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DNA-RAPD Fingerprinting and Cytogenetic Screening of Genotoxic and Antigenotoxic Effects of Aqueous Extracts of *Costus Speciosus* (Koen.)

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Abstract. The present study was conducted to investigate the genotoxic and/or antigenotoxic effect of aqueous extract of the traditional plant *Costus speciosus* (Koen.) that is used frequently for the treatment of various disorders in Saudi Arabia. Root tip meristems of *Allium cepa* were treated with elevated concentrations of *Costus speciosus* (Koen.), Samples were taken at 48h. of each concentration (2, 5, 10, 20 µg/mL) and subjected to cytogenetic and molecular genetics assays (DNA-RAPD fingerprinting). It was found that the extract has no clastogenic or mutagenic activities, mitotic index decreased mildly compared with the control. Frequency of chromosomal aberrations showed no significance in all concentrations at time of exposure. Most aberrations of the dividing cells were disturbance of chromosomes. The RAPD results demonstrated monomorphic numbers of genetic bands mostly, which were the electrophoretic products of PCR for all treatments compared with the control. Also the results exhibited the ability of *C. speciosus* as antigenotoxic and anticytotoxic potential against EMS induced DNA damage , cytotoxic and clastogenic effects in *Allium cepa* cells. The presented data showed that *C. speciosus* extract could not induce significantly genotoxic effect on *Allium cepa* cells. Furthermore, this study implies that combined treatment of *C. speciosus* has a strong inhibitory role against the genotoxic action of EMS. These results strongly suggest that the extract of *Costus speciosus* is not genotoxic, or cytotoxic but might be anti-genotoxic agent.

Introduction

Many people in developing countries use traditional drugs including medicinal herbs to meet their primary health care needs[1]. The use of medicinal plants has always been part of human culture. The World Health Organization estimates that up to 80% of the world's population relies on traditional medicinal system for some aspect of primary health care[2]. In some countries, government encourages the use of indigenous forms of medicine, because it is the first pharmacy for medicine in the history. In Saudi Arabia a large number of folkloric plants are still in use as a source of herbal therapies[3-4], *Costus speciosus* (Koen.) is one of them.

Costus speciosus contains a large amount of spirostanol glycosides, all of which yield diosgenin as steroid saponins[5], and F26G purified from rhizomes of *C. speciosus*[6-7]. Moreover, Beta-sitosterol-glucoside, tigogenin, triacontanoic-acid, curcumin, mucilage, starch and triacontanol have been previously isolated from the parts of its rhizomes and leaves[8, 9] .

This plant known under the common names of marine costua, beautiful costua, is used in folkloric medicine for the useful treatment of various disorders such as in burning sensation, constipation, leprosy, skin diseases, fever, asthma, bronchitis, inflammations and anemia and the rhizomes of *Costus speciosus* are bitter, astringent, acrid, cooling, aphrodisiac, purgative, anthelmintic, depurative, febrifuge, expectorant and tonic[10]. Despite the profound therapeutic advantages possessed by some of the plants, some constituents of medicinal plants have been shown to be potentially toxic, genotoxic and teratogenic[3, 11, 15].

However, there are no reports on the toxicological properties of this plant in literature. As part of an ongoing study on genetic safety evaluation of herbal extracts commonly used in Saudi Arabia, this study was conducted to investigate the genotoxic and antigenotoxic effects of aqueous extract of this plant on the meristemic cells of *Allium cepa.* Also this study is concerned with the verification of the ability of this plant extract as antioxidant, since some plants produce a great diversity of substances that could be of therapeutic significance in many areas of medicine. These therapeutic benefits is medicinal plants are often attributed to their antioxidant properties[16] .

Materials and Methods

Chemicals

All chemicals used in the present study were obtained from Sigma Aldrich Company.

Collection of C. Speciosus

The medicinal plant utilized in this study is *Costus speciosus* of the **Costaceae** family (some taxonomists still classify Costus and related genera in Zingiberaceae family). It is native to the Malay Peninsula of Southeast Asia, but it has naturalized in some tropical areas[17]. It was obtained from local shops of herbalists at different locations on Makkah region in Saudi Arabia and was taken to the University of Umm Al-Qura Herbarium (UAH) for identification.

Preparation of Plant Extract for Molecular and Cytogenetic Assays

Extraction was carried out as practiced locally. Briefly, Rhizomes of *C. speciosus* (2, 5, 10, 20 mg) were weighted and crushed in a mortar, the powder were soaked separately in 1 L of distill water for 360 minutes to obtain a final concentration as (2, 5,10,20 µg/mL). The extracts were filtered separately with a 2.5 m filter (Whatman® no. 42) to remove the suspended particles and the filtrate (the extract) was either used directly in the experiment or stored at 4 °C until use. Four concentrations of this stock extract were prepared (2 µg/mL, 5 µg/mL, 10 µg/mL and 20 µg/mL) to be tested for genotoxic activities.

Preparation of C. Speciosus for Antioxidants Activity

The dried powder material of *C. speciosus* was extracted with methanol in a Soxhlet apparatus. The solvent was completely removed under reduced pressure and a semisolid mass was obtained. The dried *C. speciosus* was dissolved in water and subjected to screening for antioxidant activity.

Allium Cepa

The seeds and bulbs of *Allium cepa* were obtained from local groceries in Saudi Arabia, Makkah region.

Ethyl Methanesulphonate

Ethyl methanesulphonate (EMS) is a mutagenic, teratogenic, and possibly carcinogenic organic compound with formula $C_3H_8O_3S$. It produces random mutations in genetic material by nucleotide substitution[18]. EMS is often used in genetics study for induced chromosome aberrations and mutations at statistically significant levels[19]. In this study, 6 µg/mL of EMS was used to induce genotoxic effects in *Allium cepa* cells.

Determine LD_{50} of C. Speciosus

The mature seeds of *Allium cepa* were soaked at 4 °C for 2–3 days in the distilled water, and germinated to primary roots of 2-4 mm long in a petri dish containing a filter paper of 11 cm diameter at temperature of 15-24 °C in the dark. The germinated seeds were exposed to elevated concentrations of aqueous extracts of *C. speciosus* Rhizomes (0, 2, 5, 10 and 20µg/mL) for seven days.

The length of seedling were measured followed by determining the concentration which decreased the length of plant to 50% compared to control (LD_{50}).

In Vitro Antioxidants Activity

Superoxide Anion Scavenging Activity

Measurement of superoxide anion scavenging activity of *C. speciosus* was done based on the method described by Nishimiki *et al* [20] with slight modification. About 1 mL of nitroblue tetrazolium (NBT) solution (156 µM NBT in 100 mM phosphate buffer, pH 7.4), 1 mL of NADH solution (468 µM in 100 mM phosphate buffer, pH 7.4) and 0.1 mL of sample solution of *C. speciosus* (10, 25, 50, 75, 100 and 125 µg) in distilled water were mixed and the reaction started by adding 100 µl of phenazine methosulphate (PMS) solution (60 µM PMS in 100 mM phosphate buffer, pH 7.4). The reaction mixture was incubated at 25^0C for 5 minutes, correspondingly blank contained all the reagents except extract of *C. speciosus* and the absorbance at 560nm was measured. Decreased absorbance of the reaction mixture indicated increased superoxide anion scavenging activity. Curcumin was used as reference compound. All the experiments were performed in triplicate and the results were averaged. The percentage of inhibition was determined by comparing the results of control and test samples.

In Vitro Assays for Genotoxicity/Antigeneotoxicity Cytogenetic Assay

For the evaluation of induction of chromosomal aberration, 3 onion bulbs were exposed to (LD_{50}, $2LD_{50}$, 1/2 LD_{50} and 1/4 LD_{50}) µg/mL concentrations (v/v) of the extract of *C. speciosus* Rhizomes and the control for 48h. Root tips from these bulbs were cut and fixed in ethanol: glacial acetic acid (3:1, v/v). These were hydrolyzed in 1N HCL at 60^0C for five minutes after which they were washed in distilled water. Two root tips were squashed on each slide, stained with acetocarmine for 10 min and cover slips carefully lowered on to exclude air bubble. The cover slips were sealed on the slides with clear fingernail polish as suggested by Grant[21]. Six slides were prepared for each concentration and the control (1000 cells per slide) were analyzed at ×1000 magnification for induction of chromosomal aberration, and the mitotic index was calculated as the number of dividing cells per 1000 observed cells[22, 23] The frequency of aberrant cells (%) was calculated based on the number of aberrant cells per total cells scored at each concentration[24].

DNA Extraction

After one week of growth, approximately 1.5-3.5 cm root-tips of 40 seedlings were collected, ground in liquid nitrogen, and total genomic DNA was

isolated by a CTAB method based on that of Padmalatha and Prasad[25] method with minor modifications. Purified DNA concentration and integrity of total genomic DNA in each sample were estimated fluorometrically by biophoto meter (Germany) and by observing ethidium bromide (EB)-stained band with DNA standard of 1 kb.

RAPD Fingerprinting

The conditions of DNA amplification were optimized and followed the procedure of Williams *et al.* [26] with some modifications. One of four l0-mer oligonucleotides: OPA-04 (5'-AATCGGGCTG-3'), OPA-19 (5'-CAAACGTCGG-3'), OPA-20 (5'-GTTGCGATCC-3') and OPB-11 (5' GTAGACCCGT-3') (Operon technologies Inc., Alameda, California, USA) were used for each amplification. Conditions reported by Bohanec *et al*. (1995) were improved. Each reaction (25 µL) consisted of 1 mM of MgC_{I2}, 4 mM each of dATP, dCTP, dGTP, dTTP (Boehringer-Manheim, Germany), 400 nM primer, 1.0 U of Taq DNA polymerase (Appligene-Oncor, France), 1 x reaction buffer (1 mM MgC_{I2}, 20 mM Tris HCI pH 8.0, ethylenediaminetetraacetic acid (EDTA) 1 mM, dithiothreitol 1 mM, glycerol 50%). The reaction mixture was overlaid with a drop of mineral oil and incubated in a thermal cycler (thermal cycler 480, Perkin Elmer-Cetus, USA) programmed as follows: 48 cycles of 1 mm denaturation at 94°C, 1 mm annealing at 37°C and 1.5mm extension at 72°C, followed by final extension at 72°C followed by a cooling at 4°C. Tubes containing all reaction products except template DNA were used as negative control. The reproducibility of the RAPD profiling method in detecting *C. speciosus* induced DNA changes was determined using three replicates. This experiment was also conducted to confirm if extra band appears in majority of *Allium cepa* seedlings exposed to 2, 5,10,20 µg/mL of *C. speciosus*.

PCR reaction products were mixed with one-sixth volume of gel loading buffer (analytical grade water containing 36% glycerol, 0.05% bromophenol, 30 mM EDTA and 0.05% xylene cyanol), and then separated by electrophoresis in a 2.4% agarose gel, using a Tris-borate-EDTA (TBE) system (0.5 × TBE = 45 mM Tris-base, 45 mM boric acid, and 1 mM EDTA). Agarose gel dimensions were $12 \times 6 \times 0.5$ cm^3. For comparison, DNA molecular size marker (1 kb) was used for each agarose gel.

Statistical Analysis

The data were expressed as mean ± SD. The differences between mean values and the controls were statistically investigated using *t*-tests. Genomic template stability (%) was calculated as $100 - (100\ a/n)$, where *a* was RAPD polymorphic profiles detected in each sample treated and *n* the number of total bands in the control. Polymorphism observed in RAPD profiles include

disappearance of a normal band and appearance of a new band in comparison to control RAPD profiles[27, 28], and the average was then calculated for each experimental group exposed to different *C. speciosus* treatments.

Results

Determine LD_{50} of C. Speciosus

Table 1 shows the results of the effects of the extract of *C. speciosus* on root growth of *Allium cepa*. Good root growth was achieved in the control, but at tested concentrations, root growth was highest at the 2 µg/mL concentration of *C. speciosus* extract while it was least at 50%. Inhibition of root growth was concentration dependent and statistically significant ($P < 0.05$) at 10 µg/mL of *C. speciosus* concentration was 47.9% and at 20 µg/mL of concentration was 41.6%. Inhibition of root growth which suggests mild toxicity of the plant.

Table 1. Effects of aqueous extract of *C. speciosus* on root growth of *Allium cepa*.

Conc. (µg/mL)	*Costus speciosus*		
	Mean root length±S.E.	% Length Inhibition Compare to control	LD_{50}
0	4.8±0.16	100	-
2	3.4±0.26	70.8	-
5	2.8±0.17*	58.3	-
10	2.3±0.15*	47.9	10 µg/mL
20	2.0±0.11*	41.6	-

* $P < 0.05$, level of significance of root growth inhibition compared with control.

Superoxide Anion Scavenging Activity

The superoxide anion derived from dissolved oxygen by phenazine methosulphate/NADH coupling reaction reduces nitroblue tetrazolium. The decrease in the absorbance at 560nm with the plant extract indicates the consumption of superoxide anion in the reaction mixture. As depicted in Fig. 1, *C. speciosus* at concentration from 10-125 µg/mL inhibited the production of superoxide anion radicals by 24.21-68.72%. The half inhibiting concentration (IC_{50}) value of *C. speciosus* on superoxide radical scavenging activity was found to be 84.37 µg/mL and for Curcumin 5.95 µg/mL (as reference).

Cytogenetic Assay

The effect of the extract on cell division and chromosome behavior of *Allium cepa* are presented in Table 2. There was no induced chromosomal aberration, and the mitotic index (MI) value was 32.9 in the control. With increasing concentration of the extract however, there was concentration dependent decrease in the mitotic index. Most of the treatments induced disturbances of metaphase and anaphase chromosomes (Fig. 2) at various

concentrations. It seems that the frequency of this abnormality is not concentration dependent.

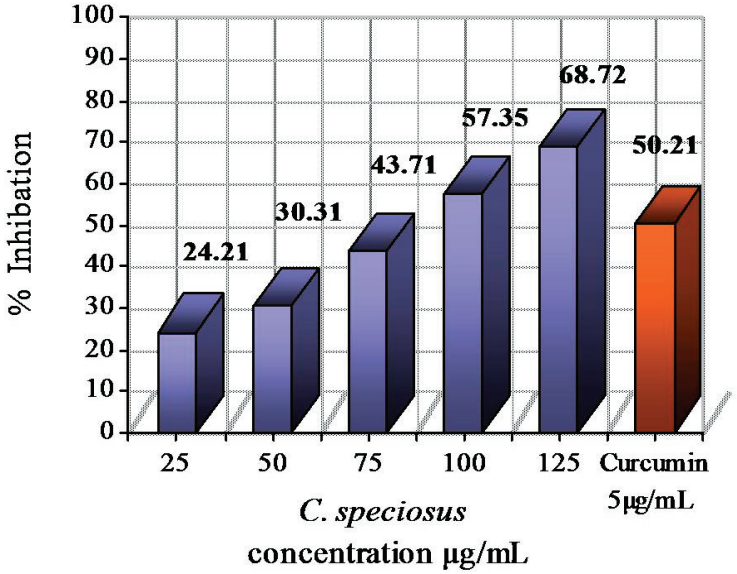

Fig. 1. Percentage Inhibition of superoxide radicals by different doses of *C. speciosus* extract and Curcumin (as a reference) on PMS/ NADH-NBT system .

Table 2. Cytogenetical of single and combined effects aqueous extract of *C. specious* and EMS on *Allium cepa* cells.

Conc. [1] (µg/mL)	*Costus speciosus*			*Costus speciosus*+EMS		
	No of dividing cells	Mitotic index	% of aberrant cells	No of dividing cells	Mitotic index	% of aberrant cells
Control	329	32.9	-	329	32.9	-
2	207	20.7*	-	187	18.7**	08.0
5	180	18.0**	-	166	16.6**	11.0
10	133	13.3**	0.20	120	12.0**	12.3
20	122	12.2**	0.40	108	10.8**	13.8
0.006 µg/mL EMS	070	07.0**	15.0	070	07.0**	15.0

[1] 1000 cells/per concentration of each extract and the control.

* $P < 0.05$, level of significance of root growth inhibition compared with the control.

** $P < 0.01$, level of significance of root growth inhibition compared with the control.

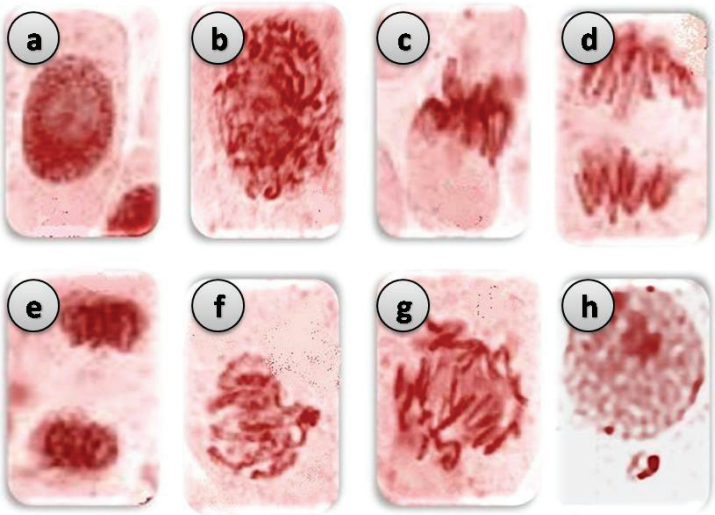

Fig. 2. Stages of mitotic division in cells of *Allium cepa* treated with water extracts of *C. specious*. Mag. 1000×. (a–e) Normal stages of mitotic division. (a) Interphase (b) prophase (c) metaphase (d) anaphase (e) telophase (f) spindle disturbance at prophase (g) spindle disturbance at anaphase (h) micronuclei cell of *Allium cepa* exposed to 20 µg/mL of *C. specious*.

Ethyl methanesulphonate (EMS) induced chromosomal aberrations such as break, fragments, bridge, and micronuclei were observed in *Allium cepa* root cells (Fig. 1). The result showed chromosome breaks, micronuclei and fragments are more common than other defects. The number of aberrant cells induced by EMS represented its genotoxic action in *Allium cepa* and it was statistically significant when compared with the control (Table 2), whereas they decrease significantly in combined treatment with *C. speciosus* at all concentrations compared with EMS single treatment, indicating that *C. speciosus* is antimutagenic potential in *Allium cepa* cells.

DNA Extraction

The suitability of the Padmalatha and Prasad (2006) methods for DNA extraction was evaluated on the basis of the DNA purity. Concentration and purity of DNA extracted are usually measured at OD_{260} and by 260 nm/280 nm absorbance ratio. The purity grade of DNA extracted from root tips of the control and the treated *Allium cepa* root cells for one week of treatment was in the range of 1.47–1.82, and the yield obtained was approximately in the range 150-80 µg.

RAPD Fingerprinting Result of RAPD profile for single treatments with *C. specious* extract was showed in Fig. 3, Table 3. Of the 4 random primers tested, only two (OPA-04 and OPA-20) gave specific and stable results. The RAPD fingerprints showed mild differences between control and exposed treatments, with apparent mild changes in the number and the intensity of amplified DNA bands. The decrease in band intensity was particularly obvious for *Allium cepa* exposed to 20 µg/mL of *C. specious* for primer 1 and 2 (Fig. 3, Table 3). The number of disappearing RAPD bands was greater at higher *C. specious* concentration for primer 1 and 2, and bands of molecular size from approximately 1200 to 1460 bp were shown to disappear after exposed to 20 µg/mL of *C. specious* extract (Fig. 2). Meanwhile, the EMS treatment gave extremely variable bands as change density (increase/decrease) and complete polymorphism bands compared with the control for primers 1 and 2 (Fig. 2). The results of RAPD profile for combined treatments with different concentrations of *C. specious* extract and EMS were showed in Fig. 3 and Table 3. It is clearly, the changes of density bands and polymorphism bands which were induced by EMS effect in *Allium cepa* cells were decreased and corrected according to the control.

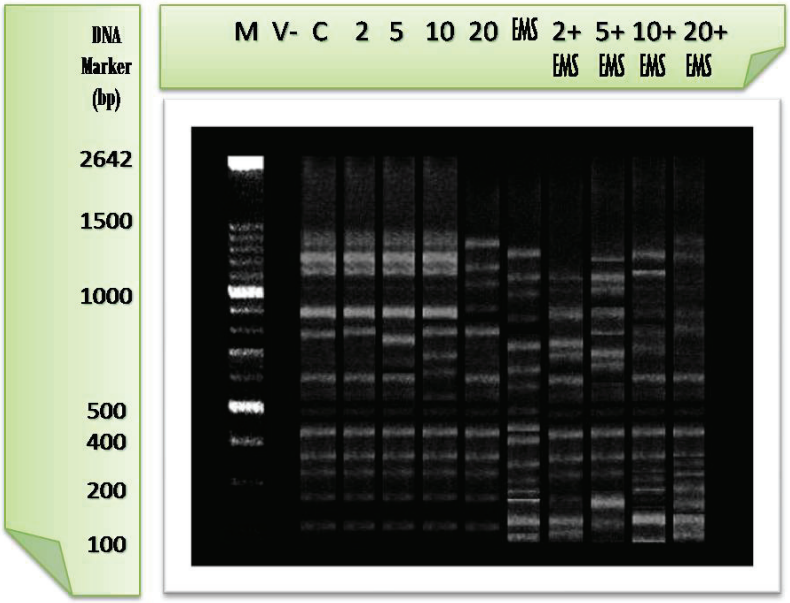

Fig. 3. RAPD profiles of genomic DNA from root cells of *Allium cepa* exposed to varying *C. speciosus* concentration in single and combined treatment with EMS. Header photo was indicated by M: DNA marker, V$^-$: negative gel control, C: control patterns, 2,5,10,20 : concentrations of *C. speciosus* (µg/mL), 2+, 5+, 10+, 20+: combined treatments of *C. speciosus* with EMS (µg/mL) using primer (OPA-20).

Table 3: Changes of total bands in control, and of polymorphic bands and varied bands in *Allium cepa* cells exposed to single and combined treatments of *C. speciosus* extract and EMS.

Primers	No. of bands in Control	Single treatments with *C. speciosus* (µg/mL)																EMS[1] (µg/mL)				Combined treatments with *C. speciosus* + EMS (µg/mL)															
		2				5				10				20				0.006				2+ 0.006				5+ 0.006				10+ 0.006				20+ 0.006			
		a	b	c	d	a	B	c	d	a	b	c	d	a	b	c	d	A	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d	A	b	c	d
Primer 1 (OPA-20)	**13**	0	0	0	0	1	0	0	0	1	0	0	0	4	2	3	1	18	13	0	0	3	3	3	1	5	2	2	2	8	3	2	2	9	4	3	2
Primer 2 (OPA-04)	**9**	0	0	0	0	0	1	0	0	1	1	0	0	2	1	2	1	21	9	0	0	2	3	1	1	3	1	2	1	4	3	1	2	4	3	1	1
Total bands	**22**	0	0	0	0	1	1	0	0	2	1	0	0	6	3	5	2	39	22	0	0	5	6	4	2	8	3	4	3	12	6	3	4	13	7	4	3
a + b		0				2				3				9				61				11				11				18				20			
a + b + c + d		0				2				3				16				61				17				18				25				27			

a: indicates appearance of new bands, b: disappearance of normal bands, c: decrease in band intensities, and d: increase in band intensities. a + b denote polymorphic bands, and a + b + c + d, varied band.

[1]: Completely variable and polymorphism bands compared with the control.

Two primers gave a total of 22 bands ranging from 110 to 1460 bp (primer 1) in molecular size in the control. Different polymorphic bands were detected at 5, 10 and 20 µg/mL of *C. specious* mildly for single treatments of *C. specious* extract. Moreover the combined treatments which decreased the number of polymorphic bands were induced by EMS. Value of polymorphic rate was $P = 0.0$, 0.09, 0.13 and 0.72 for 2, 5, 10 and 20 µg/mL of *C. specious* respectively and $P = 2.77$ at 0.006 µg/mL of EMS for single treatments. Meanwhile it was $P = 0.5$, 0.5, 0.82 and 0.91 for 2+0.006, 5+0.006, 10+0.006 and 20+0.006 µg/mL of *C. specious* + EMS respectively for combined treatments. In all cases, polymorphisms were due to the loss and/or gain of amplified bands in the treated samples compared with the control. In addition, replicated experiments confirmed that the variation in band intensities was stable and not a consequence of either a change in concentration of template DNA within a certain range or a change in PCR reagent concentration.

Discussion

The potential cytotoxic and genotoxic effects of the aqueous extract of *C. speciosus* on *Allium cepa* root cells were evaluated. The distilled water used as a control is of extremely good quality. Data that are presented in Table 1 showed the effects of extract on root growth of *Allium cepa*, that there was concentration dependent decrease in root growth. This result is consistent with the previous studies on medicinal herbs[29]. In *Allium cepa*, whenever there is root growth inhibition, there is always reduction in the number of dividing cells[23, 30, 31]. Inhibition of root growth in *Allium cepa* might be due to the presence of some heavy metals in the extract as stated in the studies of Al-Moaruf *et al*. ; Haider *et al*. [32, 33] on extracts of *Azadirachta indica*, *Mangifera indica*, *Cymbopogon citratus* and *Morinda lucida*. Some metals (zinc, copper, manganese, iron, cadmium and lead) have been implicated in inhibition of root growth in *Allium cepa*[23, 34, 35].

Free radicals have aroused significant interest among scientists in the past decade. Their broad range of effects in biological systems has drawn the attention of many experimental works. It has been proved that these mechanisms may be important in the pathogenesis of certain diseases and ageing. There are many reports that support the use of antioxidant supplementation in reducing the level of oxidative stress and in slowing or preventing the progress of complications associated with diseases[36]. Many synthetic antioxidant components have shown toxic and/or mutagenic effects, which have shifted the attention towards the naturally occurring antioxidants. Numerous plant constituents have proven to show free radical scavenging or antioxidant activity[37]. Flavonoids and other phenolic compounds (hydroxyl

cinnamic derivatives, catechines, *etc*.) of plant origin have been reported as scavengers and inhibitors of lipid peroxidation[38]. In the present study, superoxide radical reduces NBT to a blue colored formazan that is measured at 560nm. Fig. 1 shows the superoxide scavenging effect of *C. speciosus* and Curcumin on the PMS/NADH-NBT system. The decrease of absorbance at 560nm with antioxidants indicates the consumption of superoxide anion in the reaction mixture. *C. speciosus* at concentration from 10-125 µg/mL inhibited the production of superoxide anion radicals by 24.21-68.72%. This result is supported by the previous studies on antioxidant activity of *C. speciosus*[39, 41].

Chromosomal aberrations are changes in chromosome structure resulting from a break or exchange of chromosomal material. Most of the CA observed in cells are lethal, but there are many corresponding aberrations that are viable and may cause genetic effects, either somatic or inherited[42]. The results of this study did not show induction of chromosome or chromatid type of aberration in the treated cells (Table 2), but disturbances of spindle fibers at different stages of mitotic division in *Allium cepa* root cells (Fig. 2) were observed and might have been due to the presence of alkaloids or glycosides in *C. speciosus* extract. This is because extract of *C. speciosus* has been reported to contain various alkaloids, tannins, phenols, saponins, and spirostanol glycosides[8, 9]. The induction of spindle disturbances in the cell of *Allium cepa* by this extract may lead to aneuploidy and or micronucleus formation at the next stage of cell division. This usually arises from irregular separation of chromosomes at anaphase thereby making some chromosomes to reach the poles before the others. The lagging chromosome(s) or fragments may be lost or form nuclear membrane around itself thereby forming micronucleus[21]. The reduction in the number of dividing cell at tested concentrations suggests that extract of this plant has mitodepressive effect on the cell division of *Allium cepa*. Mitodepressive effects of some plant extracts, the ability to block the synthesis of DNA and nucleusproteins had earlier been reported[43, 44]. They may not even allow the initiation of their biosynthesis and such action occurring in the interphase nucleus apart from influencing the ultimate structure of the chromosome during cell division could also cause reduction of number of other stages. The results here suggest that the tested extract possess inhibitory, mitodepressive effects mildly on root growth, cell division and chromosomes behavior of *Allium cepa* cells.

Ethyl methanesulphonate (EMS) is a mutagenic, teratogenic, and possibly carcinogenic organic compound with formula $C_3H_8O_3S$. It produces random mutations in genetic material by nucleotide substitution; specifically by guanine alkylation. This typically produces only point mutations. It can induce mutations at a rate of $5x10^{-4}$ to $5x10^{-2}$ per gene without substantial killing. The ethyl group of EMS reacts with guanine in DNA, forming the abnormal base O-

6-ethylguanine. During DNA replication, DNA polymerases that catalyze the process frequently place thymine, instead of cytosine, opposite O-6-ethylguanine. Following subsequent rounds of replication, the original G:C base pair can be altered to A:T pair. This change in genetic information, is often harmful to cells, and can result in disease. Many mutagens cause a wide variety of cancers in humans[18]. EMS is often used in genetics study to induced chromosome aberrations and mutations at statistically significant levels[19]. The results in the present investigation exhibited the ability of *C. speciosus* as antigenotoxic and anticytotoxic potential against EMS induced DNA damage and CA in *Allium cepa* cells. Similar result observed by Qari[45] was exhibited of *O. marjorana* effects against sodium azide which induced micronucleus, number of aberrant cells and different kinds of chromosomal aberrations in *Vicia faba* cells. The protective effect of *C. speciosus* is due to its antioxidant action, trapping of free radicals, formation of complex with mutagens[46]. The mode of action of anti-mutagenesis may act as modulation of mutagen metabolism by absorbing the xenobiotics, or inhibition of SOS (superactive oxygen species) functions or by altering the activation and detoxification of toxic agents as suggested by similar results obtained by Premkumar *et al*.; Oda; and Goud *et al*., [47, 49] respectively in their studies with curcumin, saffron and garlic. Also, the stabilization of the formed phenoxy free radicals is responsible for its free radical scavenging activity and chemopreventive effect mutagens[50]. The modulatory role of *C. speciosus* in inhibiting mutagenicity and/or cytotoxicity need more studies to understand the mechanism of antigenotoxic action.

DNA based assays such as RAPD and AFLP are the most widely used tools for assessment of the genetic variation due to use of RAPD for the detection of DNA damage presents a number of advantages[51]. The assay of RAPD is suitable for any extracted DNA of sufficient quality, allows rapid analysis of a large number of samples. As arbitrary primers are used, specific details of DNA damage or the genome sequence in organisms are not needed. Furthermore, no radioactivity or enzymatic degradation of PCR products is required prior to analysis[27]. Previous studies have shown that changes in DNA fingerprint (*i.e.* band patterns) observed reflect DNA alterations in genome from single base changes (point mutations) to complex chromosomal rearrangements[27, 52] and that DNA fingerprinting offers a useful biomarker assay in assessment of genotoxicity[53, 54]. But, in this study, DNA variation was induced mildly by *C. speciosus* on *A. cepa* cells, meanwhile, the treatments with EMS was clearly reflected by changes in RAPD profiles as variation in band intensity, disappearance of bands, and appearance of new PCR products occurred in the profiles.

In this study, no change in band intensity occurred for the three lower *C. speciosus* concentration (2, 5 and 10 µg/mL) for primer 1 and 2 whereas mild decrease in band intensity was particularly obvious for *Allium cepa* exposed to 20µg/mL of *C. speciosus* for primer 2. Also, disappearance of PCR products was obvious for *Allium cepa* cells exposed to the higher concentration of *C. speciosus* (20µg/mL). The disappearance of PCR products mainly affected the high molecular weight bands because the chances of obtaining DNA damage increased with the length of the amplified fragment, this suggesting random interaction between DNA and *C. speciosus*. Whereas EMS induced decrease in band intensity and disappearance/appearance of PCR products for primer 1 and 2.

EMS and high concentration of *C. speciosus* could induce DNA damage such as single- and double-strand breaks, modified bases, basic sites, DNA-protein cross-links, oxidized bases and even huge adducts *etc*. in organisms[55-58]. The presence of the above types of DNA lesions and mutations may also induce important structural changes that can significantly affect the chemical reaction of PCR events[59] Appearance of new PCR products occurred because some oligonucleotide priming sites could become accessible to oligonucleotide primers after structural change or because some changes in DNA sequence have occurred due to mutations (resulting in new annealing events), and/or large deletions (bringing two pre-existing annealing sites closer), and/or homologous recombination (two sequences that match the sequence of the primer)[27]. The appearance of extra bands occurred mostly with EMS and high concentration of *C. speciosus* (20µg/mL) for differing primers. Apparent bands may also be the results of genomic template instability related to the level of DNA damage, the efficiency of DNA repair and replication[27].

In conclusion, the data presented here show, that *C. speciosus* extract could not induce a significant genotoxic effect in *Allium cepa* cells. Furthermore, this study implies that combined treatment of *C. speciosus* with EMS has a strong inhibitory role against the genotoxic action of EMS. More investigations are necessary to determine the components can act as antimutagenic agent.

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الكشف عن السمية الوراثية ومقاومة السمية الوراثية للمستخلص المائي لنبات القسط العربي باستخدام البصمة الوراثية بتقنية DNA-RAPD والمؤشرات الخلوية الوراثية

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المستخلص. الهدف: لقد ركزت الدراسة الحالية على فحص تأثير المستخلص المائي لنبات القسط العربي لإحداث السمية الوراثية أو مقاومتها، والذي عادة ما يستخدم لعلاج العديد من الاختلالات المرضية في المملكة العربية السعودية. الطرق: تم تعريض الخلايا الإنشائية في القمم النامية لجذور نبات البصل لتراكيز متصاعدة من المستخلص النباتي (٢، ٥، ١٠، ٢٠ ميكروجرام/مل) لمدة ٤٨ ساعة، وقد هيئت هذه المعاملات للاختبارات الخلوية الوراثية والجزيئية من خلال تطبيق البصمة الوراثية بتقنية الـــRAPD. النتائج: لقد أظهرت النتائج أن المستخلص النباتي لم يظهر تأثيرا طفورياً آو سام كروموسوميا بدرجة معنوية، فقد كان هناك انخفاض طفيف في دالة الانقسام مقارنة بالتجربة الضابطة، كما أن الاختلالات الكروموسومية المستحدثة غير معنوية عند جميع المعاملات، ومعظم الاختلالات الخلوية المسجلة كانت على هيئة تشتت كروموسومي. كما أظهرت نتائج البصمة الوراثية أنه لا تغير معنوي في المادة الوراثية لخلايا البصل من جراء التعرض للمستخلص النباتي. في حين أن النتائج أوضحت أن المستخلص المائي لنبات القسط العربي استطاع مقاومة طفور الاختلالات الخلوية المستحدثة بفعل مادة ايثايل ميثانوسلفونيت EMS. الخلاصة: أوضحت البيانات المعروضة في هذه الدراسة أن

مستخلص القسط العربي المائي ليس له تأثير سام وراثيا بشكل معنوي عند الجرعات المستخدمة في هذه الدراسة، وفي الوقت ذاته، فإن المستخلص المائي لنبات القسط يمتلك العديد من المؤشرات على أنه عامل مقاوم للطفور والتسمم الذي قد يلحق بالجهاز الوراثي.