Medicine and Community Health Archives

E-ISSN: 3023-3011

Available at: https://mchapub.com/index.php/mcha

Original Research

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Antifungal, Antioxidant (Peroxynitrite scavenging, Hydroxyl radical scavenging and Nitric oxide radical scavenging) and Screening of Bioactive Chemical Compounds of Colocynth (Citrullus colocynthis) Using GCMS Technique

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Article info:
Received: 10 March 2024
Revised: 29 March 2024
Accepted: 25 April 2024
Published: 10 May 2024

Abstract:

Background: Inflammation of the joints, acne, hepatitis, urticarial infections, bowel irregularities, constipation, and bowel disturbance are some of the conditions that *citrullus colocynthis* helps alleviate. The glycosides and phenolic acids found in Citrullus colocynthis are extremely diverse in composition. Consequently, the purpose of this research was to examine the antifungal effects of *Citrullus colocynthis* fruit extract and to screen for bioactive components using GCMS.

Materials and Methods: The local plant parts that constitute the *Citrullus colocynthis* fruit and other plants were provided by provincial market of Babylon. The remaining plants were then sent for washing and separation from foreign bodies to the College of Science, the University of Babylon, to be studied intimately in the specialized Botanical laboratory. I guess these were some baked beans. “Everything here is completely smooth.” With counting the constants, we evaluated different components of spectra, and then the percentage relative peak area allowed us to determine all. These compounds were tentatively identified by comparing their respective kinetic retention times and the mass spectrum of the data obtained by GC-MS system, this system was purchased as the NIST and Wiley libraries. The diameter of the inhibition zone which is described in millimeters (mm) is the indicator of the antifungal activity of the fruit extracts.

Results: The secondary metabolites detected by GC-Mass technology are: 9-Octadecenoic acid, trans-Zeaxanthin, tetraneurin-A, Lycoxanthin, N-(4-Hydroxyphenyl-d4)retinamide,1-tert-Butyl-4,4-diphenylpiperidine, Thiocarbamoylthioacetic acid, Lucenin-2, 4-(2,3-Diphenylcyclopropyl)phenol, 5-Aminoisothiazol-3-yl)methanol, 4,8,12,15,19,21-tetracosahexaenoic acid, and Ethyl trans-2-phenylcyclopropanecarboxylate. Antifungal activity of secondary metabolites of *Citrullus colocynthis*: Bioactivity of the fruit extract (Ethyl acetate, and Ethanol) of Citrullus colocynthis and standard antibiotics against six fungi and yeast. Alternaria alternaria (14.09 ± 0.28 and 17.61 ± 0.32), Aspergillus flavus (22.30 ± 0.45 and 23.09 ± 0.47), Trichophyton rubrum (16.00 ± 0.49 and 20.74 ± 0.41), Fusarium oxyporum (24.08 ± 0.48 and 15.11 ± 0.49), Cladosporium herbarum (14.79 ± 0.28 and 19.00 ± 0.36), Cladosporium herbarum (6.10 ± 0.02 and 7.05 ± 0.04), Candida albicans (21.00 ± 0.43 and 15.11 ± 0.49), and standard antibiotics (23.31 ± 0.48 and

27.09 ± 0.51) for Voriconazole (VCZ) and Amphotericin B (AmB) respectively. *Citrullus colocynthis* metabolites was very highly-active against *F. oxyporum* (24.08 ± 0.48).

Conclusion: Based on the findings of this study, Citrullus colocynthis could be a great plant to look for if you're looking for active chemicals that can help with fungal infections.

Keywords: Antifungal, Antioxidant, Bioactive Chemical Compounds, Citrullus colocynthis, GCMS.

Introduction:

The huge majority of the current study on the antifungal drugs hits towards either the synthetic or the natural substances from plants. Research designing traditional medicine drug targets portion on identification of the antifungal activity of different plants, herbs, and remedies since their extracts have capability to provide antibacterial action in vitro and in vivo [1]. This bitter gourd, a common mediterranean vine with the mediterranean basin and west-asia as source area; harbours various popular names, which includes its arabic name _{('Abu Jahl's melon)}_, colocynth, bitter apple, bitter cucumber, egusi, vine of Sodom, and wild gourd. It is a kind of squash of the Cucurbitaceae family. The fruit has a sharp acidic flavor and has a rough round and smooth with the diameter of 5 to 10 cm. Immaturity only the calyx contains the fruit, the photo of yellowish behaves before the development of this pattern. The seeds which are carried in the mesocarp of hard-shelled nut during propagation activity are white, dry, small, crunchy, and spongy. These three is six bean in every one of them. Frequency and quality of the fruits is between fifteen and thirty, with each fully grown plant. At length, the therapeutic use of the fruit from colocytus tree has been reported to have a health benefits. Traditionally, the fruits from the Citrullus colocynthis plant were used for medicinal purposes to treat kidney and bladder problems, cancer, coughs, fevers, malaria, rheumatism, and sore throats, as well as to address fungal diseases, ulcers, tumors, and urogenital disorders [3, 4]. Mainly, it is the extract from edible Citrullus colocynthis and the processed results which may be used in pharmaceutics or fuel production as well. g. , oilseed and biofuel). Several monuments of antiquity consist of the semblance of the proteinous seed of the squash in the Eurasian Near and northern Africa.

The assessment of structural diversity of phytoconstituents in plant extracts can be done by recognition of chemical compounds gas chromatography-mass spectrometry. The incomparably great caduceus potency of the technique with which it conducts tests and generates chemical fingerprints, with their unmatched accuracy and precision. Apart from this, GC-MS allows qualitative and quantitative data as well as the mass spectrum in relation to the coupled database. This is highly applicable in the pharmacology field to know which active substance creates which effect. Research on C. colocynthis revealed that it has calotropin, tannins, terpenoids and flavonoids present in its whole plant extract in addition to coumarins [8]. The aim of this job was the discovery of unique phytoconstituents by ethanolic seeds extract of Citrullus colocynthis, to clarify the pharmacological activities, including antifungal and antioxidant, which can be used to explain the curative properties of the plant of this. GC-MS was used in this experiment. Here, we will focus on some of the potential benefits and challenges associated with implementing AI in healthcare.

Materials and Methods:

Samples Collection

The Citrullus colocynthis fruit and other dried plant parts were sourced from Babylon Province's local markets. Following washing and the isolation of foreign substances, they were studied in the sophisticated Botanical laboratory at the College of Science, University of Babylon. After they were ground to a powder using an electric grinder, the powder was sealed in polythene bags and stored at room temperature until needed.

Compounds Extracts

Gather 100 grams of each plant and put them in a glass beaker. Then, using a reflex condenser, add 400 mL of 2% acetic acid to make citrullus

colocynthis fruit. It is left to cool when the extraction with the solution is complete. After that, a rotary evaporator was used to condense the top layer, and the dry material was stored in a refrigerator at 4°C until it was needed. To make the concentration, alcoholic extracts were dissolved to a concentration of 100 mg/mL. To combat fungus, the final concentration of each solvent will be 100 mg/mL.

Gas chromatography – mass spectrometry analysis (GC-MS)

An GC-MS carried the detection of components through their separation and identification. A fused silica capillary column (30 m, 0.25 μm) is employed for chromatographic separation due to its high-performance and low bleed properties. 251 mm, 0. In the GC-MS analysis study, 0.25 mm film thickness) from Thermo Scientific were employed. An application of a Trace GC Ultra/ISQ Single Quadruple MS, TG-5MS was used for these tests. A 70 eV electron ionizer and a helium flow-rate of 1 mL/min served as gas chromatography-mass spectrometry detector's carrier gases. This is for one sample volume of 1μL in which injection is thought to be. We set the injector and MS transfer line temperature to 280 ◦C (433 ◦F). The temperature program was as follows: starting at 40 ◦C (3 minutes) and the final temperature at 280 ◦C (5 minutes), the oven temperature was programmed to increase at 5 ◦C per minute until it reached 280 ◦C [8, 9]. With the help of the percentage relative peak area, we were able to measure and determine the amount of each component in the sample that was successfully drawn out. The chemicals had been tentatively identified by comparing their respective retention times and mass spectra with a comparison datasource from the library of GC-MS system, and came from the NIST and Wiley libraries.

Study of the Antifungal Efficacy of Fruit Extracts

Ethyl acetate and ethanol fruit extract's antifungal activity has been investigated using Sabouraud Dextrose Agar (SDA) and the mixing method. From each concentration, 0.1 mL was transferred to a Petri dish. Following the addition of the SDA medium and the subsequent drying of the dishes, a sterile cork borer was used to extract a 5 mL disc from each fungus, which was then placed on top of the growth medium. For a duration of 7 days, the petri dishes are kept at a temperature of 25°C ± 2. The diameter of the inhibitory zone, measured in millimeters (mm), is used to determine the antifungal activity of the fruit extracts.

Percentage inhibition of diameter growth percentage inhibition of diameter growth PIDG (%):

Diameter of sample – Diameter of control / diameter of the control × 100

Antioxidant (Peroxynitrite scavenging, Hydroxyl radical scavenging and Nitric oxide radical scavenging) activity

Peroxynitrite scavenging

An upper pre-frost precipitate was collected to prepare the peroxynitrite solution and its concentration was determined at 302 nm the wavelength (ε = 1670 M-1 cm-1). In the process of ascertaining the peroxynitrite scavenging activity, an Evans Blue bleaching assay was employed. A slightly modification was done to the ordinary carried through the method of the assay. 5 μM EB, and several plant extract concentrations (0-200 μg/ml) and 1 mM peroxynitrite. We made a measurement at 611 nm wavelength after 30 minutes of incubation at 25 ° C. The comparison between ONOO- % scavenging in both the sample and control was used to make these determinations. Ind fewer than 6 runs did the experiments. Gallic acid was the reference material selected for this task.

Hydroxyl radical scavenging

The system consisting of Fe3+, ascorbate, EDTA, and H2O2 (the Fenton reaction) was used to produce hydroxyl radicals [11]. The final volume of the combination was 1 ml. Prior to adding 1 ml of 1% aqueous TBA and incubating the mixture at 90°C for 15 minutes to develop the color, 0.5 ml of the reaction mixture was added to 1 ml of 2.8% TCA after 1 hour of incubation at 37°C.

Absorbance at 532 nm relative to a suitable blank solution was measured after cooling. We ran each test six times. As a control, we utilized mannitol, which is a classic OH scavenger. A comparison was made between the test and blank solutions in order to determine the percentage inhibition.

Nitric oxide radical scavenging

Since an aqueous sodium nitroprusside (SNP) solution reacts with nitric oxide at a pH level prevailing in the organism to form nitrite ions, these can be detected and evaluated using the Griess-Illosvoy reaction [12]. The final volume of basophilic staining featured phosphate buffered saline with a neutral pH of 7. (a) a high concentration range of the test solution from 0-70µg/ml, (b) 10 mM of SNP. Then, an approximately 1 ml volume of sulfanilamide solution (clearly 0.33% in a 20% acetic acid of glacial composition) was added, after 150 minutes of incubation interval at room temperature, and the mixture was permitted to stand for 5 min. NED (2 ml) is added to the mixture prior to leaving it to stand for 30 minutes at 25°C, after which the crimson balls show precipitation of the protein. 1% w/v) was added. Blank assay was employed to prove the spectrophotometric absorption at 540 nm with respect to the produced pinsky chromophore which involves nitrite ions diazoniation with sulphanilamide followed by subsequent coupling with NED. For each test, we repeated every trial 6 times. What is curcumin, Curcumin was employed as a reference element.

Statistical analysis

The mean ± SD of six measurements is used to represent all data. We used KyPlot 2.0 beta 15 (32 bit) for our statistical analysis. The method $Y = 100*A1/(X + A1)$ was used to determine the IC50 values. Here, A1 is the IC50, Y is the response ($Y = 100\%$ when $X = 0$), and X is the inhibitory concentration. Paired t tests were used to compare the IC50 values. Significant results were defined as $p < 0.05$.

Results and Discussion:

Solvent extraction method as well as how much active principle is in the crude extracts are key factors determining the antifungal activity. C. colocolioce sus. The plant extracts, as alcoholic preparation, contain high levels of flavonoids and tannins [13]. The role of flavonoids and alkaloids is also widely recognized in the defense against bacteria. Through production of chemical substances, bacteria plasmolysis, both cellular wall and content damage, and finally cell death are accomplished. However, the fungal development of resistance to active plant substances is rendered either possible due to the enzyme produced by a fungus, the removal of all active chemicals, the fungicide target molecule being insensitive or the modification of genes that encode fungicide target. Combined with these substances are substances with a propensity to cross the cytoplasmic membrane thereby disrupting the microbial cells' replication capabilities by binding to the active sites of the enzymes. The secondary metabolites detected by GC-Mass technology are: 9-Octadecenoic acid, trans-Zeaxanthin, tetraneurin-A, Lycoxanthin, N-(4-Hydroxyphenyl-d4)retinamide, 1-tert-Butyl-4,4-diphenylpiperidine, Thiocarbamoylthioacetic acid, Lucenin-2, 4-(2,3-Diphenylcyclopropyl)phenol, 5-Aminoisothiazol-3-yl)methanol, 4,8,12,15,19,21-tetracosahexaenoic acid, and Ethyl trans-2-phenylcyclopropanecarboxylate. The bioactivity of Citrullus colocynthis fruit extract (Ethyl acetate and ethanol) and conventional antibiotics against six fungus and yeast: into the antifungal activity of secondary metabolites is a study.

Alternaria alternaria (14.09 ± 0.28 and 17.61 ± 0.32), Aspergillus flavus (22.30 ± 0.45 and 23.09 ± 0.47), Trichophyton rubrum (16.00 ± 0.49 and 20.74 ± 0.41), Fusarium oxyporum (24.08 ± 0.48 and 15.11 ± 0.49), Cladosporium herbarum (14.79 ± 0.28 and 19.00 ± 0.36), Cladosporium herbarum (6.10 ± 0.02 and 7.05 ± 0.04), Candida albicans (21.00 ± 0.43 and 15.11 ± 0.49), and standard antibiotics (23.31 ± 0.48 and 27.09 ± 0.51) for Voriconazole (VCZ) and Amphotericin B (AmB) respectively. With an activity level of 24.08 ± 0.48, the metabolites of Citrullus colocynthis were extremely effective against Fusarium oxyporum.

Dangerous to natural processes is creation of pharmaceuticals from natural sources mostly caused by pathogenic fungus due to growth of resistance to antibiotics. Hence, a P. expansus and A. flavus cultures were provided with C. colocynthis fruit extract, and as a result achieved a growth inhibition [14]. Studies of C. colocynthis prove anti-inflammatory, antibacterial, and antifungal properties henceful for this. The recorded fatality rate from these fungi has been on a trouble rise over the recent years. This has been because of the increasing resistance to medications and crossing over from the resolved species in research. These antifungal drugs are complacently effective, unfortunately in spite of the fact that the number of antifungal medicines are in dire need of expansion. The most commonly found species are Candida and Aspergillus, two resistant fungus, give rise to their fascinating changing patterns of susceptibility to drugs. Therefore, the treatment of the fungal infection by using the conventional therapy is now just a wish.

Water-soluble glucosides and resins compounds which are spatially-bound, and dissolved in water may downregulate the membrane-induced enzymatic activity. The extract from its fruits also contains alkaloids like colocynthidin and colocynthin. This allergenic substance, tannins and flavonoids, astrays the cell membranes leads to change in cell's fabric by binding to and depositing on proteins [16, 17]. This makes components to release out from the cell and the cell itself ends in dying. A potential consequence of chemical exposure may be the inhibition of pathways leading to the enzyme activity, such as protein binding or DNA/RNA formation-preventing the latter.

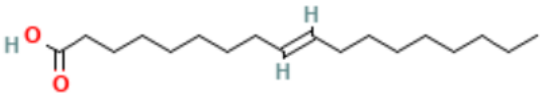
Citrullus colocynthis fruit extract (in ethanol, ethyl acetate, and standards) and its antioxidant activity against peroxynitrite, hydroxyl radicals, and nitric oxide. A variety of extract types were documented, including crude, ethyl acetate fraction, ethanol fraction and standard recorded 700.67±35.91, 645.51±32.82 and Gallic acid (standard) 856.08±37.05 respectively of Peroxynitrite scavenging. Although peroxynitrite (ONOO-) is not very unstable on its own, it becomes the extremely reactive peroxynitrous acid (ONOOH) when protonated. Oxidative stress and tissue damage result from the production of an excess of ONOO-. By oxidizing it, peroxynitrite makes Evans Blue bleachable. The current findings indicate that the plant extract has a higher activity than the reference gallic acid in inhibiting Evans Blue bleaching by scavenging peroxynitrite. **Figure 7.** While recorded 301.82±27.25, 236.03±20.19, and Mannitol (standard) 549.15±31.27respectively Hydroxyl radical scavenging potential **Figure 8.**

At the same time record 39.12± 2.58, 22.89± 2.16, and Curcumin (standard) 75.73±4.94 respectively Nitric oxide radical scavenging potential. Through the pie graph in Figure 9, compared to the standard Curcumin, crude and other fractions demonstrated the remarkable high percentage of the inhibition of nitric oxide radical scavengers activities ($P<0.05$). Of course, people are very familiar about nitric oxide as it is one of the most important factors in different kinds of inflammation. As this compound is highly toxic, it causes uterus vasculature dysfunction at certain amounts, which leads to septic shock, and additionally it has been documented that it is associated with colon cancer and other inflammatory pathologies including ulcerative colitis, type 1 diabetes, multiple sclerosis, arthritis, and type 1 diabetes. On the NO's interaction with the superoxide radical anion O2-•NOO- is being formed, which is a very reactive anion •Toxicity of NO is increased by 2 orders. Through the process of oxygenation, the nitric oxide that sodium nitroprusside forges goes through the chemical transformation that results in the production of nitrite. Nitric oxide is one of the by-products of metabolism. This natural process of converting nitrates to nitrates is known as a nitric oxide process. By inhibiting oxygen from taking part in this process [23–26], the extract helps prevent the generation of nitric oxide. This evaluation showed that the under study extract was having much better active scavenging of nitric oxide than active nutricurmin alone

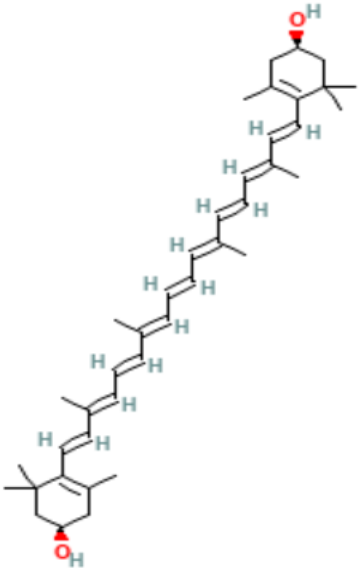
Table 1. Screening of bioactive compounds of *Citrullus colocynthis* using GCMS Technique.

Compound	Molecular Formula	Molecular Weight
9-Octadecenoic acid	$C_{18}H_{34}O_2$	282.5 g/mol
trans-Zeaxanthin	$C_{40}H_{56}O_2$	568.9 g/mol
tetraneurin-A	$C_{17}H_{22}O_6$	322.4 g/mol
Lycoxanthin	$C_{40}H_{56}O$	552.9 g/mol
N-(4-Hydroxyphenyl-d4)retinamide	$C_{26}H_{33}NO_2$	395.6 g/mol
1-tert-Butyl-4,4-diphenylpiperidine	$C_{21}H_{27}N$	293.4 g/mol
Thiocarbamoylthioacetic acid	$C_3H_5NO_2S_2$	151.21 g/mol
Lucenin-2	$C_{27}H_{30}O_{16}$	610.5 g/mol
4-(2,3-Diphenylcyclopropyl)phenol	C21H18O	286.4 g/mol
5-Aminoisothiazol-3-yl)methanol	$C_4H_6N_2OS$	130.17 g/mol
4,8,12,15,19,21-tetracosahexaenoic acid	$C_{24}H_{36}O_2$	356.5 g/mol
Ethyl trans-2-phenylcyclopropanecarboxylate	$C_{12}H_{14}O_2$	190.24 g/mol

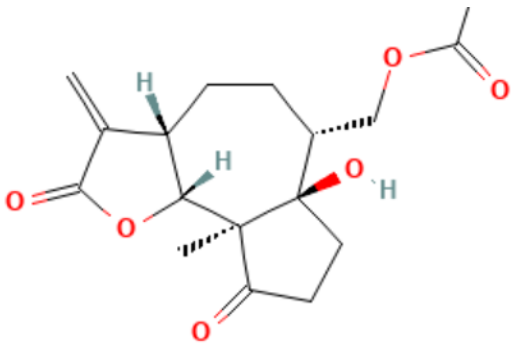
9-Octadecenoic acid



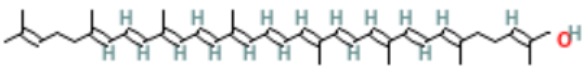
trans-Zeaxanthin



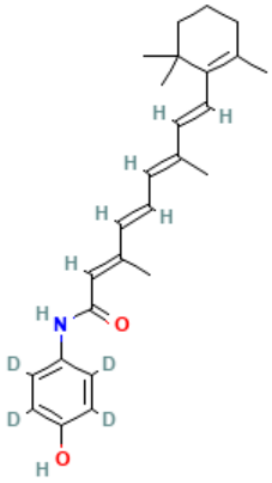
tetraneurin-A



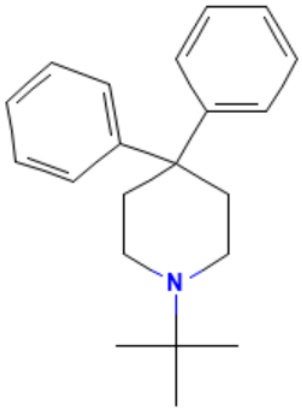
Lycoxanthin



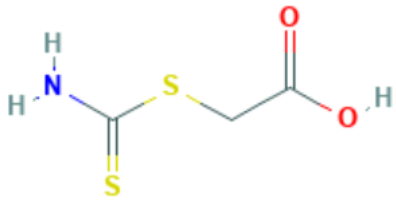
N-(4-Hydroxyphenyl-d4)retinamide



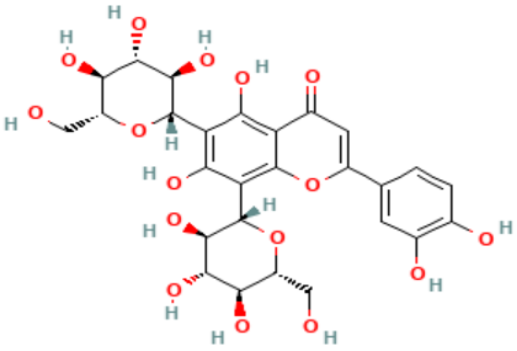
1-tert-Butyl-4,4-diphenylpiperidine



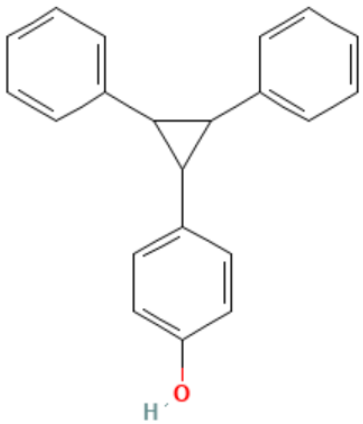
Thiocarbamoylthioacetic acid



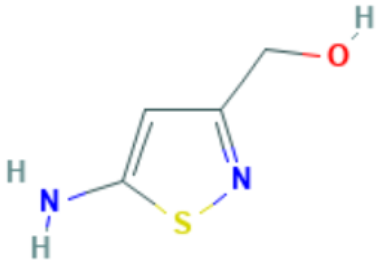
Lucenin-2



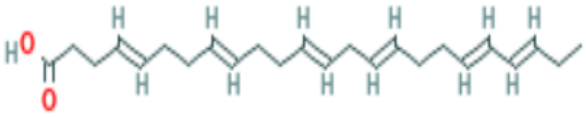
4-(2,3-Diphenylcyclopropyl)phenol



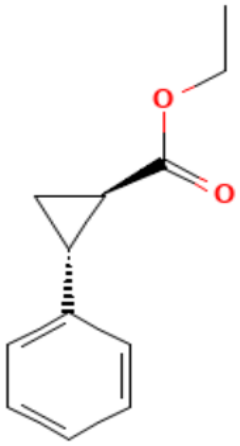
5-Aminoisothiazol-3-yl)methanol



4,8,12,15,19,21-tetracosahexaenoic acid



Ethyl trans-2-phenylcyclopropanecarboxylate



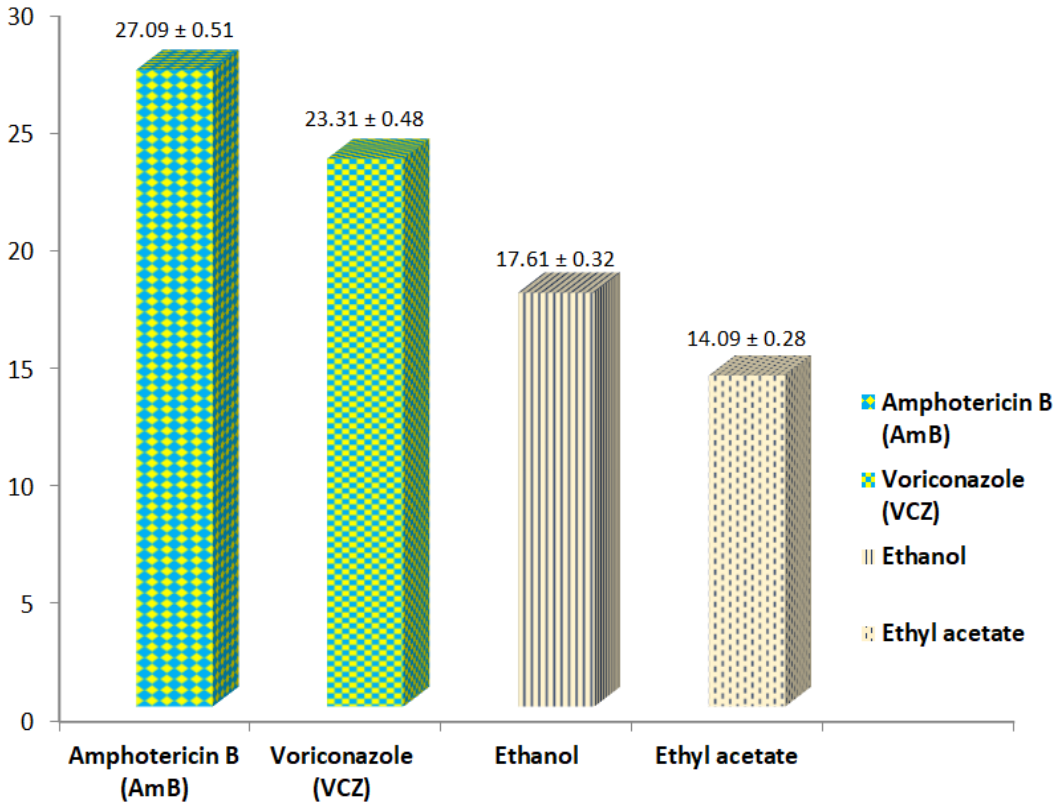


Figure 1. Anti-Fungal activity of secondary metabolites compounds derived from fruit extracts of *Citrullus colocynthis* against *Alternaria alternaria*

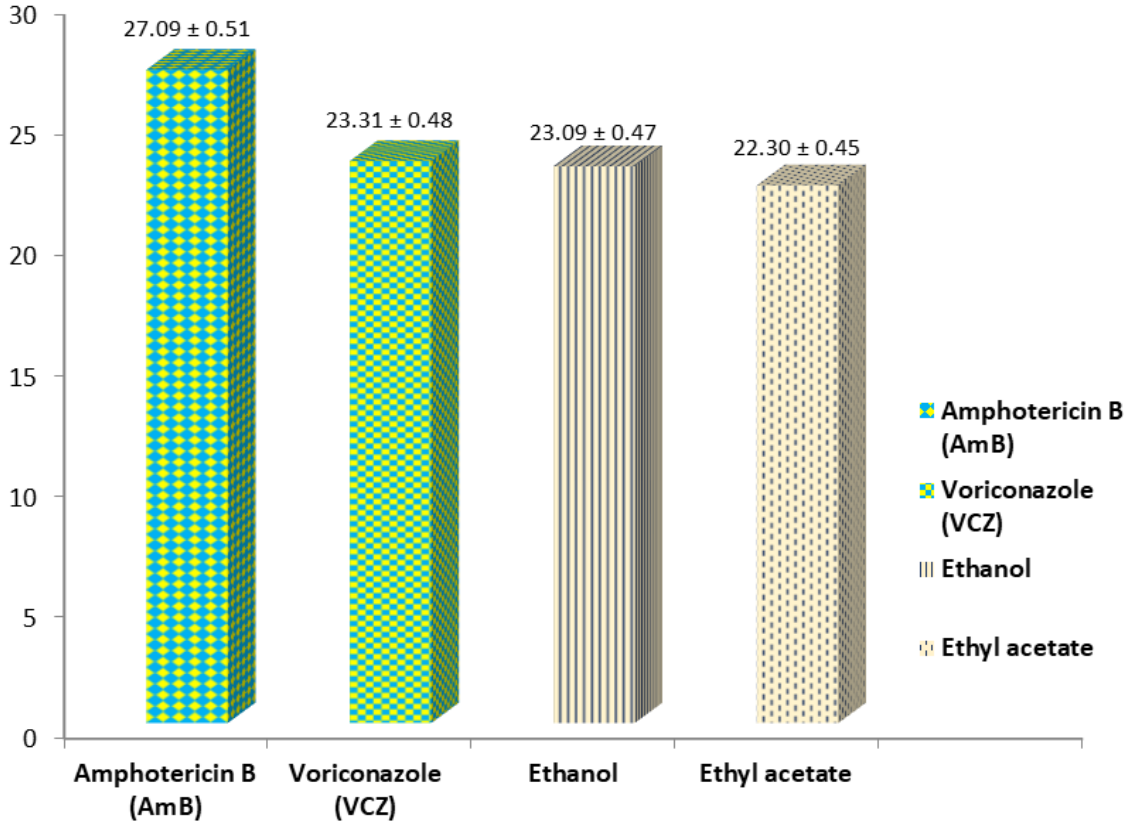


Figure 2. Anti-Fungal activity of secondary metabolites compounds derived from fruit extracts of *Citrullus colocynthis* against *Aspergillus flavus*

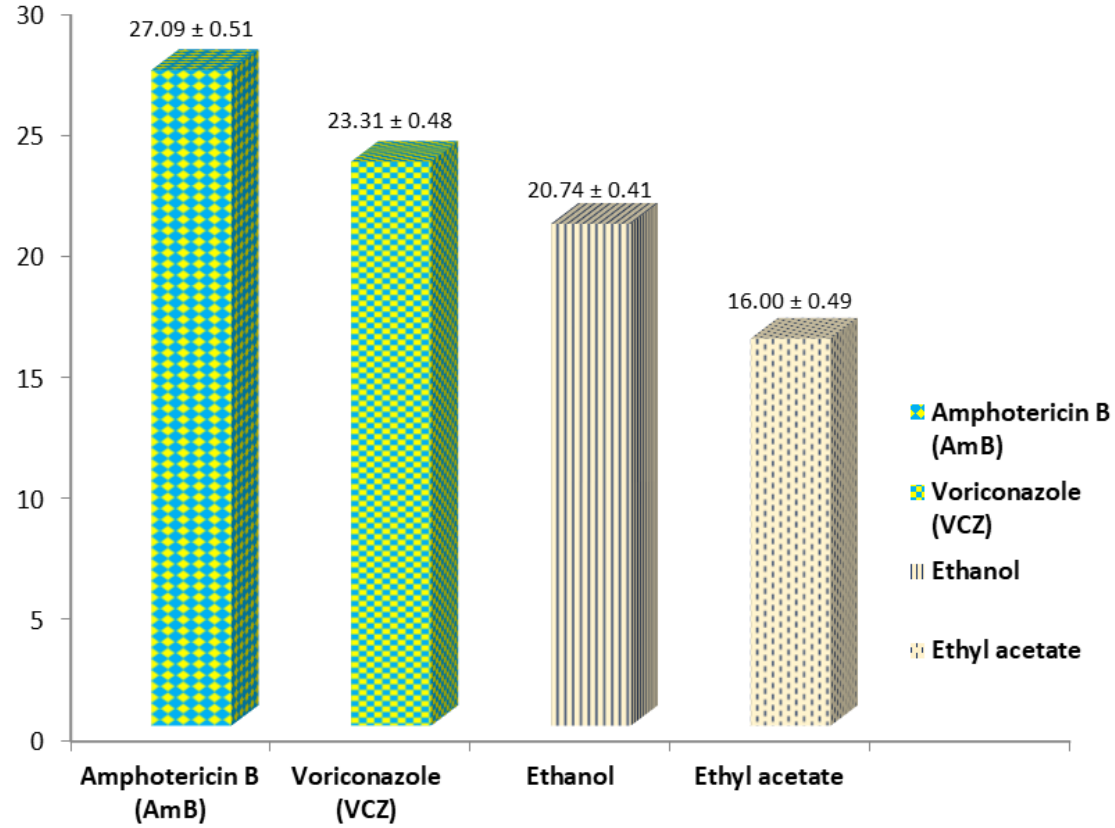


Figure 3. Anti-Fungal activity of secondary metabolites compounds derived from fruit extracts of *Citrullus colocynthis* against *Trichophyton rubrum*

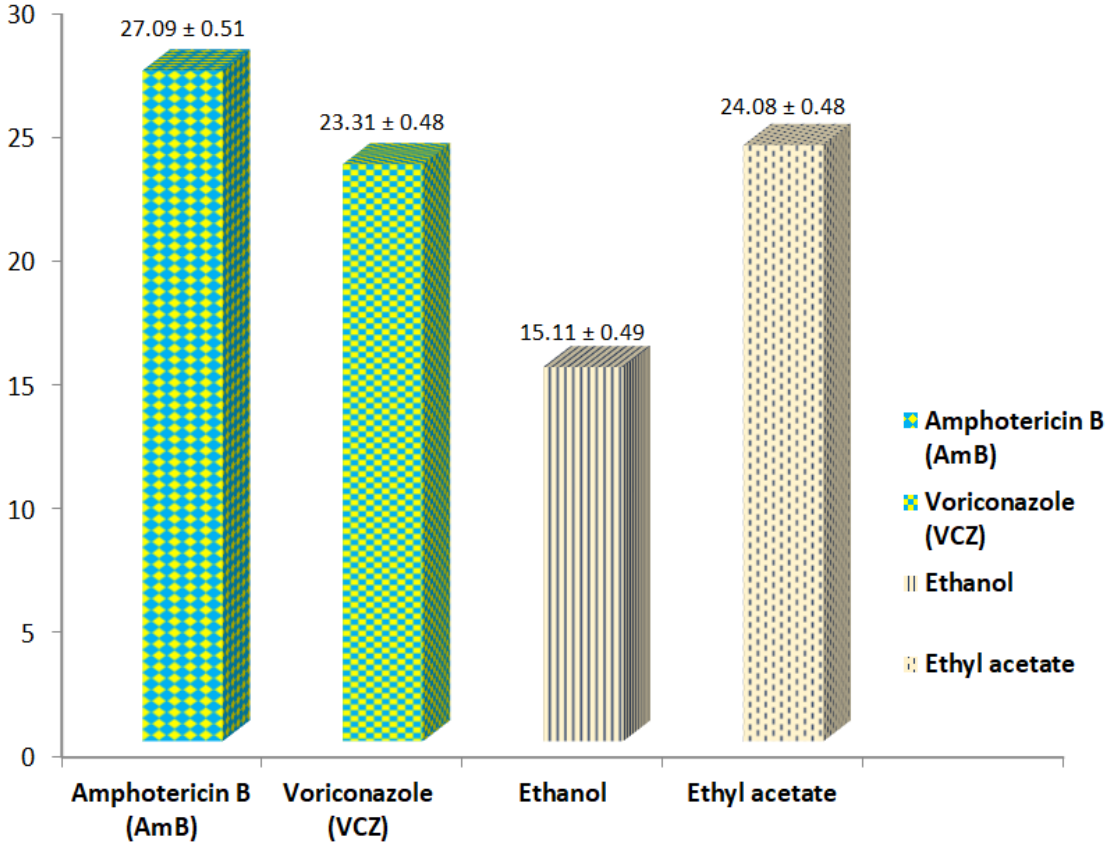


Figure 4. Anti-Fungal activity of secondary metabolites compounds derived from fruit extracts of *Citrullus colocynthis* against *Fusarium oxyporum*

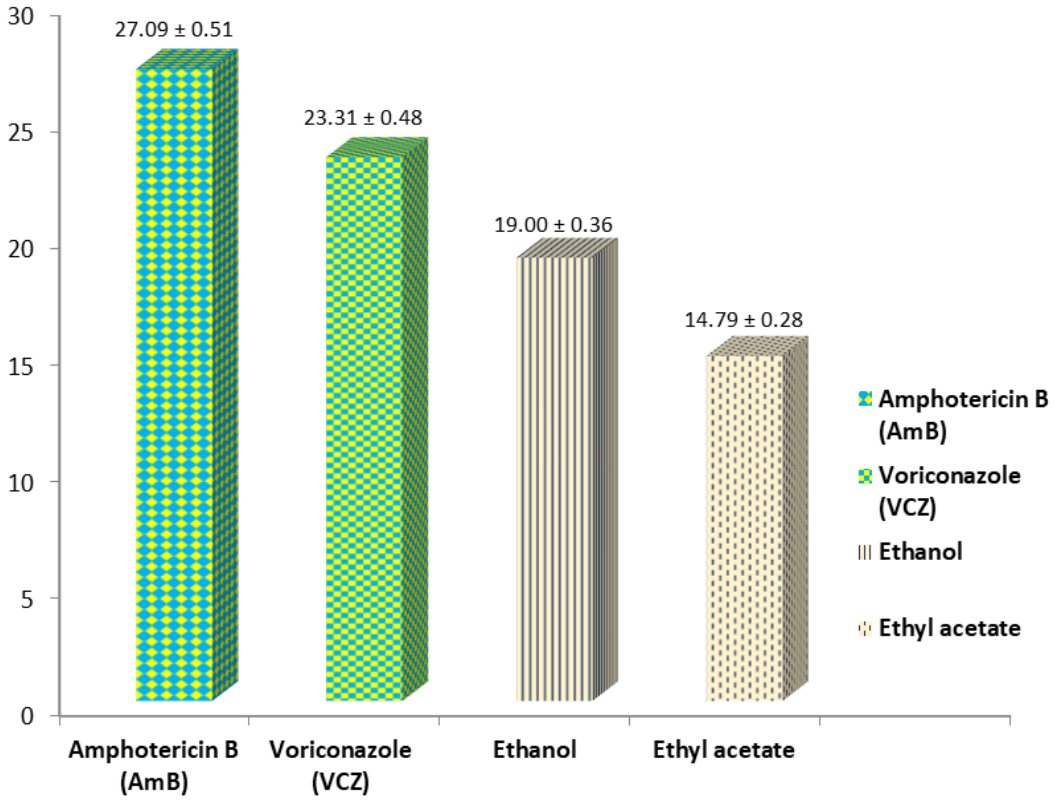


Figure 5. Anti-Fungal activity of secondary metabolites compounds derived from fruit extracts of *Citrullus colocynthis* against *Cladosporium herbarum*

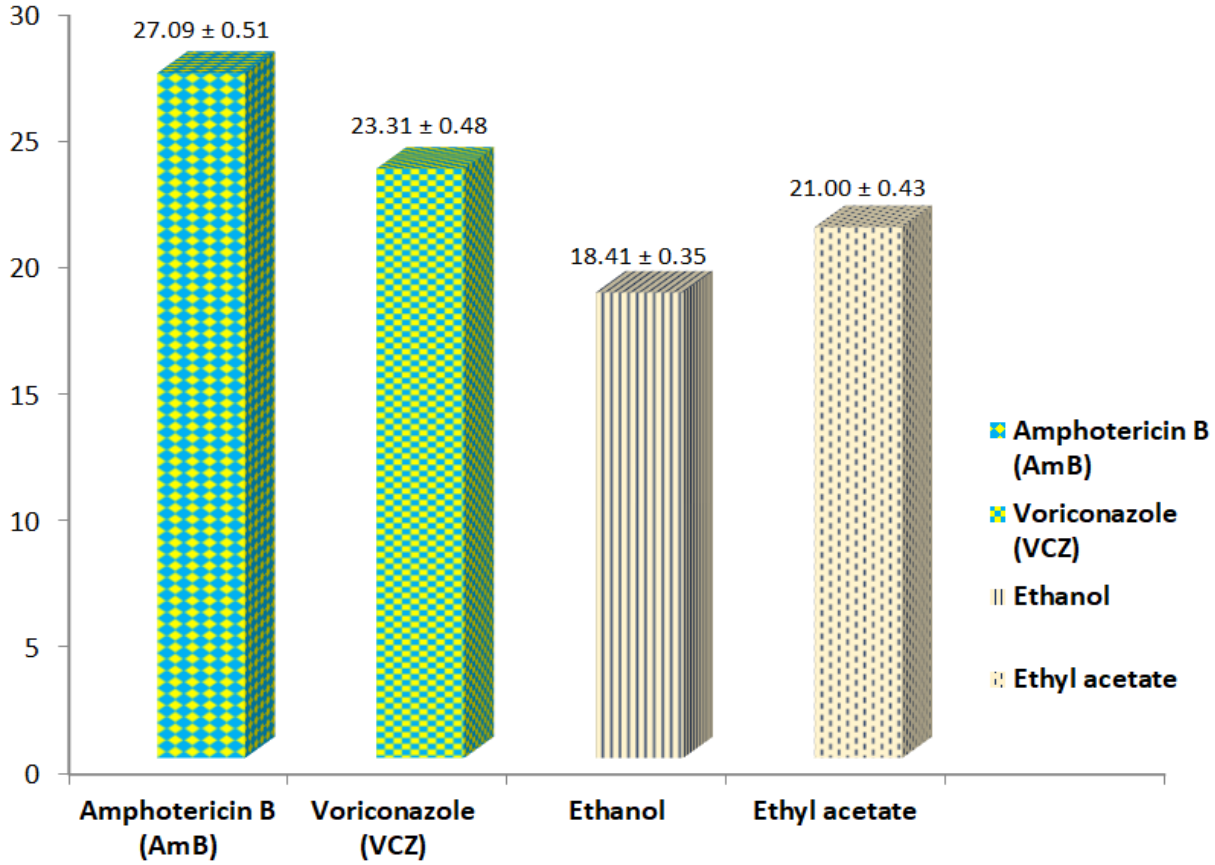


Figure 6. Anti-Fungal activity of secondary metabolites compounds derived from fruit extracts of *Citrullus colocynthis* against *Candida albicans*

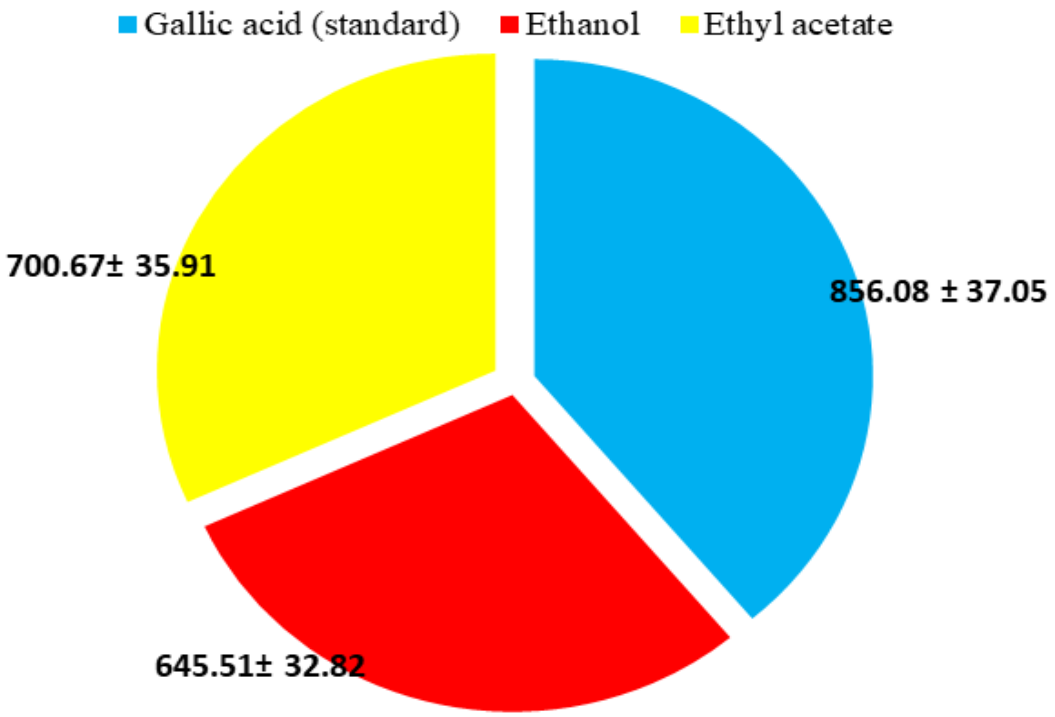


Figure 7. Antioxidant activity (Peroxynitrite scavenging) of fruit extract (Ethyl acetate fraction, Ethanol fraction and Gallic acid (standard)) of *Citrullus colocynthis*

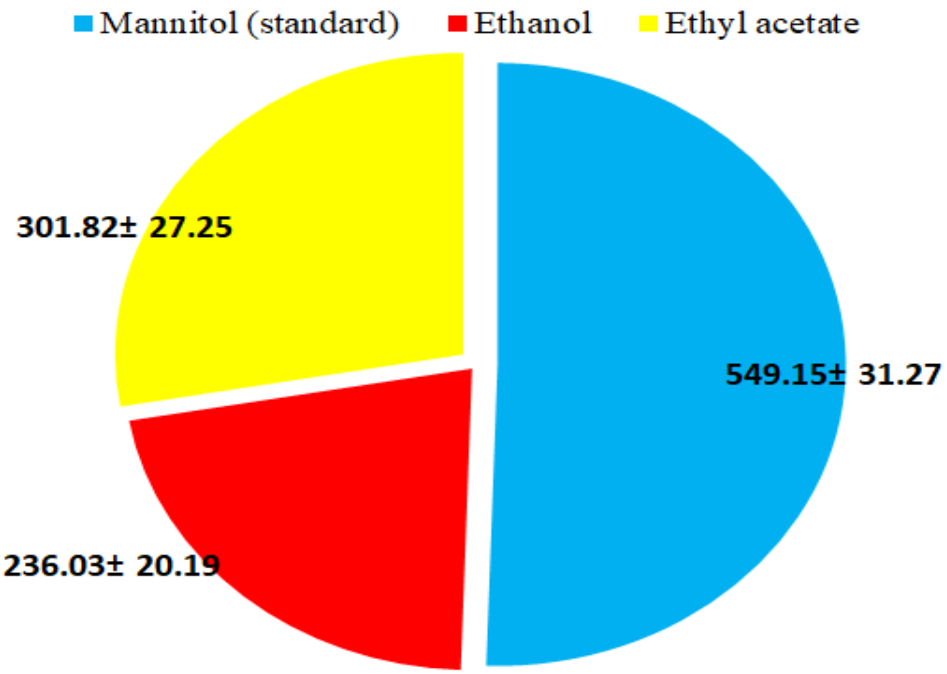


Figure 8. Antioxidant activity (Hydroxyl radical scavenging) of fruit extract (Ethyl acetate fraction, Ethanol fraction and Mannitol (standard) of *Citrullus colocynthis*

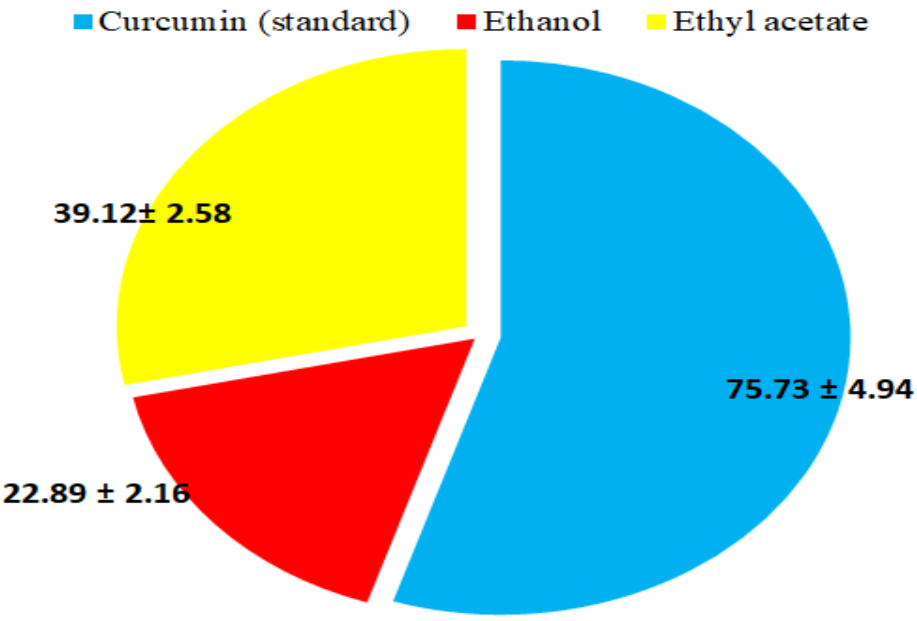


Figure 9. Antioxidant activity (Nitric oxide radical scavenging) of fruit extract (Ethyl acetate fraction, Ethanol fraction and Curcumin (standard)) of *Citrullus colocynthis*

Conclusion:

According to our study, C. colocynthis demonstrates antifungal activity towards pathogenic fungi, and maybe these compounds can be of benefit during treatment of fungal infections in the future. Citrullus oxypromium metabolites were equally as good in the fight against Fusarium oxyporum, with scores of 24 as well. 0.8 ± 0.048 The concluding part of the paper demonstrates that a Citrullus colocynthis is a

likely plant, which has medical value extractives to being used in the therapy of diverse fungal infections. Besides, these discoveries suggest that such substances be taken in consideration into further screenings with a view to understand their pharmacological potential. That would mean distinguishing a new drug.

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