

## Biofunctional molecules from *Citrullus colocynthis*: An HPLC/MS analysis in correlation to antimicrobial and anticancer activities

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### Abstract

**Background:** *Citrullus colocynthis* belongs to Family *Cucurbitaceae*. It grows widely in Egypt and Sudan and it has been used in folk medicine of Sudan and many other African countries as anti inflammatory, anti diabetic, and antioxidant agent. **Objectives:** To evaluate the antibacterial, antifungal, antiviral, anticancer activities of ethanolic crude extracts of the fruits, leaves, seeds and roots of this plant, as well as identifying them HPLC/MS. **Materials and Methods:** Dried fruits, seeds, leaves and roots of *C. colocynthis* were powdered and passed through a 40- mesh, then, the powders were extracted with 95% ethanol in a soxhlet apparatus. The residues were dried under reduced pressure in rotary evaporator. Crude extract from different plant parts were evaluated biologically and phytochemically. **Results:** All extracts showed good antifungal activities with inhibition zone ranges between  $15.1 \pm 0.32$  to  $25.6 \pm 0.16$  mm. In terms of plant organ, fruits were the most active. In term of fungal strain *Aspergillus fumigatus* and *Geotricum candidum* were the most sensitive. Against tested Gram +ve, all extracts showed good activities except roots, while antibacterial activity against Gram -ve showed that the fruits extract have good activity as it was the sole extract with activity against *Pseudomonas aeruginosa*. Test for antiviral activities showed moderate to weak inhibitions of cytopathic effect (CPE). Anticancer activities of different crude extracts showed that fruits had significant antitumor activities against all tested cell lines, the IC<sub>50</sub> values were 24.6, 16, 18.5 and 19.7  $\mu\text{g/ml}$  for HCT-116, MCF-7, Hep-G2 and Caco-2 respectively. Seeds extract was only active on HCT-116 and Hep G2 with IC<sub>50</sub> =21.2  $\mu\text{g/ml}$  for HCT-116 and 22.4  $\mu\text{g/ml}$  for Hep G2. Leaves extract was only active against Hep G2 cancer cell line with IC<sub>50</sub> 19.7  $\mu\text{g/ml}$ . Roots extract show weak antitumor activity on tested cell lines (IC<sub>50</sub> values > 30 $\mu\text{g/ml}$ ). HPLC/MS qualitative and quantitative analysis of different organs extracts revealed the presence of 21 compounds identified as fourteen cucurbitacins, three flavonoids, three tannins, and one sterol. The presence of cucurbitacins can explain most of the biological activities. **Conclusion:** The biological activities of colocynth different parts are due to the presence of secondary metabolites mainly cucurbitacins in addition to flavonoids and tannins. These activities prove the use of this plant in folk medicine and deserve much more future exploration targeting their discovery in unexplored sources and their derivatives for improving their anticancer and antimicrobial abilities.

**Keywords:** *Citrullus colocynthis*, crude extracts, antifungal, antibacterial, antiviral, anticancer, HPLC/MS

### 1. Introduction

Infectious diseases caused by bacteria, fungi, viruses and parasites are still a major threat to public health, despite the tremendous progress in human medicine. Their impact is particularly large in developing countries due to relative unavailability of medicine and emergence of widespread drug resistant microorganisms (Okeke et al., 2005). Therefore search for new antimicrobial substance must be continued and all possible strategies should be exploring (Clardy and Walsh, 2004). Rational drug design does not always yield effective antimicrobials. In past, potent enzyme inhibitors have been successfully designed and synthesized but they had only moderate antimicrobial activity (Silver and Bostian, 1990).

Current research on nature of molecules and products primarily focused on plant since they can be sourced more easily and be selected on the basis of their ethanomedicinal uses (Verpoorte et al., 2005). The use of ethanopharmacological knowledge is one of the attractive ways to enhance the probability of success in new drug finding efforts (Cordell and Colvard, 2005; Patwardhan, 2005). At the same time, discovery and identification of new antitumor drug with low side effects on immune system has become an essential goal in many studies of immuno-therapies (Xu et al., 2009). Despite many efforts, multi drug resistance is still considered as a major drawback in chemotherapy of cancer which has been the subject of exhaustive experiments recently (Gottesman et al., 2002).

Medicinal plants contain several active principles with specific therapeutic effects. They represent a source of chemical compounds such as tannins, flavonoids, saponins, resins and alkaloids with curative properties, often not provided by synthetic chemical compounds (Fabricant and Farnsworth, 2001). *Citrullus colocynthis* belongs to the family of Cucurbitaceae. Members of this family are generally dioeciously herbs

which may be prostrate or climbing by means of tendrils. *Citrullus colocynthis* had been used medically since ancient times. Traditionally fruit of was used for the treatment of microbial diseases, jaundice, inflammation, ulcer, diabetes and urinary diseases in Asian and African countries (**Saganuwan, 2010**) and as insecticidal agent (**Rahaman et al., 2008**). The aqueous extract of *Citrullus colocynthis* possesses an antidiabetic effect and antioxidant activity (**Gurudeeban and Ramanathan, 2010**). It had been used as a strong laxative to treat refractory edema, amenorrhea, nerves pain fever, snake bite, antiparasite (worms), muscle pain in hand and feet effects are its other important features. Recent research shows that consumption of *C. colocynthis* with radioactive radiations has effects on growth of cancerous tumors such as larynx cancer **lazar(Abdel-Hassan et al., 2000; Al-Zahrani and Al-Amer, 2006; DELAZAR et al., 2006; Memon et al., 2003)**.

The objectives of this work are to demonstrate the antibacterial, antifungal, antiviral and anticancer activities in correlation to phytochemical constituents of ethanolic crude extracts from different organs of *C. colocynthis*.

## 2. Materials and methods

### 2.1 Plant material:

*C. colocynthis* fruits, seeds, leaves and roots were collected from Omdurman, Sudan ([15°39'N 32°29'E](#)), and were used to prepare the ethanolic crude extracts from different organs.

### 2.2 Bacterial and fungal strains:

Nine bacterial strains; *Staphylococcus aureus* (RCMB 010027), *Staphylococcus epidermidis* (RCMB 010024), *Streptococcus pyogenes* (RCMB 010015), *Neisseria gonorrhoeae* (RCMB 010076), *Proteous vulgaris* (RCMB 010085), *Klebsiella pneumoniae* (RCMB 0010093), *Shigella flexneri* (RCMB 0100542), *Pseudomonas aeruginosa* (RCMB 010043) and *Escherichia coli* (RCMB 010056) and four fungal strains; *Aspergillus fumigatus* (RCMB 02564), *Candida albicans* (RCMB 05035), *Geotricum candidum* (RCMB 05096) and *Trichophyton mentagrophytes* (RCMB 0925) were obtained from the Regional Center of Mycology and Biotechnology Antimicrobial Unit (RCMB), Cairo, Egypt.

### 2.3 Cells and viral strains:

Vero cells (African green monkey kidney cell line, Hepatitis A virus (HAV), Herpes Simplex Virus type I (HSV-I) and Herpes Simplex Virus type II (HSV-II) were obtained from virology center, Faculty of Medicine (Girls), Al-Azhar University. Vero cells were maintained in MEM supplemented with 10% Fetal Bovine Serum (FBS), 25 µg/ml gentamicin and 200 mM L-glutamine.

### 2.4 Cancer Cell lines:

Colon adenocarcinoma (HCT-116), breast adenocarcinoma (MCF-7), liver carcinoma (Hep-G2) and intestinal adenocarcinoma (Caco-2) cell lines were obtained from National Institute of Cancer, Cairo University, Cancer biology department, pharmacology unit, Cairo, Egypt. Cells were routinely cultured in DMEM (Dulbecco's Modified Eagle's Medium), which was supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, containing 100 units/ml penicillin G sodium, 100 units/ml streptomycin sulphate, and 250 mg/ml Amphotericin B.

### 2.5 Preparation of plant crude extracts:

500 grams of each plant organ (fruits, leaves, seeds and roots) were powdered and passed through a 40-mesh sieve, extracted with 95 % ethanol in a soxhlet apparatus, and then the solvent was removed and dried at 40°C under reduced pressure with a rotary evaporator.

### 2.6 Evaluation of Antimicrobial Activity of *C. colocynthis* crude extracts:

The antibacterial activity of ethanolic crude extracts of different parts of *C. colocynthis* was evaluated by the Kirby-Bauer method (**Bauer et al., 1966**). Briefly, sterile paper discs (6 mm) were loaded with 25 µL of *C. colocynthis*/SNPs or the tested plant extracts. Several isolated colonies of each standard strain was selected from a culture of 12–18 hrs on nutrient agar (Oxoid, UK) and dissolved in sterile normal saline solution. The suspension was adjusted to match a solution of 0.5 McFarland turbidity standards at 600 nm using spectrophotometer Shimadzu dual beam UV–visible spectrophotometer, model UV-1650 (Kyoto/Japan). The surface of MHA plates (Oxoid, UK) or Sabouraud dextrose agar plates (Oxoid, UK), were inoculated with the adjusted suspension of bacteria and fungi respectively. Impregnated discs were placed on the surface of the inoculated medium and incubated at 37 °C for 18–24 hrs and at 30–32 °C for 48 hrs for bacteria and fungi respectively, except for *Trichophyton mentagrophytes* fungus which was incubated for 14 days. The diameter of the inhibition zone was measured in millimeter, and was recorded as mean ± SD of the triplicate experiment. Ampicillins (10µg), Gentamicin (10µg) discs for bacteria, and Amphotericin B (5µg) for fungi were used as positive controls. Cultured species producing halos equal to or greater than 13 mm were considered susceptible.

## 2.7 Antiviral Assay:

The antiviral activities were tested by measuring the cytopathic effect (CPE). Briefly; Vero cells were plated in a 96-well plate at a density of 10,000 cells/well in DMEM medium with 10% FBS and incubated O/N at 37°C in a humidified incubator with 5% CO<sub>2</sub>. The following morning, the medium was changed and the cells were challenged with 10<sup>-4</sup> TCID<sub>50</sub>/ml and were simultaneously treated with two-fold serial dilutions of the tested extract and incubated at 37°C for 3 to 6 days. The infection control and untreated Vero cells control in the absence of the tested samples were included. Six wells were used for each concentration of the tested sample. The plates were observed every 24 hours under the inverted microscope until the virus in the control wells showed complete CPE. The monolayers were fixed with formalin and then stained with a 0.1% crystal violet solution. The antiviral activity was assessed by the inhibition of the cytopathic effect on the tested cell culture. Inhibition of the CPE was scored under light microscopy as negligible (less than 25%); weak (25% to less than 50%); moderate (50% to less than 75%) and strong (more than 75%). CPE was measured in three independent experiments, and each experiment was performed in triplicates, (Dargan, 1998; Vijayan et al., 2004).

## 2.8 Evaluation of in vitro cytotoxic activity of the crude extracts on tested cell lines:

MTT assay was performed to determine the cytotoxic property on HCT-116, MCF-7, Hep-G2 and Caco-2 cell lines. Briefly cell lines were seeded in 96-well tissue culture plates. Appropriate concentrations of stock solution were added and incubated for 48 hours at 37°C. Non-treated cells were used as control. Incubated cultured cell was then subjected to MTT (3-(4, 5 Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide, a tetrazole) colorimetric assay. The tetrazolium salt 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide (MTT) is used to determine cell viability in assays of cell proliferation and cytotoxicity. MTT is reduced in metabolically active cells to yield an insoluble purple formazan product. Cells were harvested from maintenance cultures in the exponential phase and counted by a hemocytometer using trypan blue solution. The cell suspensions were dispensed (100µl) in triplicate into 96-well culture plates at optimized concentrations of 1 × 10<sup>5</sup>/well for each cell lines, after a 24 hours recovery period. Assay plates were read using a spectrophotometer at 520 nm. The spectrophotometrical absorbance of the samples was measured using a microplate (ELISA) reader. The cytotoxicity data was standardized by determining absorbance and calculating the correspondent extracts concentrations. Data generated were used to plot a dose-response curve of which the concentration of extract required to kill 50% of cell population (IC<sub>50</sub>) was determined.

$$\text{Cell viability (\%)} = \text{Mean OD/ control OD} \times 100$$

The IC<sub>50</sub> values (concentration at which 50% of cells were death) against colon adenocarcinoma (HCT-116), breast adenocarcinoma (MCF-7), liver carcinoma (Hep-G2) and intestinal adenocarcinoma (Caco-2) cell lines are reported as mean± standard deviation of three independent experiments. IC<sub>50</sub> values that were greater than 30µg/ml considered insignificant, and vice versa.

## 2.9 HPLC/MS:

### 2.9.1. Chemicals:

Methanol, acetonitrile and formic acid HPLC grade were purchased from Sigma-Aldrich- Fluka (Madrid, Spain).

### 2.9.2 HPLC/MS analysis:

The chromatographic system consisted of a Dionex Ultimate 3000 HPLC (Bremen, Germany) composed of a quaternary pump with an on line degasser, a thermostated column compartment, a photodiode array detector (DAD), an auto sampler, and Chromelon software. The HPLC separation was performed on Zobrax SB-C18 column (150 mm×4.6 mm, 1.8 µm, Agilent Company, USA), at flow rate of 0.8 ml/min. The column oven temperature was set at 30°C and the injected volume was 5, 10 and 10 µL of leaves, inflorescences and stems respectively. Mobile phase consisted of two solvents, (A) methanol and (B) 0.2 % formic acid. Separation of compounds was carried out with gradient elution profile: 0 min, A: B 10:90; 36 min, A: B 70:30; 50 min, A: B 100:0; 60 min.

The HPLC/MS system consisted of electrospray ionization (ESI) interfaced Bruker Daltonik Esquire-LC Amazon SL Ion Trap Mass spectrometer (Bremen, Germany) and Dionex Ultimate 300 (Germany), mentioned above. The ionization parameters were as follows: positive ion mode; capillary voltage 4000 V, end plate voltage -500 V; nebulizing gas of nitrogen at 50 p.s.i.; drying gas of 10 l/min nitrogen at 350 °C.

## 3. Results

### 3.1 Antimicrobial activity

Antibacterial and antifungal activities were carried out for ethanolic crude extracts of the fruit parts, leaves, seeds and roots of *C. colocynthis*. These materials were tested for their ability to inhibit growth of *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Neisseria gonorrhoeae*, *Proteus*

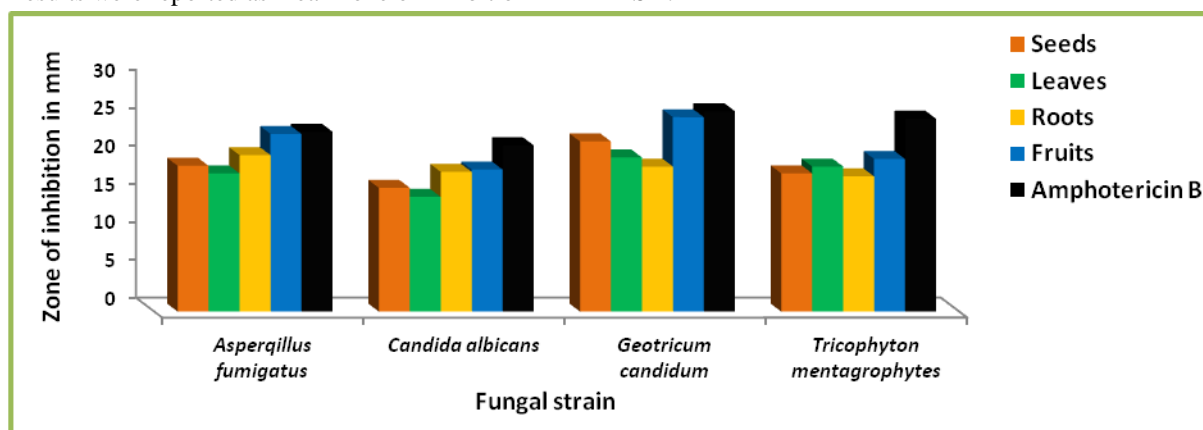
*vulgaris*, *Klebsiella pneumoniae*, *Shigella flexneri*, *Pseudomonas aeruginosa* and *Escherichia coli* and four fungal strains; *Aspergillus fumigatus*, *Candida albicans*, *Geotricum candidum* and *Trichophyton mentagrophytes*. Amphotericin B, Ampicillin and Gentamycin were used as reference drugs for fungi, gram +ve and gram –ve bacteria respectively.

From the results, all extracts showed good antifungal activities with inhibition zone ranges between  $15.1 \pm 0.32$  to  $25.6 \pm 0.16$  mm. It was found that the fruits extract of was the more effective as antifungal agent, especially against *Aspergillus fumigatus* and *Geotricum candidum* (Table 1, Fig. 1). Antibacterial activities against Gram +ve showed the activity of different part except roots extract. In term of plant organ seeds extract was the most active, while in term of strain *S. aureus* was the most sensitive (Table 2, Fig. 2). Activities against Gram –ve showed that fruits extract showed good activity, it was the sole extract exhibited anti pseudomonal activity. Roots extract wasn't active against *Proteous vulgaris* and *Neisseria gonorrhoeae*, whereas seeds extract also wasn't active against *Proteous vulgaris* (Table 3, Fig. 3)

**Table (1): Antifungal activities of crude extracts from different organs of *C. colocynthis***

Tested fungi	Ethanollic crude extract of				Amphotericin B
	Seeds	Leaves	Roots	Fruits	
<i>Aspergillus fumigatus</i>	19.2±0.34	18.2±0.28	20.6±0.29	23.4±0.24	<b>23.7±0.10</b>
<i>Candida albicans</i>	16.3±0.52	15.1±0.32	18.4±0.18	18.7±0.62	<b>21.9±0.12</b>
<i>Geotricum candidum</i>	22.4±0.31	20.3±0.25	19.1±0.35	25.6±0.16	<b>26.4±0.20</b>
<i>Trichophyton mentagrophytes</i>	18.2±0.19	19.1±0.21	17.8±0.11	20.1±0.42	<b>25.4±0.16</b>

Results were reported as mean zone of inhibition in mm ± SD.

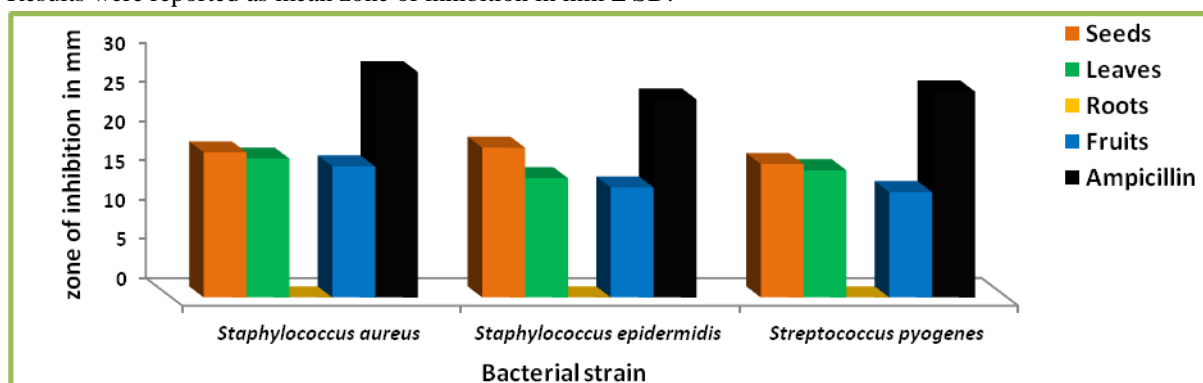


**Figure (1): Antifungal activities of crude extracts from different organs of *C. colocynthis***

**Table (2): Antibacterial activities of crude extracts from different organs of *C. colocynthis* on Gram +ve bacteria.**

Tested Gram +ve	Ethanollic crude extract of				Ampicillin
	Seeds	Leaves	Roots	Fruits	
<i>Staphylococcus aureus</i>	18.6±0.21	17.8±0.33	NA	16.8±0.28	<b>28.9±0.14</b>
<i>Staphylococcus epidermidis</i>	19.2±0.29	15.3±0.12	NA	14.1±0.39	<b>25.4±0.18</b>
<i>Streptococcus pyogenes</i>	17.1±0.42	16.3±0.39	NA	13.5±0.41	<b>26.4±0.34</b>

Results were reported as mean zone of inhibition in mm ± SD.



**Figure (2): Antibacterial activities of crude extracts from different organs of *C. colocynthis* on Gram +ve bacteria**

Table (1): Antibacterial activities of crude extracts from different organs of *C. colocynthis* on Gram-ve bacteria.

Tested G -ve	Ethanollic crude extract of				Gentamycin
	Seeds	Leaves	Roots	Fruits	
<i>Neisseria gonorrhoeae</i>	15.1±0.51	15.9±0.29	NA	19.4±0.13	<b>19.9±0.18</b>
<i>Proteous vulgaris</i>	NA	11.9±0.33	NA	17.7±0.42	<b>23.4±0.30</b>
<i>Klebsiella pneumoniae</i>	17.4±0.39	20.6±0.19	18.5±0.62	22.9±0.31	<b>26.3±0.15</b>
<i>Shigella flexneri</i>	15.8±0.28	16.3±0.17	16.7±0.55	22.4±0.28	<b>24.8±0.24</b>
<i>Pseudomonas aeruginosa</i>	NA	NA	NA	14.2±0.40	<b>25.3±0.18</b>
<i>Escherichia coli</i>	19.7±0.37	18.5±0.42	17.2±0.17	17.6±0.13	<b>17.3±0.12</b>

Results were reported as mean zone of inhibition in mm ± SD.

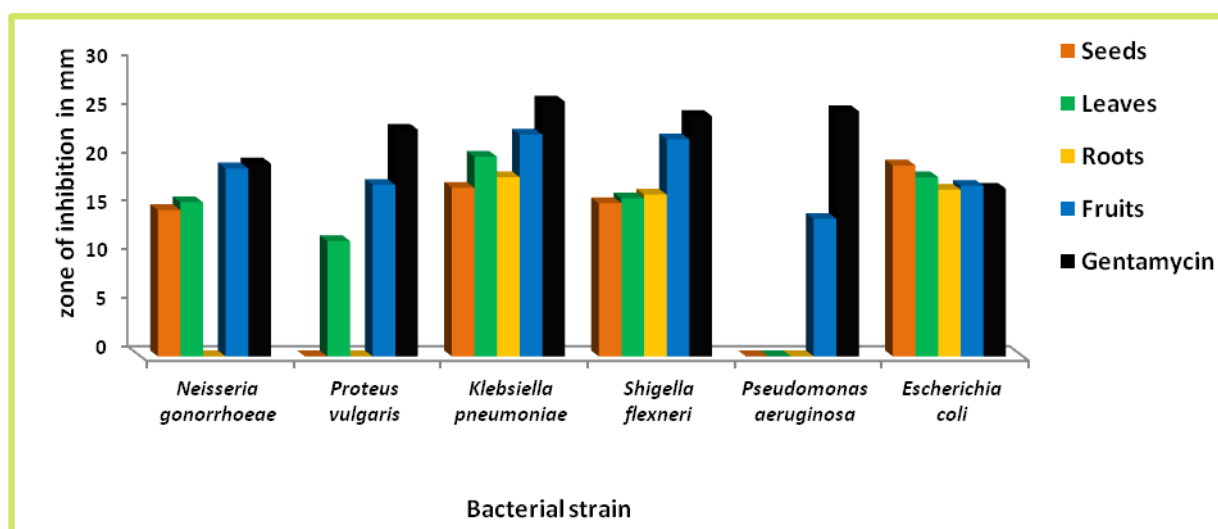


Figure (3): Antibacterial activities of crude extracts from different organs of *C. colocynthis* on G –ve bacteria.

### 3.2 Antiviral activity

Antiviral activities of different crude extracts were assessed by measuring the cytopathic effect (CPE). Antiviral evaluation showed moderate (50-75%) to weak (25-50 %) inhibitions of CPE (Table 4). Leaves extract didn't show antiviral activity on HSV-1 and HSV-2 strains. Root extracts showed the highest activity as compared to other plant organs. HSV-2 was the most sensitive, whereas, HAV was the most resistant.

Table (4): Antiviral activities of crude extracts from different organs of *C. colocynthis*

Extract	Antiviral effect on		
	HSV-2	HSV-1	HAV
<b>Fruits</b>	31%	28%	29%
<b>Leaves</b>	-ve	-ve	34%
<b>Seeds</b>	38%	41%	32%
<b>Roots</b>	52%	54%	43%

**HAV:** Hepatitis A virus; **HSV-I:** Herpes simplex type I virus; **HSV-II:** Herpes simplex type II virus. Weak (25% to less than 50%); moderate (50% to less than 75%) and strong (more than 75%).

### 3.3 Anticancer activity

Fruits extract showed the best antitumor activities against all tested cell lines (IC<sub>50</sub> Values < 30µg/ml). The IC<sub>50</sub> values were 24.6, 16, 18.5 and 19.7 µg /ml for HCT-116, MCF-7, Hep-G2 and Caco-2 respectively (Fig. 4a). Seeds extract was only active on HCT-116 & Hep G2 with IC<sub>50</sub> =21.2 µg for HCT-116 and IC<sub>50</sub> = 22.4 µg for Hep G2 (Fig. 4b). Leaves extract was only active against Hep G2 cancer cell line with IC<sub>50</sub> 19.7 µg/ml (Fig. 4c). Roots extract show weak antitumor activity on tested cell lines (IC<sub>50</sub> values > 30µg/ml) (Fig. 4d).

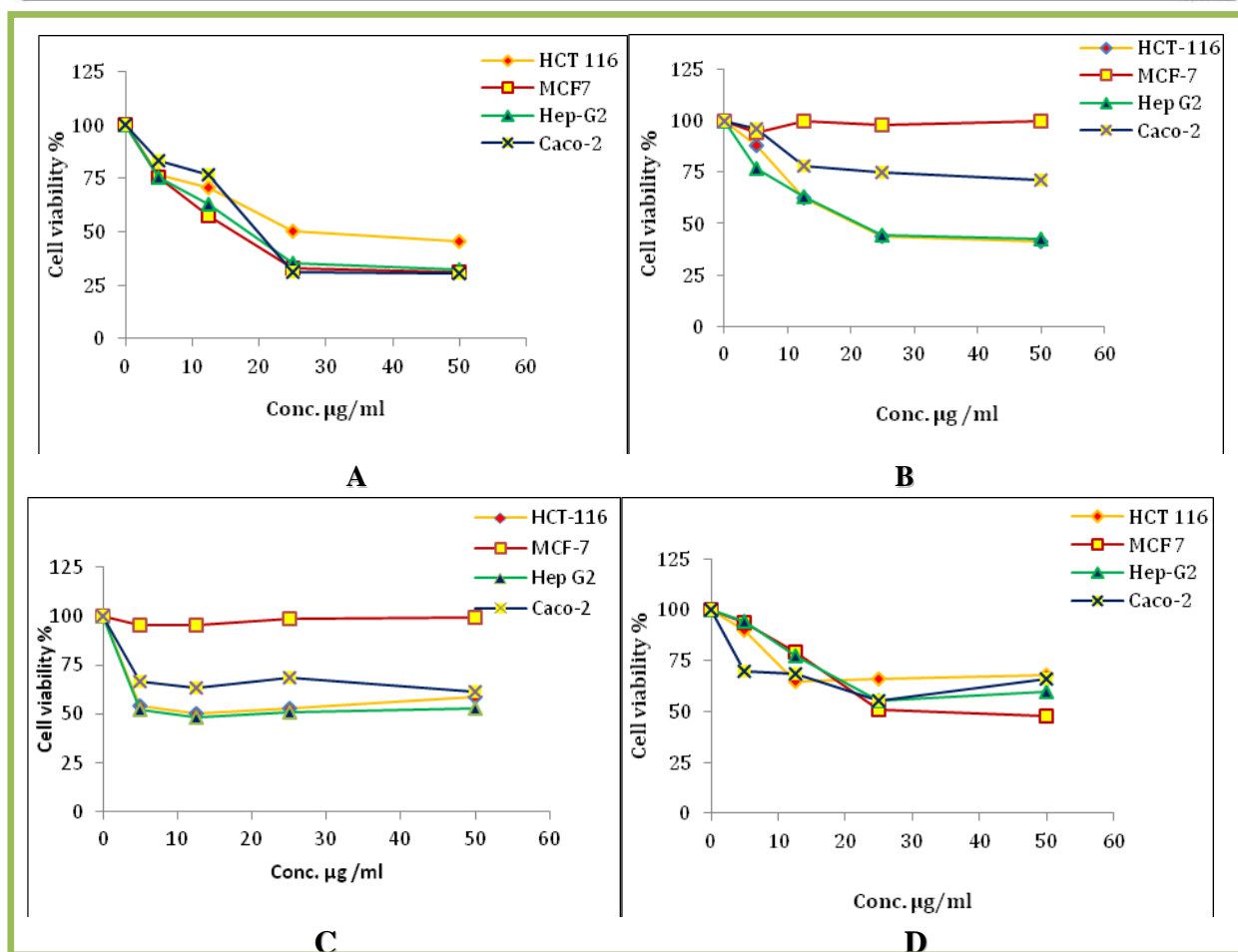


Figure (4): Anticancer activities of ethanolic crude extracts of fruits (A), seeds (B), leaves (C) and roots (D) of *C. colocynthis* on HCT-116, MCF-7, Hep G2 and Caco-2 cancer cell lines.

### 3.4 HPLC/MS analysis:

HPLC/MS analysis was used for determination of the major secondary metabolites in crude extracts of *C. colocynthis* plant organs.

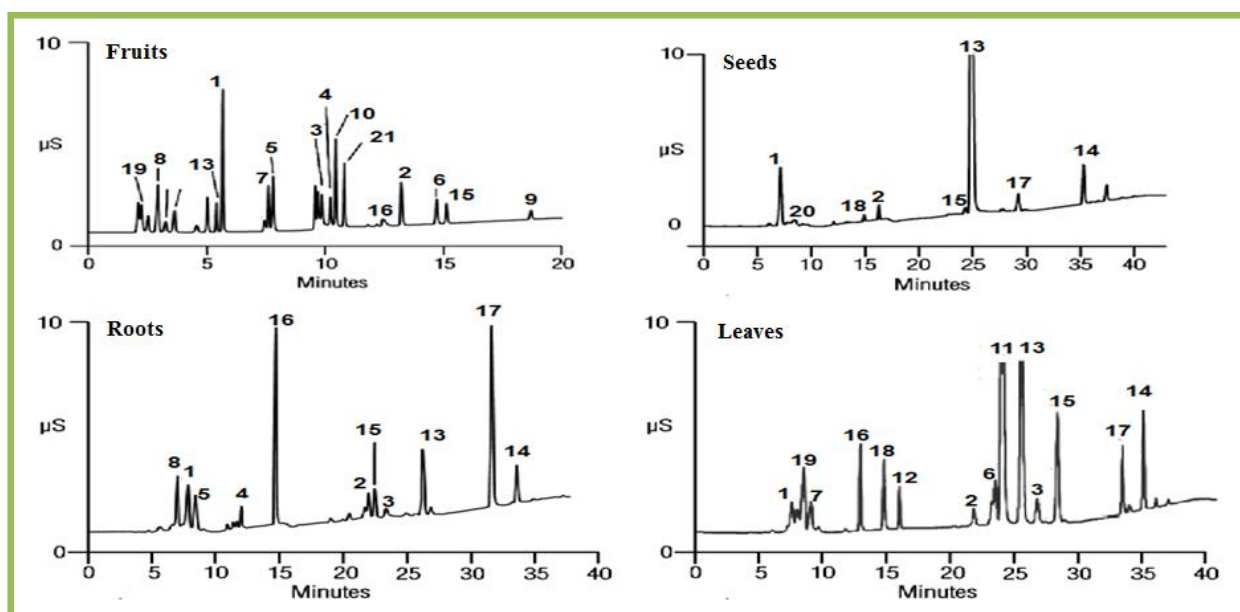


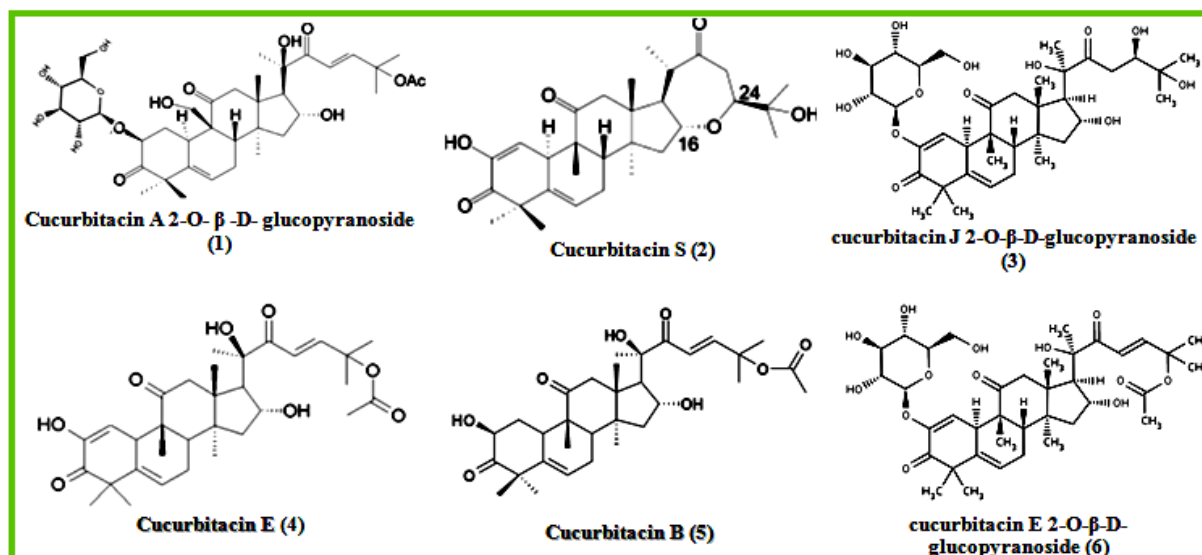
Figure (5): HPLC chromatograms of identified different organs of *C. colocynthis*

Fourteen cucurbitacins and cucurbitacin glucosides were identified as cucurbitacin A 2-O-  $\beta$ -D- glucopyranoside [1], cucurbitacin S [2], cucurbitacin J 2-O-  $\beta$ -D- glucopyranoside [3], cucurbitacin E [4], Cucurbitacin B [5], cucurbitacin E 2-O-  $\beta$ -D- glucopyranoside [6], dihydrocucurbitacin B [7], cucurbitacin K 2-O-  $\beta$ -D- glucopyranoside [8], Cucurbitacin L glucoside [9], Cucurbitacin I glucoside [10], Cucurbitacin I 2-O-  $\alpha$ -D- glucopyranoside [11], cucurbitacin E 2-O- $\alpha$ -D-glucopyranoside [12] besides colocynthoside B [19] and colocynthoside A [20]. In addition, two flavonoids were also identified, namely isovitexin [14] and quercetin [15] four tannins were also identified corilagin [16], gallicocatechin [17], 3-O-Caffeoylquinic acid [18] and Gastrodin [21], beside spinasterol which is classified as a plant sterol [13], as shown in (Table 5, Fig. 5).

**Table (5): HPLC/MS identified compounds in different parts of *C. colocynthis*:**

Identified compounds by HPLC/MS	(m/z)	Relative %			
		Fruits	Seeds	Roots	Leaves
1 Cucurbitacin A 2-O- $\beta$ -D- glucopyranoside*	736.3	9.6	6.7	2.7	1.9
2 Cucurbitacin S*	498.2	5.4	1.8	1.6	1.3
3 Cucurbitacin J 2-O- $\beta$ -D- glucopyranoside	693.3	2.8	--	0.7	1.1
4 Cucurbitacin E	556.3	3.4	--	1.2	--
5 Cucurbitacin B	558.3	4.8	--	3.1	--
6 Cucurbitacin E 2-O- $\beta$ -D- glucopyranoside	718.3	2.3	--	--	2.6
7 Dihydrocucurbitacin B	560.0	4.1	--	--	3.0
8 Cucurbitacin K 2-O- $\beta$ -D- glucopyranoside	694.3	4.3	--	3.7	--
9 Cucurbitacin L 2-O- $\beta$ -D- glucopyranoside	791.0	1.1	--	--	--
10 Cucurbitacin I 2-O- $\beta$ -D-glucopyranoside	676.3	5.9	--	--	--
11 Cucurbitacin I 2-O- $\alpha$ -D-glucopyranoside	676.3	--	--	--	5.4
12 Cucurbitacin E 2-O- $\alpha$ -D-glucopyranoside	718.3	--	--	--	6.7
13 Spinasterol	412.6	3.6	8.5	5.3	9.1
14 Isovitexin	432.1	--	4.8	3.1	8.1
15 Quercetin	302.2	2.6	--	2.9	7.9
16 Corilagin*	634.4	1.1	--	8.4	6.2
17 Gallicocatechin	306.2	--	2.1	7.9	4.1
18 3-O-Caffeoyl quinic acid	354.1	--	0.9	--	4.4
19 Colocynthoside B	806.4	3.1	--	--	4.1
20 Colocynthoside A	734.3	--	0.7	--	--
21 Gastrodin	286.2	5.1	--	--	--

\*: Newly identified compound in *C. colocynthis*



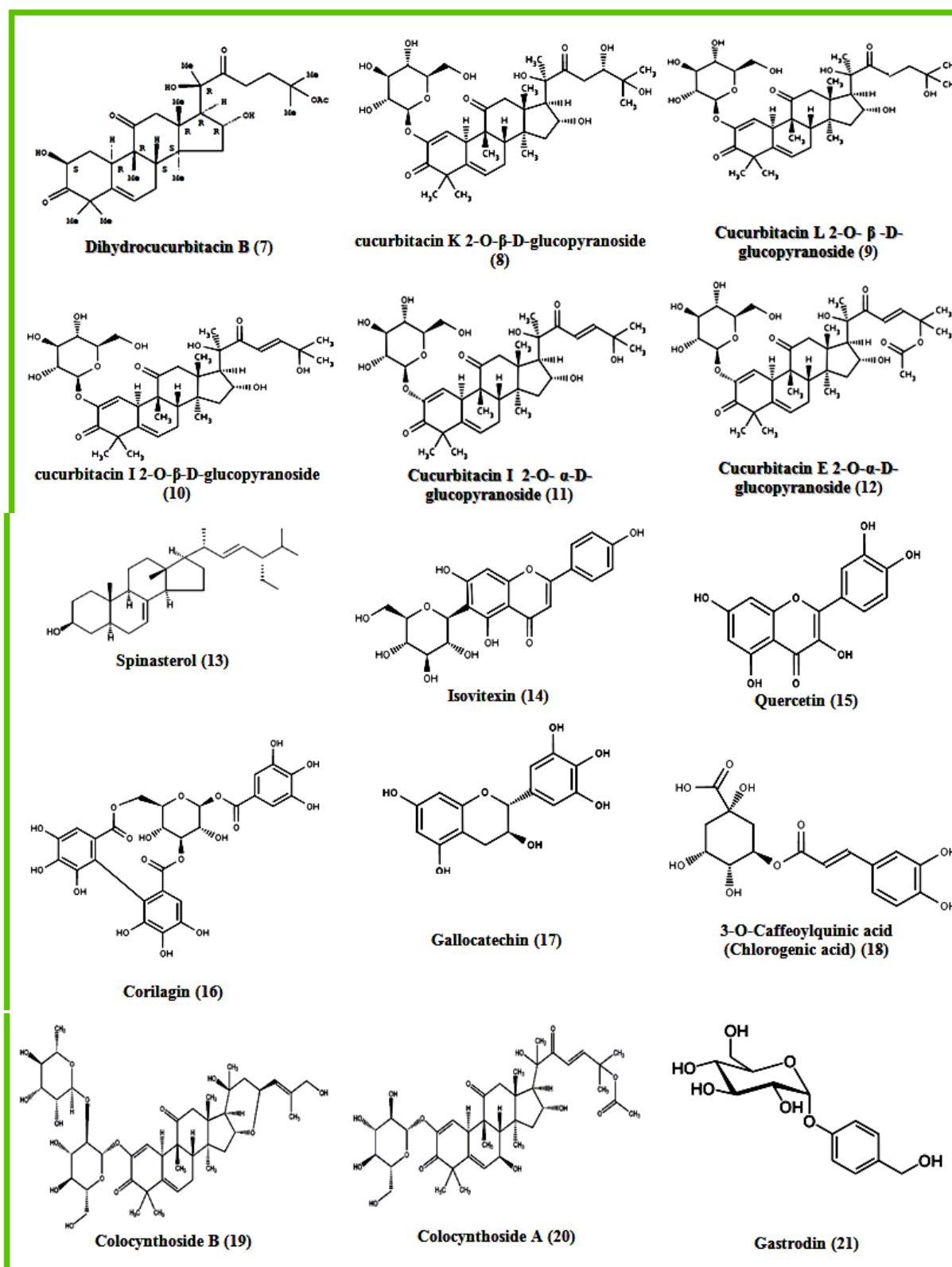


Figure (6): Chemical structures of HPLC/MS-identified compounds

#### 4. Discussion

Medicinal plants have been used for ages in the treatment of diseases. In recent years, herbal medicines have increasingly been used to treat infections difficult to manage, but their use as food preservative was rarely



studied. This study has provided multifaceted results as made obvious by the antifungal, antibacterial, antiviral, anticancer as well as phytochemical screening of ethanolic crude extracts from fruits, seeds, leaves and roots of *C. colocynthis*.

The phytochemical screening reported in this paper revealed that the different parts of *C. colocynthis* are rich in bioactive substances which are responsible for their biological activity. Identification of chemical metabolites of the colocynth plant indicate that there are some variation as far as the type of the secondary metabolite is concerned and the part of the colocynth plant tested, the result obtained indicated some variation in their quantities. Among all tested extracts fruits was the most biologically active, which is in agreement with the study of (Marzouk et al., 2009). Phytochemical screening showed the presence of many secondary metabolites namely; cucurbitacins, cucurbitacin glucosides, flavonoids, tannins, and sterols. Cucurbitacins and cucurbitacin glucosides are known antitumor compounds, the richness and availability percentage of these compounds in such plant part reflect its anticancer potency (Lee et al., 2010). It was also found that extracts of plants containing cucurbitacins were shown to act as bactericides and fungicides (Duke, 1992).

Cucurbitacins exert their antimicrobial and anticancer activities through inhibition of specific signal transduction pathways. Also they non-specifically bind protein targets by forming thio-ether bonds, this will lead to inhibit or disrupt the targeted protein (Chen et al., 2005).

Cucurbitacin structures are characterized by the tetracyclic cucurbitane nucleus (triterpenes) with a variety of oxygen substitutions at different positions. Because of the possession of hydrophobic properties and poorly soluble water, polymeric micellar systems exhibited improved antitumor efficacy because of a better solubilization and targeting after local and/or systemic administration.

Studying the structure activity relationship of cucurbitacins; a class of highly oxidized tetracyclic triterpenoids; and their glucosides (Figure 7) revealed that the organ; with special focus on fruits; in which the side chain contains 'OAc' group, or adjacent 'double bonds' at carbon no. 22, 23 and/or at carbon no. 1, 2 and 'carbonyl group' at carbon no.21 are more biologically active while those containing 'glucose sugar moiety' are less active. This can explain the superior activity of fruits rather than other plant organs and this in agreement with Chen et al., 2012; Bartalis and Halaweish 2005.

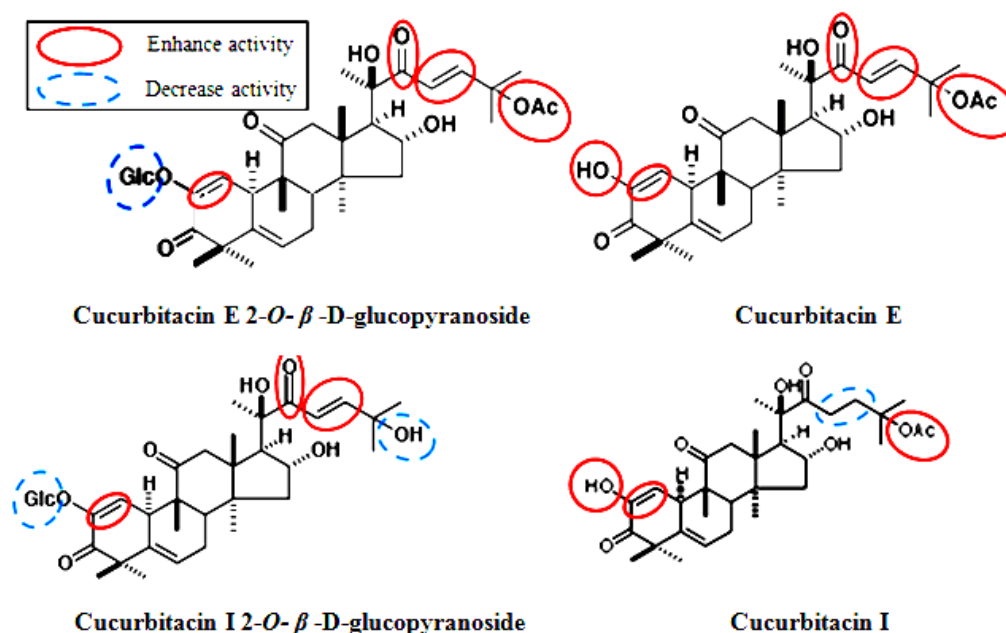


Figure (7): Biofunctional groups in correlation to biological activities

Cucurbitacins E and B result from the acetylation of cucurbitacins I and D, a feature that increased hydrophobicity and cytotoxicity (Bartalis and Halaweish, 2005). Cucurbitacins E and I differ, respectively, from cucurbitacins B and D by the presence of a double bond between C1 and C2 which increases both the hydrophobicity and the cell toxicity. Because of the possession of hydrophobic properties and poorly soluble water, polymeric micellar systems exhibited improved antitumor efficacy because of a better solubilization and targeting after local and/or systemic administration (Bartalis and Halaweish, 2005). Cucurbitacins deserve

much more future investigations targeting their discovery in unexplored sources and their derivatives for improving their anticancer and antimicrobial abilities. Moreover, preclinical and clinical studies using combined treatment composed of cucurbitacins and standard chemotherapy immunoassays should be planned for.

Tannins and flavonoids as well are presently a major core of research because they are considered as potent antioxidants, anti-inflammatory, antibacterial, antiviral and anticancer agents (Benariba et al., 2013). flavonoids exert their antimicrobial effect through formation a complex with cell wall and affect its integrity, they are responsible for the scavenging process or chelators and may disrupt microbial membranes, whereas the antimicrobial activity of tannins may be attributed to the their astringent property and highly oxygenated nature which may induce complexation with enzymes or substrates (Cushnie and Lamb, 2005; Scalbert, 1991).

## 5. Conclusion

This study concludes that colocynth plant organs were highly effective as antifungal, antibacterial, antiviral and anticancer agent. Therefore, there has been a growing interest in the use of colocynth as a promising source of more efficient new therapeutic anticancer drug. More investigation needed on the location of active ingredients, their physical and chemical properties, and their antimicrobial potentialities as well as further research at the molecular level.

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