and graph plots were analyzed using BioStat software and Microsoft Excel.

Results and Discussion

Results of the bioactive compounds present in *Citrullus colocynthis* ethanol extracts based on GC-MS analysis are presented in (Table 1 GC-MS Analysis of *Citrullus colocynthis* ethanolic extract.) along with the reported biological activity. The similarity search was based on each peak to find compounds using the NIST08 library database and the PubChem database using the (NCBI). Nineteen components were identified. The antioxidant ability of the chemicals identified in the GC-MS analysis were further reduced to only 28 compounds with known antioxidant properties that had been established in Citrullus colocynthis extracts before through PubChem and ChEBI databases coupled with literature search. The prevalent bioactive compounds were 1H-Pyrrole-2-carboxaldehyde, 2'-Hydroxy-5'-methylacetophenone, 3-Nonyn-2-ol, spiro[3H-chromene-2,1'-cyclobutane]-4-one, 4-

propanoylphenyl 2,2-dimethylpropanoate, 6-Hydroxyhexanoic acid lactone, Octadecanoic acid, tetradecyl ester, 4-(3-Hydroxy-1-propenyl)-2-methoxyphenol, 4-Phthalimidobutyric acid, n-Tetradecanal, 3-Trifluoroacetoxydodecane, octahydropyrrolo[1,2-a]azocin-5(1H)-one, (R)-alpha-Hydroxy-gamma-butyrolactone, N-(4-Methoxyphenyl)-acetylacetamide, 1-Pentanol, 2,3-dimethyl, Glutaraldehyde, 3-methyl, 3,3-Diphenyldihydrofuran-2(3H)-one, Catechol diethyl ether, 1H-Indole, 2,3-dihydro-2-methyl and 9,12-Octadecadienoic acid, ethyl ester.

Table 1 GC-MS Analysis of *Citrullus colocynthis* ethanolic extract.

No	compounds	MF	MW	No	compounds	MF	MW
1.	1H-Pyrrole-2-carboxaldehyde	C_5H_5NO	95.1 g/mol	2.	2'-Hydroxy-5'-methylacetophenone	$C_9H_{10}O_2$	150.17 g/mol
3.	3-Nonyn-2-ol	$C_9H_{16}O$	140.22 g/mol	4.	spiro[3H-chromene-2,1'-cyclobutane]-4-one	$C_{12}H_{12}O_2$	188.22 g/mol
5.	4-propanoylphenyl 2,2-dimethylpropanoate	$C_{14}H_{18}O_3$	234.29 g/mol	6.	6-Hydroxyhexanoic acid lactone	$C_6H_{10}O_2$	114.14 g/mol
7.	Octadecanoic acid, tetradecyl ester	$C_{32}H_{64}O_2$	480.8 g/mol	8.	4-(3-Hydroxy-1-propenyl)-2-methoxyphenol	$C_{10}H_{12}O_3$	180.2 g/mol
9.	4-Phthalimidobutyric acid	$C_{12}H_{11}NO_4$	233.22 g/mol	10.	n-Tetradecanal	$C_{14}H_{28}O$	212.37 g/mol
10.	3-Trifluoroacetoxydodecane	$C_{14}H_{25}F_3O_2$	282.34 g/mol	11.	octahydropyrrolo[1,2-a]azocin-5(1H)-one	$C_{10}H_{17}NO$	167.25 g/molv
12.	(R)-alpha-Hydroxy-gamma-butyrolactone	$C_4H_6O_3$	102.09 g/mol	13.	N-(4-Methoxyphenyl)-acetylacetamide	$C_{11}H_{13}NO_3$	207.23 g/mol
14.	1-Pentanol, 2,3-dimethyl	$C_7H_{16}O$	116.2 g/mol	15.	Glutaraldehyde, 3-methyl	$C_6H_{10}O_2$	114.14 g/mol
16.	3,3-Diphenyldihydrofuran-2(3H)-one	$C_{16}H_{14}O_2$	238.28 g/mol	17.	Catechol diethyl ether	$C_{10}H_{14}O_2$	166.22 g/mol
18.	1H-Indole, 2,3-dihydro-2-methyl	$C_{26}H_{19}N_3O_3$	421.4 g/mol	19.	9,12-Octadecadienoic acid, ethyl ester	$C_{20}H_{36}O_2$	308.5 g/mol

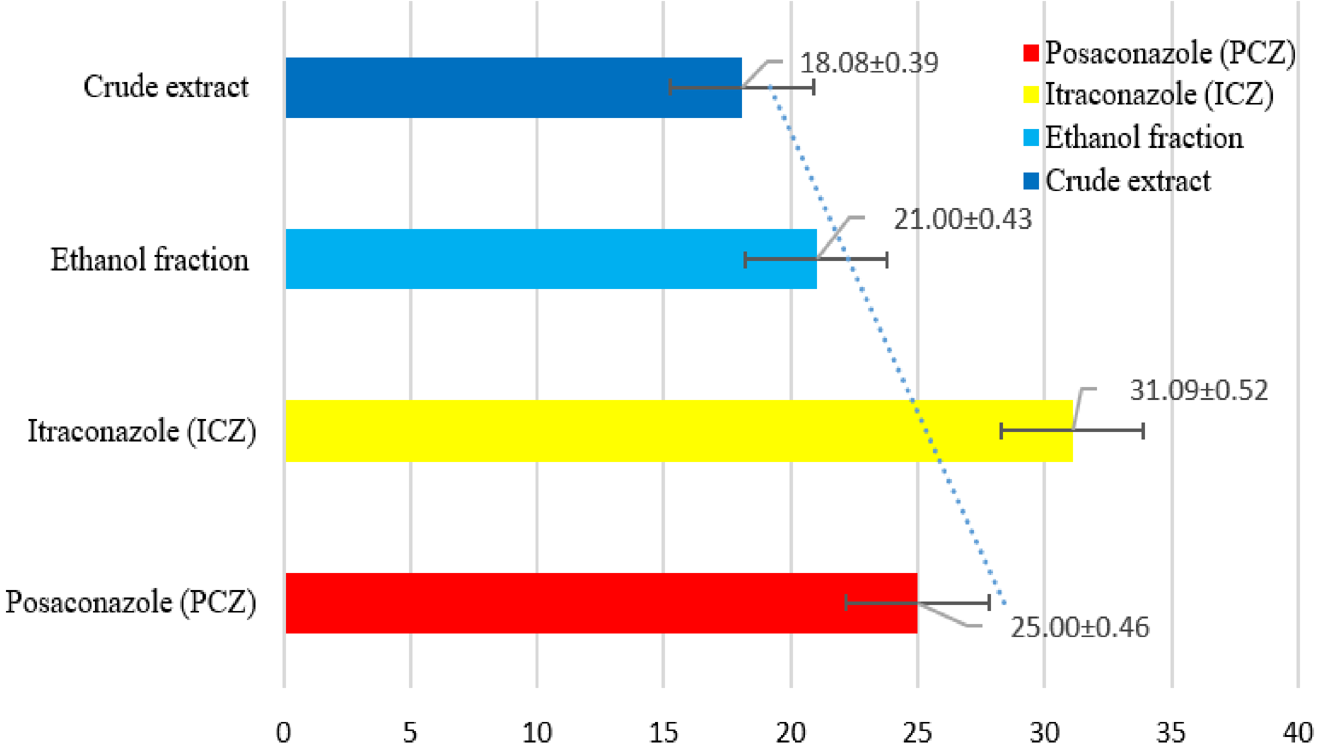

Figure 1. Antifungal activity of secondary metabolites of Hollyhock (*Citrullus colocynthis*) against *Aspergillus flavus*.

Figure 1

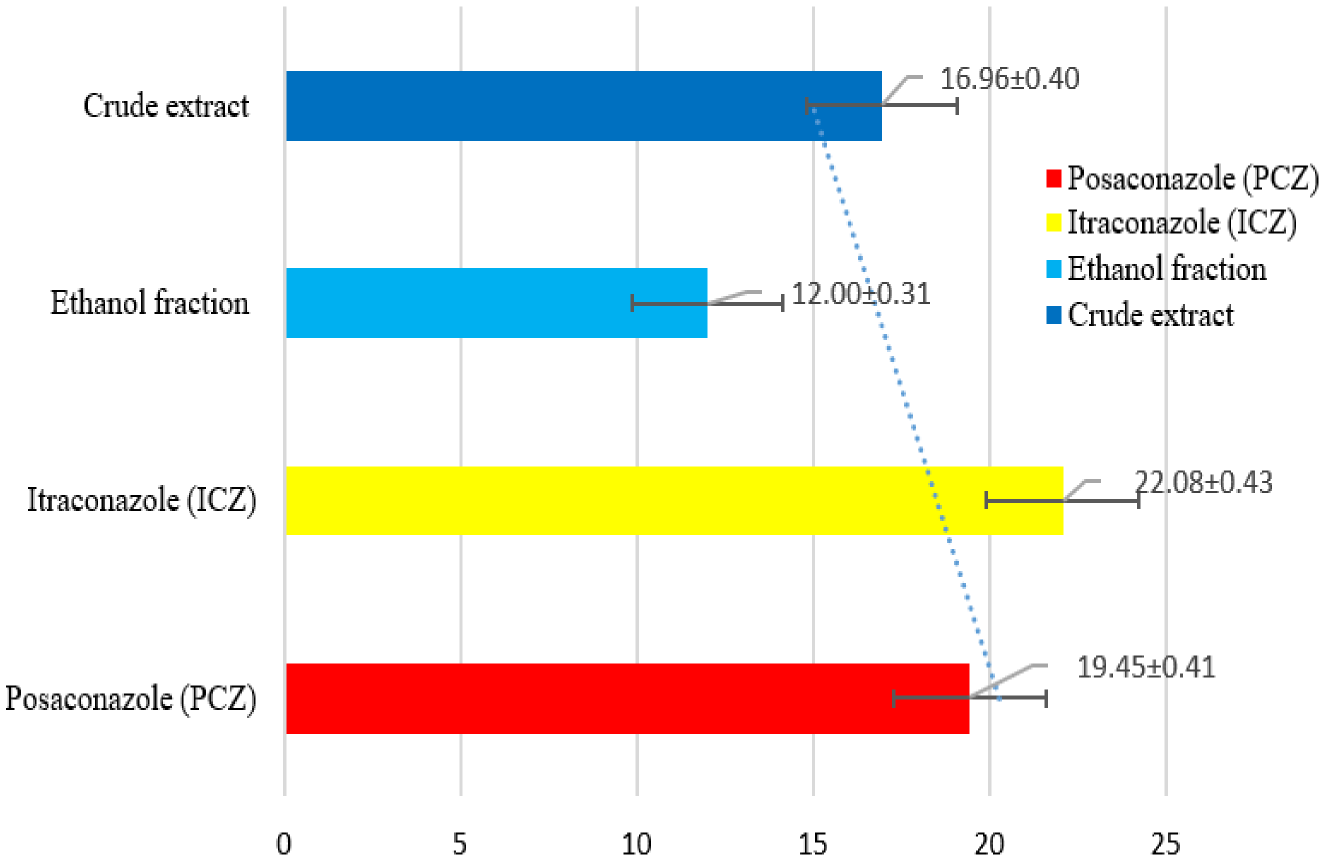

Figure 2. Antifungal activity of secondary metabolites of Hollyhock (*Citrullus colocynthis*) against *Fusarium oxyporum*.

Figure 2

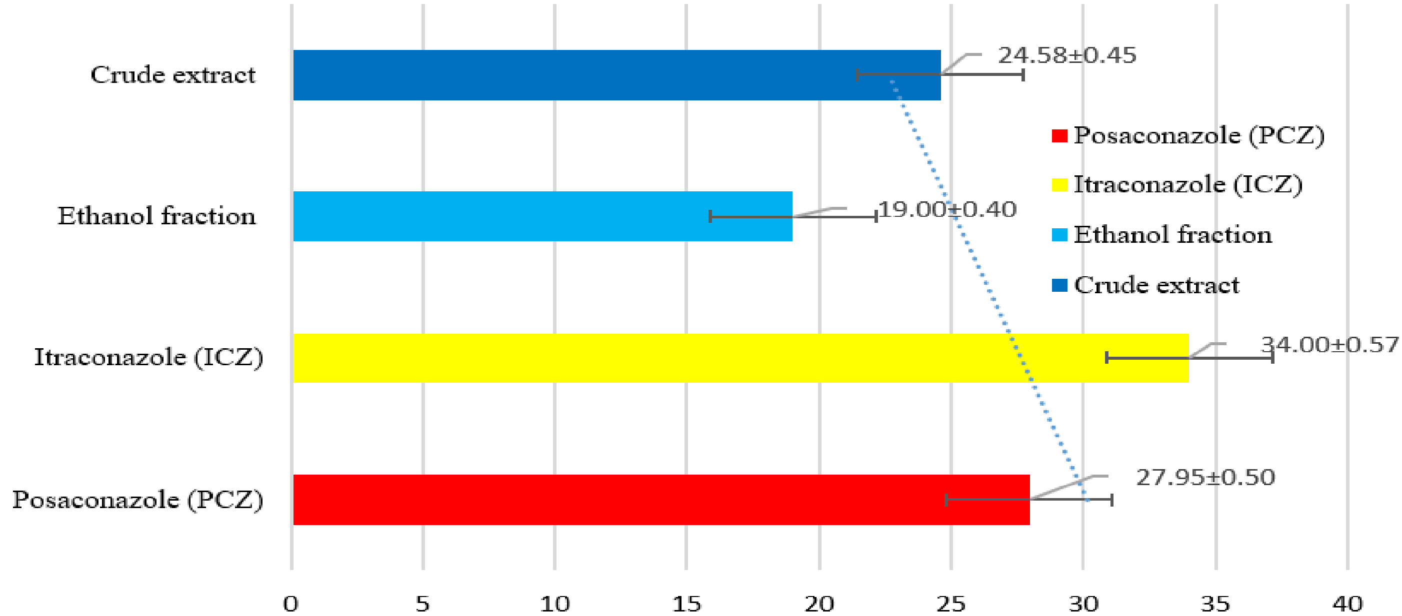

Figure 3. Antifungal activity of secondary metabolites of Hollyhock (*Citrullus colocynthis*) against *Cladosporium herbarum*.

Figure 3

The methanolic crude extract and ethanol fraction of Hollyhock (*Citrullus colocynthis*) in comparison to Itraconazole (ICZ) and Posaconazole (PCZ) on three fungi. Observed on *Aspergillus flavus* (18.08±0.39, 21.00±0.43, 31.09±0.52, and 25.00±0.46). Monitored on Fusarium oxyporum (16.96±0.40, 12.00±0.31, 22.08±0.43, and 19.45±0.41). It was reported against *Cladosporium herbarum* on the same time (24.58±0.45, 19.00±0.40, 34.00±0.57, and 27.95±0.50). The metabolites of *Citrullus colocynthis* had a very high percentage of activity (24.58±0.45) against *Cladosporium herbarum*. Bitter apples have the same type of phytochemicals. The phytochemicals have been reported to enhance human health in numerous pharmacological, biochemical and antibacterial effects. The antimicrobial properties of *Citrullus colocynthis* extracts to the test organisms can be attributed to the phytocompounds present in them[16,17]. It is reported that alkaloids have the ability to alter the activity and ability to multiply of a microorganism by developing complexes with the DNA. The antimicrobial properties of flavonoids which inhibits bacterial enzymes occur only when they react with their cell walls amino acids present in microorganisms. Tannins disorient respiratory process, and disrupt the enzyme activity of bacteria by blocking their entering into the necessary substrates that can assist their growth[18,19,20]. The ethanolic extract of *Citrullus colocynthis* had the highest zone of inhibition when compared to the aqueous extract in regards to the test organism in terms of antibacterial sensitivity[21,22] of the fruit epicarp or seeds in different proportions with P. aeruginosa being the least and the Bacillus cereus the most. This has been congruent with the findings of the previous studies which established that the water extract of *Citrullus colocynthis* did not significantly or marginally inhibit *Aspergillus flavus* and *Cladosporium herbarum* but the ethanolic extract did. This could be due to the fact that ethanol is a volatile solution as compared to water and is more likely to elute active chemicals in the samples.

All these phytochemicals have been proved to enhance the health of human beings in a range of pharmacological, biochemical and antibacterial effects. It was the phytocompounds that existed in the test organisms that led to the antibacterial effect which the Citrullus colocynthis extracts exhibited. Reportedly, alkaloids are able to alter the activity and ability of a microorganism by creating complexes with the DNA. The tannins inhibit the accessibility of microbes to their required substrates to grow and therefore interrupt the process of respiration, as well as inhibit the working of the enzymes of the germs. *Citrullus colocynthis* extracts have secondary metabolites such as cinnamic acid, linolenin, farnesyl acetone, n-tetratetracontane, and 7,8-dimethyltocol among others which are pertinent to the antioxidant activities as adopted by FRAP and DPPH assays. All extracts were effective against all strains[23,24,25]. Although, the extracts of *C. colocynthis* displayed the lowest antibacterial and anticandidal activity, the fruit extracts demonstrated the highest MICs and MBCs/MFCs (0.10mg/mL against *C. albicans* and *C. glabrata*, 0.20mg/ml against *P. aeruginosa* and *E. coli*). This means that the ethanolic extract of the fruit epicarp is not as effective as the seed one[26]. The efficacies of bitter apple seeds can be explored in the development of drugs and preservatives in the food and pharmaceutical-industries respectively.

Conclusion

Strong antibacterial properties of plants have been necessitated by the resistance of the antibiotics. This paper compared and contrasted the antifungal activity of *Citrullus Colocynthis* ethanolic extract, which prevents the attack of pests on household fruits. The ethanolic extract of the fruit epicarp had more phytocompounds. This gave the ethanolic extract of the fruit epicarp. The findings of the present research point to the fact that ethanol extracts of *Citrullus colocynthis* are an excellent source of bioactive compounds with interesting antibacterial activity. Nineteen bioactive chemicals were discovered with the help of GC-MS analysis. The predominant bioactive compounds of ethanolic extracts were 1, 1-Pentanol, 2,3-dimethyl, Glutaraldehyde, 3-methyl, 3,3-Diphenyldihydrofuran-2(3H)-one, catechol diethyl ether, 1H-Indole, 2,3-dihydro-2-methyl and 9,12-Octadecadienoic acid.

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