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Cytotoxicity, DNA Cleavage and Antimicrobial Activity of Citrullus Colocynthis Plant Extracts

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Abstract

In this study, various parts of cucurbitacin rich plant Citrullus colocynthis extracts were assayed the biological activities like antimicrobial, cytotoxicity in Allium sativum root tips, MTT assay with MDA-MD-231cancer cells and DNA cleavage. Antimicrobial activity, high concentration of root extract was found to be more effective on both gram-positive and gram-negative bacteria, almost similar results to low concentration of standard cucurbitacin E, whereas other extracts showed a moderate effect. Cytotoxicity was observed in Allium sativum root tips with all extracts in both durations (4 hrs and 24 hrs) with increasing duration of treatment. More chromosomal aberrations in mitotic cell division were observed with root and fruit pulp extracts. At higher concentrations, root extract showed positive results in MTT assay, whereas no effect of other extracts was observed. Most effective DNA cleavage was recorded with root extracts in both concentrations. The cumulative biological effect was observed by root extract while comparing with other extracts.

Keywords: MTT assay; DNA cleavage; Root extracts; Allium sativum

Introduction

Citrullus colocynthis is a medicinally valuable plant source that has been traditionally used for several applications. Different plant parts and fruits were a good source for the bioactive compounds that exhibit antimicrobial properties. The ancient practice of using Citrullus colocynthis immature fruits and seeds as an antimicrobial agent demonstrates the geographical distribution, influence on the chemical composition and antimicrobial efficiency [1].

In vitro antibacterial activity of aqueous and dilute acetone extracts of Citrullus colocynthis was determined from different plant parts at different maturation stages. The flavonoids of Citrullus colocynthis plant were considered as microbial inhibitors which are resistant to antibiotics. The ethanolic extracts of Citrullus colocynthis prevent the diseases caused by Staphylococcus aureus and solve the drug resistance problem. The fruits and roots of Citrullus colocynthis (Cucurbitaceae family) have long been utilized in oriental herbal medicine for their anti-inflammatory and anti-diabetic effects [2].

Cucurbitacin are of great attention because of the worldwide range of biological activity exhibited in plants and animals. Many compounds of this group have been investigated for their cytotoxic, hepatoprotective, anti-inflammatory, cardiovascular effects and as kairomones for diabroticite beetles. Cucurbitacin are classified into cucurbitacin A, B, C, D, E, F, I, L, 23, 24-dihydro cucurbitacin F, as well as three acetylated derivatives. The effects of cucurbitacin B on various GMB cell lines was found to possess strong antiproliferative effects, whereas cucurbitacin E inhibits the proliferation of prostate cancer cells and causes disruption of the cytoskeleton structure of actin and vimentin.

Cucurbitacin B, D, E, and I inhibited the growth of several cancer cell lines and inhibited COX-2 enzyme rather than COX-1. Fruits of Citrullus colocynthis in brine shrimp assay exhibited strong cytotoxicity towards Artemia salina nauplii, moderate reduction cell viability in MCF-7 at low concentration for a long period of time and has significant growth-inhibitory action on human breast cancer and hepatoma cells. The hydroalcoholic extracts of C. colocynthis had significant anti-proliferative effect on MCF7 and AGS cell lines in 72hrs a dose-dependent and apoptosis induced mechanism [3].

The intrinsic capacity of substances like drugs or toxins to change one or more chemical or physiological functions of a cell, tissue, organ or organism is considered to be the biological activity of that particular compound. Along with this activity, its concentration and duration of cellular exposure to the substance is also determined. Biological activity may reflect the "domino effect," in which the modification of one function disrupts the normal activity of one or more other functions [4,5].

A special characteristic of higher angiosperms plants is their capability to produce a large number of organic chemicals of high structural assortment. The so-called secondary metabolites are grouped based on their functions into diverse categories like chemotherapeutic, bactercteriostatic, bactericidal and antimicrobial. Screening of medicinal plants for their

phytochemicals and antibacterial activities is important to find out the potential compounds of therapeutic use. An antibacterial is an agent that interferes with the growth and reproduction of bacteria. The potentially important antibacterial source is a traditional medicine that can be used for the development of chemotherapeutic agents. Although currently millions of chemical structures are available for screening of therapeutic value, natural products, particularly of plant origin remain the most important sources of new drugs. Hence plants continue to be the source of drugs for most of the world's population. Over the past three decades, it has become immensely popular for the herbal industry and naturally derived products. Among the many plants used as antibacterial, the members belonging to Apocynaceae are reported to have better effect against pathogens and these plants are being locally available [6].

Cancer is the cause of foremost deaths worldwide. The number of cancer deaths is 171.2 per 100,000 men and women per year. The rate of cancer continues to increase largely due to ageing, the growth of the world population and the increasing of cancer-causing behaviours particularly, smoking in developing countries. Apart from all causing cancers, breast cancer is the most common cancer and the foremost cause of cancer deaths among women of all races. Cancer is generally treated with surgeries like chemotherapy, radiation but however side effects may occur during treatments. The scientific mark on efficiency and safety remains limited. As possibly, a variety of purified compounds from herbs have been investigated as sources of new anticancer drugs by Biochemists and Biologists. In both In vitro and in vivo, many herbal families have been purified, among them some exhibit antiproliferative and cytotoxic anticancer activity including activity against carcinomas of prostate, colon, breast, lung and central nervous systems [7].

The cytotoxicity assay deals with the cell viability or changes in the regular process like the arrest of cells at various stages of the cell cycle, changes in membrane integrity, metabolic activity and disappearance of monolayer. In higher eukaryotes, few plants like Pisum sativum, Tradescantia paludose, Allium cepa and Allium sativum are being used as test systems for studying mutagenesis and cytotoxic effect. Allium sativum is frequently used in the cytological study due to appropriate chromosomes, apparent elucidation of aberrations and genetically uniform cloves. The chromosomal abnormalities like the chromosomal bridge, nuclear lesion, sticky chromosome and abnormal metaphase can be observed after treatment of Allium sativum roots with a test sample if the compound contains cytotoxic activity. Allium cepa L. roots can also be used to study the inhibition of the root growth and cytotoxic effect of garlic aqueous extract [8].

The ability of plant extract to cleave the nucleic acids was analyzed by comparing the DNA banding pattern of control and plant extracts. The cleavage of substrate DNA was thought to occur by the oxidation of deoxyribose or oxidation of nucleobases. The ultimate consequence of cleavage at any level specified above is to damage the nucleobases or the pentose sugar and hydroxyl radical species of O2 (OH) are found to be involved in the general mechanism. The nucleophilic attack is the widely accepted mechanism of DNA hydrolysis reaction. Metal ion mediated hydrolysis of nucleic acids can be exploited in the design of artificial restriction endonucleases. Hydrolytic cleavage of nucleic acids is preferable over redox-mediated cleavage reactions, as the information during the hydrolytic reaction is preserved. The fragments thus produced are useful in gene manipulation and in understanding the roles of metal ions in metalloenzyme catalysis. The data obtained from the phytochemical screening of plant extracts and fractionation analysis also authenticating the occurrence of several steroids flavonoids, and glycosides those consisting of hydroxyl groups in their structures may influence them to proceed through oxidative cleavage of DNA. Methanolic and n-hexane extracts of Acacia franetiane has shown the DNA cleavage activity Methanolic extracts of Celastruspaniculatus L., Picrorhizakurroa L.and Withania somnifera L. showed a dose-dependent free radical scavenging capacity and a protective effect on DNA cleavage [9].

MTT assay is the most commonly used calorimetric method used to determine cell viability. When compared to dead or inactive cells, the healthy and rapidly growing cells exhibit high rates of MTT reduction. Only live cells are able to take up the tetrazolium salt. The enzyme (succinate dehydrogenase) present in the mitochondria of the live cells is able to convert internalized tetrazolium salt to formazan crystals, which are purple in colour. The cucurbit extracts were analyzed with MTT assay, which revealed in a concentration-dependent manner by decreasing the viability of HT-29 and HCT- 15 cells substantially.

The present investigation was performed to study the different biological activities of Citrullus colocynthis like antibacterial activity, cytotoxicity study and DNA cleavage [10].

Materials and Methods

Preparation of plant extract

Different plant parts (root, fruit peel, fruit pulp and tendrils) of C. colocynthis were collected, shade dried and ground to a coarse powder. 10 gm of each sample was extracted into 100ml of different solvents like methanol and ethanol separately by cold maceration technique. The extracts were filtered through Whatman No.1 filter paper and kept in an incubator at 370C till the solvent evaporated.

Antibacterial testing

The agar well diffusion method was employed for the determination of the antimicrobial activities of plant extracts of C. colocynthis. The dried plant extracts were dissolved in DMSO and used for the assay of antimicrobial activity against gram-negative and gram-positive microbes. The gram-negative bacteria include Escherichia coli, Salmonella typhi and gram-positive organisms include Staphylococcus aureus, Bacillus subtilis. All bacterial strains were maintained on freshly prepared nutrient agar media [11].

Cytotoxicity effect

The MDH- MD- 231 cells were plated separately in 96 well plates at a concentration of 1X 104 cells/well. After 24 h, cells were washed twice with 100µL of serum-free medium and starved for an hour at 37 0C. After starvation, cells were treated with different concentrations of C. colocynthis plant extracts (10, 30, 60, 120µg/ml) for 48 h. At the end of the treatment period, the medium was aspirated and serum-free medium containing MTT (0.05 mg/ml) was added and incubated for 4 h at 37 0C in a CO2 incubator. After incubation, a medium containing MTT was discarded and the cells were washed with PBS (200 µl). The resultant crystals were dissolved in 100 µl of DMSO and mixed thoroughly. Spectrophotometric absorbance of the purple-blue formazan dye was measured in a microplate reader at 570 nm. The assay is based on the reduction of soluble yellow tetrazolium salt to insoluble purple formazan crystals by metabolically active cells [12].

The uniform size scales of Allium sativum were selected and their discs were dipped in tap water for obtaining roots. Different concentrations (250, 500, 750 and 1000 µg/ml) of various plant extracts of Citrullus colocynthis were prepared and root tips thus obtained were dipped in these extracts and incubated for two different periods like 4 hrs and 24 hrs. Roots tips are treated in a similar way without any extract for control. After 24hrs of fixation (alcohol: acetic acid in 3:1 ratio is used as a fixative), they are transferred to 70% ethanol for preservation. Root tip squashes were prepared in aceto- orcein after hydrolysis with 1N HCl at 600C. The root tips were examined under a phase-contrast microscope for cytotoxicity study.

DNA Cleavage

DNA cleavage ability of the complexes was evaluated using super coiled plasmid DNA pBR322 (100 ng/µL). The DNA was incubated with varying concentrations of plant extracts (50 and 100 µg/ml DMSO), in Tris-HCl buffer (pH 7.2) for 1 hr at 37°C. It was observed that both the complexes are able to cleave DNA without any added reagents or light as they contain nucleophiles such as coordinated water molecules and/or hydroxyl groups in the ligands, indicating the hydrolytic cleavage of DNA.

Agarose gel electrophoresis method

Cleavage products were analyzed by the agarose gel electrophoresis method.20 µL of DNA sample (mixed with bromophenol blue dye at 1:1 ratio) was loaded carefully into the electrophoretic chamber wells along with standard DNA marker containing TAE buffer and loaded into agarose gel with constant 50 V of electricity for 30 min. Gel was removed and stained with 10 µg /mL ethidium bromide for 10–15 min. The electrophoresed gel was observed under the UV transilluminator to determine the extent of DNA cleavage and the results were compared with standard FeSO4.

Results and discussion

In the present investigation, the biological activities like antibacterial, cytotoxicity on Allium sativum root tips, MTT assay, and DNA cleavage with methanolic extracts of different parts (root, fruit peel, fruit pulp and tendril) of Citrullus colocynthiswas evaluated.

Antibacterial

In vitro antibacterial activity of methanol extracts of different parts of C. colocynthis was investigated. Antibacterial activity of plant extract against two gram-positive Bacillus megaterium, Bacillus subtillis and two gram-negative Escherichia coli, Proteus vulgaris were examined. The microbial cultures were procured from the Microbial Type Culture Collection (MTCC), Chandigarh, India.

The different concentrations of plant extracts (25, 50, 100, 150 and 250 µg/ml) were screened for antibacterial activity. Among all the concentrations tested, 250 µg/ml were seen to be effective against both gram-negative and gram-positive bacteria. Root extracts were found to be more effective compared to other extracts. Root extract exhibited a maximum zone of inhibition of 0.6 cm against Escherichia coli, 0.7 cm against Proteus vulgaris, 0.7 cm against Bacillus megaterium and 0.6 cm against Bacillus subtillis. This was almost equal to pure cucurbitacin E.

Fruit peel and fruit pulp extract were also showing almost similar results to that of root extracts, tendril extract was seen to be least effective in comparison. Streptomycin 25 µg/ml was taken as standard antibiotic and all the results were compared against 25 µg/ml cucurbitacin E standard. The methanolic extract has excellent antimicrobial activity against Bacillus subtillis and Escherichia coli when compared to other extracts in Cucurbitaceae species viz. Cucurbita pepo, Momordica charantia, Coccinia indica. The methanol and dichloromethane extracts of Cucumis sativus pulp showed the strongest activity with MIC values 2.43- 3.15 mg/ml [13].

Cytotoxicity

The different plant extracts of C.colocynthis were responsible for the inhibition of mitosis. The mitotic inhibition increased with the increase in the concentration of extract and duration of treatment. Chromosomal breakages and extreme fragmentation were the most common abnormalities (Fig. 1). Other abnormalities at metaphase are unoriented chromosomes. At anaphase, fragmentation of chromosomes was most frequent followed by laggards (Fig. 2), cytokinesis inhibition and formation of binucleate cells were prominent mitotic abnormalities observed at higher concentrations of extracts. As a consequence, multinucleated conditions were observed. The mitotic index decreased with the increase in concentrations of extracts and duration of exposure. The inhibitory effect may be due to the blockage of DNA synthesis.

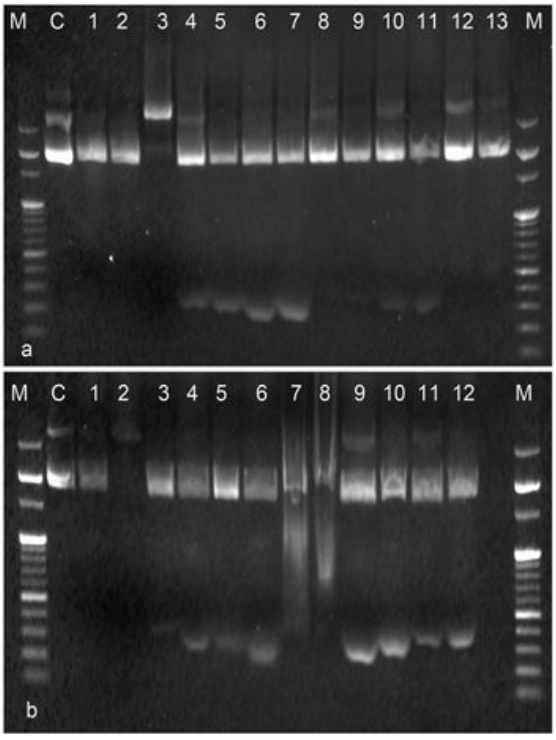
Among the different extracts tested, root extract (Fig. 1) was shown to be more effective, followed by fruit pulp extract.

Inhibition of mitosis and the remaining extract shown a moderate effect. Inhibition of mitosis was observed to be moderate four hours treatment and highly effective in the twenty-four hours treatment. Minimum mitotic inhibition was

observed at 250 µg/ml, whereas the maximum effect was observed at a 1000 µg/ml concentration of extract. The increase in the concentration of the extract resulted in the increase of inhibition effect. Similar results were reported by sadaqa et al.,in garlic extract on root tips of Allium cepa that the cytotoxic effect depends on the concentration and exposure time. Aqueous extract of Limonium globuliferum showed chromosomal aberrations like sticky chromosomes, anaphase bridges, laggard chromosomes, and anaphase – telophase disorders especially at high concentrations of the extract.

Sticky chromosomes at metaphase(b)Clumped and disturbed metaphase(c)Spindle abnormalities at anaphase (d) Vagrant chromosome in anaphase (e) Anaphase bridge (f) Chromosome break with unoriented chromosomes g) Anaphase bridge with laggards h) Cross bridges at anaphase with laggards i) Telophase bridge (Fig. 2)

Figure1: Chromosome aberrations in Allium Sativum root tips treated with fruit pulp extracts of Citrullus colocynthis (L.) Schard



MTT assay

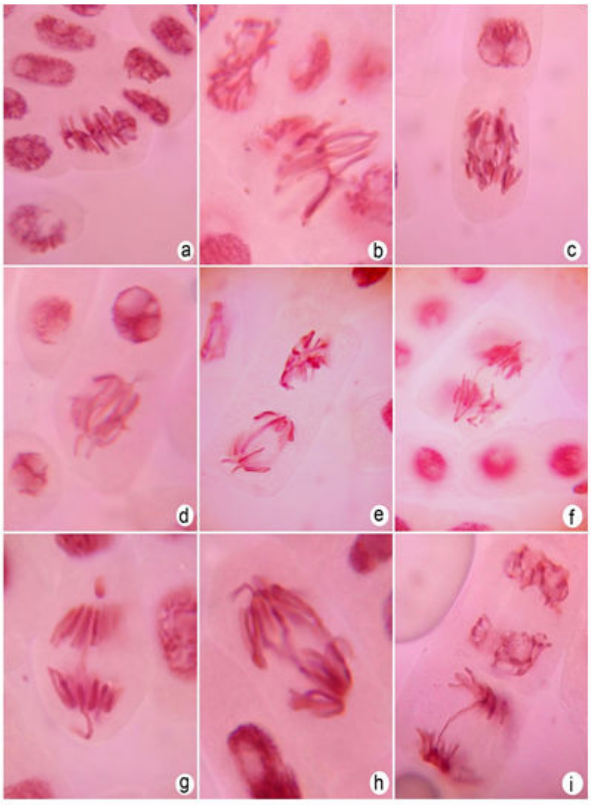
The effect of different methanolic extracts of C. colocynthis on the proliferation of breast cancer cell lines (MDA-MD-231) was evaluated by measuring the cell viability at 250, 500 and 1000 µg/ml concentrations of the test compounds for 72h, respectively. The maximum percentage of inhibition obtained for MDA-MD-231 cells was 46% with root extract at the concentration of 1000 µg/ml. At 250 µg/ml concentration there is no effect on the cell growth, but a moderate effect on cell growth was observed with 500 µg/ml concentration. The remaining other extracts have not shown any effect on cell growth even at 1000 µg/ml concentration.

Additionally, the anti-proliferative activity of standard cucurbitacin E, checked against these cancer cells has shown more than 63% of inhibition at 250 µg/ml concentration. Similarly, Acacia franesiana was reported to possess antiproliferative activity in n-hexane extract with 21.70% inhibition activity against MCF-7 cell line and methanol extract with 23.3% inhibition activity against K562 and HePG2 cell lines. Few other researchers applied different plant extracts on MDA-MD-231 cell lines and reported inhibition of cell growth, which include black turtle has been extracted with 50µg/ml Ruta graveolens, Ferulahermonis, Alcerosea and Convolvulus arvensi plant extract

DNA cleavage activity

The DNA cleavage activity of different plant extracts of C. colocynthis was determined using the agarose gel electrophoresis method. DNA cleavage is controlled by relaxation of supercoiled circular form into nicked circular form and linear form. When circular plasmid DNA is subjected to electrophoresis, relatively faster migration will be observed than the intact supercoiled form. The pictures of the gels are presented (Fig. 3).

Figure2: DNA cleavage with different parts extracts of Citrullus colocynthis (L.) Schard



- (M) Marker (C) control (1,2) DMSO treated (3) Fecl2 treated (4) 5µl treated leaf extracts (5) 10 µl treated leaf extract (6) 5 µl treated stem extract (7) 10 µl treated stem extract (8) 5 µl treated tendril extract (9) 10µl treated tendril extract (10) 5 µl treated fruit peel extract (11) 10 µl treated fruit peel extract (12) 5 µl treated seed extract (13) 10 µl treated seed extract

- (M) Marker (C) control (1) DMSO treated (2) Fecl2 treated (3) 5µl treated leaf extracts (4) 10 µl treated leaf extract (5) 5 µl treated stem extract (6) 10 µl treated stem extract (7) 5 µl treated root extract (8) 10 µl treated root extract (9) 5 µl treated fruit pulp extract (10) 10 µl treated fruit pulp extract (11) 5 µl treated fruit peel extract (12) 10 µl treated fruit peel extract

The electrophoresis gel exhibited complete DNA cleavage in the case of standard FeSO4 (10 mg/1 mL). Compared to standard FeSO4, root extract has been shown to completely cleaved the DNA even at 50 µg /mL and cleavage at 100 µg /mL. The remaining compounds have partially cleaved the DNA. As of these results, we deduce that the presence of pyranone moiety on thiazole core, 4-hydroxy phenyl and 4-hydroxy-3-methoxyphenyl groups on the pyrimidine core has enhanced the DNA cleavage activity. Methanolic extracts of CelastruspaniculatusL., Picrorhizakurroa L. and Withaniasomnifera L. were showing the effect on DNA cleavage induced by H2O2 UV-photolysis. The various solvents like n-hexane, DCM and methanol extract of Acacia farnesiana Linn. Shown significant DNA cleavage at concentrations of 25, 50 and 100 µg by fractions and methanolic extracts [14,15].

Conclusion

The biological activities like antimicrobial, cytotoxicity in Allium sativum root tips, MTT assay with MDA-MD-231cancer cells and DNA cleavage results indicated that all extracts of Citrullus colocynthis (L.) Schard were effective. In antimicrobial activity, a high concentration of root extract was found to be more effective on both gram-positive and gram-negative bacteria, almost similar results to low concentration of standard cucurbitacin E, whereas other extracts showed a moderate effect. Cytotoxicity was observed in Allium sativum root tips with all extracts in all durations, with increasing duration of treatment. More chromosomal aberrations in mitotic cell division were observed with root and fruit pulp extracts. At higher concentrations, root extract showed positive results in MTT assay, whereas no effect of other extracts was observed. Most effective DNA cleavage was recorded with root extracts in both concentrations. The cumulative biological effect was observed by root extract while comparing with other extracts.

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