Deregulation of Mitosis Progression and Cytotoxic Effect Triggered in *Allium Cepa* L. Roots by *Rubus Sancatus* Schreber Extract

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Abstract: Background and Aims: Medicinal plant extracts have been traditionally used in curing several diseases in folk medicine but several of them proved to cause cellular changes in many vital systems. Family Rosaceae demonstrated different biological activities in several previous investigations. The present study aims to assess the cytological and cellular effect of different concentrations of *Rubus sanctus* Schreber aqueous alcoholic extract (*Rs*AAE) on *Allium cepa* L. root meristems using different techniques. **Material and Methods**: Cytological and cellular effect of different concentrations (1, 5& 10 mg mL^{-1}) of *Rs*AAE for different duration of administration (3, 6& 12h) on *A. cepa* L. root cells were studied using mitotic, ultrastructural and biochemical analyses. **Result:** The administration of different concentrations of *Rs*AAE for different periods of exposure exhibited suppression of the mitotic and cell cycle progression on *A. cepa* root meristems. Several mitotic abnormalities were recorded. *Rs*AAE enhanced deleterious changes on the cellular organelles of *A. cepa* root meristems leading to autolysis of the cells. Gene expression was changed as 10 *de novo* bands were produced as well as several bands were disappeared, these alterations were associated with a drastic inhibition of protein content. **Discussion**: The present study declared the prevalence mitostatic and cytotoxic effect of *Rs*AAE on *A. cepa* root meristems. In spite of *R. sanctus* has valuable effects as a medicinal herb, it can enhance severe complications and destruction on the normal cells when it is used indecorously.

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1. Introduction

The decision of cell to enter and/or exit cell division or differentiate depends on several cellular events and signals. These cellular events have long been of great interest to understand the bases of each one. Several agents stimulate the mitotic activity, while others cause mitodepressive and toxic activity. These mitodepressive drugs are effective against cells that are proliferating and some of them produce cytotoxic effect either by damaging the DNA during the S-phase of the cell cycle or by blocking the formation of the mitotic spindle in M-phase **(Gali-Muhtasib and Bakkar, 2002; Cuyacot *et al.,* 2014).** The ever increase usage of medicinal plant extract in curing diseases instead of the synthetic drugs has been established so long ago, but recent *in vitro* and *in vivo* studies have explored the mutagenic and the cytotoxic effect of many plant extracts used as food or in traditional medicine **(Komal, 2013; Asthana and Kumar, 2014).** These findings raise the concern about the potential mutagenic and/or the cytotoxic hazards resulting from the wrong or the extra usage of such plant extracts. Most of the antimitotic compounds derived from plant affect the microtubule dynamics of the cell and induce persistent modification of biological processes and signaling pathways that ultimately lead to cell death **(Mollinedo and Gajate, 2003).** Inhibition of mitotic activity is linked to disturbance of some basic process in the correct cell cycle progression as well as the mitotic index (MI) ratio. Mitotic index is a characteristic ratio for particular tissue, so any change in this ratio reflects the influence on the biochemical events inside the cell **(Çelik and Aslantürk, 2010).** Some phenolic compounds present in plant extract, such as catechin and quercetin, interfere with DNA as well as protein synthesis and thereby exhibit strong cytotoxic activity **(Wong and McLean, 1999).**

Rosaceae family has been traditionally used for various therapeutic purposes. One of the most important genera among this family is Rubus genus. The leaves extract of *Rubus ulmifolius* has been traditionally used for the treatment of diarrhea, wounds healing, liver diseases and diabetes mellitus **(Dall'Acqua *et al.,* 2008).** The polyphenol extract of *Rubus idaeus* exhibited anticancer activity against the colon cancer *in vitro* **(Coates *et al.,* 2007)**, while the total saponin extract of *Rubus parvifolius* displayed antitumor effect on malignant melanoma **(Zheng *et al.,* 2007).** The radical scavenging activity of *Rubus*

idaeus ethanol extract has been proved by **Venskutonis *et al.* (2007).** *Rubus coreanus* can be used to improve visual sensitivity and other retinal functions. It can also be used to delay the lipid peroxidation damage to eye sight and may stop blindness caused by lipid peroxidation **(Wahid *et al.*, 2011). Cai*et al.* (2012)** proved the antibacterial activity of the volatile oil extracted from *Rubus parvifolius* leaves against wide range of Gram positive and negative bacteria**.** Our objective plant *Rubus sancatus* Schreber has been proved to have a protection property against carbon tetrachloride-induced toxicity in rats liver **(Badr *et al.*, 2009)** as well as an antioxidant and wound healing properties **(Serteser *et al.*, 2008; Peşin *et al.*, 2009).** Additionally, it has been shown to have an anti-inflammatory activity using carrageenan-induced hind paw edema on mice and potent antinociceptive activity **(Akcos *et al.*, 1998; Erdemoglu *et al.*, 2003).** *Rubus sanctus* Schreber grows wild in Saint Katherine and it is used for wound healing in folk medicine (self-communication)**.** The plant is a shrub with 1–2 m of a prostrate or arching stem. It has ternate or palmate leaves with 5 leaflets. Flowers are numerous, while fruits with several black, glabrous and juicy drupelets **(Boulos, 1999).** Although the several previous studies, there have been no investigations concerning the evaluation of the cellular and cytological effects of *Rs*AAE, therefore the purpose of the present study was to evaluate the cytotoxic and antimitotic potential of *Rs*AAE *in vivo* using standard assay methods utilizing *Allium cepa* L. root meristem model **(Sharma, 1983),** the results from the present study will enhance understanding of cellular and cytological toxicity mechanism**.**

2. Material and methods

2.1 Plant materials and preparation of extracts

Onion bulbs (*Allium cepa* L., 2*n* = 16, var. Giza 6 Mohassan) were obtained from The Agriculture Research Center Giza, Egypt. The dried outer scales were carefully removed then the bulbs were used for the bioassay according to standard procedures **(Rank and Nielsen, 1993).***Rubus sancatus* Schreber aerial parts were collected from Wadi Gebal, Saint Katherine, Egypt in May 2010. It was authenticated by Saint Katherine Protectorate members.

A weight of 100 g of air dried aerial part of *R. sancatus* was extracted five times in 500 mL of 70% ethyl alcohol at room temperature. The collected extracts were filtered and concentrated under reduced pressure. After the lyophilization, the lyophilized extract was dissolved in distilled water forming concentrations of 1, 5 and 10mg mL^{-1}.

2.2 The phenolic profile of Rubus sancatus aqueous alcoholic extract

Identification of individual phenolic compounds of *Rs*AAE was performed according to **Soliman (2002)**. A weight of 0.1 g of dried extract was extracted in 3mL of spectral grade methanol. HPLC analysis was performed on a Hewlett-Packard HPLC (Model 1100) using a hypersil C18 reversed-phase column. A constant flow rate of 1mL min^{-1} was used with two mobile phases: (A) 0.5% acetic acid in distilled water at pH 2.6, (B) 0.5% acetic acid in 99.5% acetonitrile. The elution gradient was linear starting with (A) and ending with (B) over 35min using UV detector at λ= 254nm. Phenolic compounds were identified by comparing their relative retention time with those of the standards mixture chromatogram. The percentage of each compound was calculated on the basis of the area peak percentage.

2.3 Mitosis analysis

Allium cepa bulbs were grown in tap water for 48h at room temperature. Five germinated bulbs were suspended on each concentration (1, 5& 10mg mL^{-1}) of *Rs*AAE and distilled water for the control for 3, 6&12h. Five root meristems per each experimental group were fixed in Carnoy's fixative, hydrolyzed in 1N HCl at 58-60ºC for 5min then stained with Schiff's reagent. Squashed specimens were analyzed at 40x10 magnification using Olympus Microscope (BX50). Mitotic index (MI) was calculated as the percent ratio between dividing cells and total number of cells. While, the proportion of mitotic phases was determined as a percent ratio between cells in specific phase and the dividing cell according to **Fiskesjö (1985; 1997)**, moreover the frequency of abnormalities was scored as the percent ratio between aberrant cells and the dividing cell **(Gabara *et al.*, 2006).** The most frequent abnormalities were pictured using Panasonic digital camera CP230.

2.4 Ultrastructure analysis

Treated and control specimens of *A. cepa* root meristems were fixed in 2% glutaraldehyde in 0.05mol L^{-1} sodium cacodylate buffer (pH 6.9), for 2h at 4ºC. Specimens were washed in sodium cacodylate buffer and post fixed in 4% osmium tetraoxide for 1h at 4ºC. Afterward, specimens were dehydrated in ethanol water series starting with 50% followed by passage through a graded propylene oxide ethanol series, root meristems were gradually infiltrated with Epon 812 resin by placing them for 3h in each of series of resin/propylene oxide mixture, ending by 100% resin. Finally, specimens were embedded in freshly prepared resin, and polymerized in oven at 60°C for 48h **(Glińska and Gabara, 2000).** Sections (1 μm) were cut with Reichert Ultra-microtome, mounted on copper grids and stained with 0.5% uranyl acetate for 30min and lead citrate for 30min as described by **Reynolds (1963)**. Observations were carried out using JEOL TEM 1010 transmission

electron microscope at 80 kV, Electron Microscope Unit, Center for Mycology and The Regional Biotechnology, Al Azhar University.

2.5 Biochemical analysis

Estimation of total soluble protein content in treated and untreated *A. cep* roots was carried out using 0.8g of fresh tissue. The tissues were air dried, frozen in liquid N_2 and ground to a fine powder. Powdered tissues were extracted by 1mL 80% ethyl alcohol, precipitated for 15min at 4000 rpm at -5°C and dissolved in 1mL phosphate buffer (pH 7.0). The protein extract were stained using Coomassie Brilliant Blue G-250 (Sigma-Aldrich Product Code 27815) and the absorbency was recorded photometrically at 595nm using spectronic 21D according to **Bradford (1976)**.

Protein pattern was detected using sodium dedocylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) of 12.5% and 0.75mm thick by means of Hoefer (SE 245) dual vertical minigel. A weight of 1g of each treatment was powdered by liquid nitrogen, extracted with 1mL of extraction buffer (1.21 g TrisHCl, 1 mL 10% SDS, 0.5 mL β-mercaptoethanol & 5g sucrose completed to 50 mL distilled water - pH 8.0), precipitated, then the pellets were dissolved with sample buffer (1.2mL TrisHCl, 2mL 10% SDS, 1mL glycerol, 0.5mL 0.4% bromophenol, 0.5mL β-mercaptoethanol& 4.8mL distilled water) following protocol of **Gallagher and Smith (1995).** The homogenate was boiled in water bath for 90 sec, loaded on gel and allowed to run at 70 volts, 40 mA. Finally, the gel was stained with Coomassie Brilliant Blue stain and destained according to **Laemm Li (1970)** and **Wilson (1983).** Protein bands were identified by comparing their molecular weights with those of wide range Sigma marker (Product Code C3437**)**. Gel was photographed and scanned for analysis using Gene Tools syngene ver. 4.00(a) gel documentation system software.

2.6 Statistical analysis

Statistical analysis was carried out using GraphPad (Version 4.0) with five replicates for each group. Data were expressed as mean ± standard error (s.e.) calculated by one way analysis of variance (ANOVA) and significance was analyzed using Tukey's and Dunnett's tests where significance was accepted at $p \leq 0.05$ & $p \leq 0.01$.

3. Result

3.1 The phenolic profile of Rubus sancatus aqueous alcoholic extract

The HPLC chromatogram of the phenolic profile of *Rs*AAE declares that the extract contains mixture of phenolics as can be shown in Fig. 1. Protocatechuic as a simple tannin was observed in peak number 4 with retention time (RT) 12.6 and some phenolic acids (hydroxy benzoic acid and chlorogenic acid) were also shown in peaks 8 and 9 with RT of 14.6 and 15.5 respectively. The phenolic compounds such as phenol and vanillin (peak 10 and 13) were present in *Rs*AAE with RT of 17.3 and 19.4, while only 1 flavonoid compound (quercetin) was exist in peak 18 with RT of 32.7. Figure 1 reveals the presence of some unidentified compounds. The highest contribution is shown by chlorogenic acid (27.30%), whereas the lowest one (0.65%) is the phenol compound (Table 1).

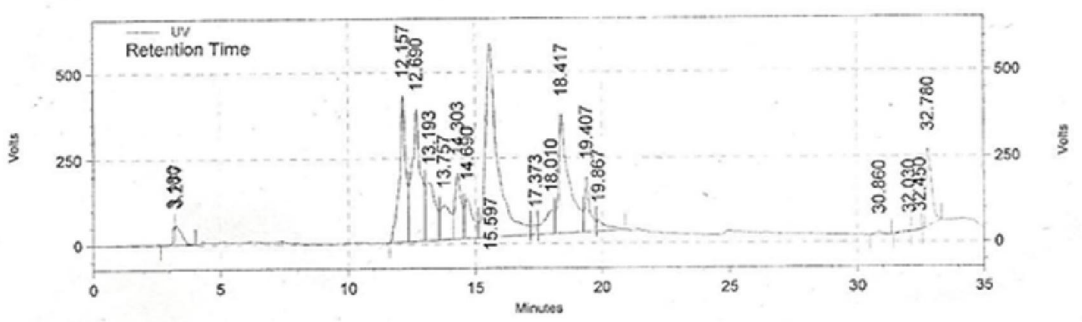


Figure 1. HPLC chromatogram of *R. sancatus* aqueous alcoholic extract

Table 1. Phenolic compounds of *Rubus sancatus* aqueous alcoholic extract identified using HPLC

Peak No.	RT	Compound	Percentage
4	12.6	Protocatechuic	13.14
8	14.6	Hydroxy benzoic acid	4.01
9	15.5	Chlorogenic acid	19.26
10	17.3	Phenol	0.89
13	19.4	Vanillin	5.45
18	32.7	Quercetin	7.69

3.2 Mitosis analysis

Cytological analysis revealed the depression of mitotic indices (MI) of treated roots as compared with those of their respective controls at all durations and concentrations tested. This statistically significant reduction was concentration and time dependent. The toxic effect of *Rs*AAE was clearly observed after treatment with 5mg $mL^{-1}$and 10mg mL^{-1} for 12h as cell division was completely inhibited. The lowest ratio of MI (0.88) was achieved after treatment with 10mg mL^{-1} *Rs*AAE for 6h, while 2.95 was the highest MI ratio which was achieved after treatment with 1mg mL^{-1} *Rs*AAE for 3h (Table 2).

Table 2. Effect of *Rubus sancatus* aqueous alcoholic extract on mitotic index and percentage of mitotic abnormalities of *Allium cepa* root meristems

	Total No. of cells	Mitotic index ± s.e.	% of Mitotic abnormalities ± s.e.
Control			
3h	5030	4.33±0.24	10.17±1.53
6h	5035	4.42±0.11	12.22±1.65
12h	5049	4.82±0.10	12.69±1.59
1mg mL^{-1}			
3h	5140	2.95±0.05**	15.04±2.01
6h	5080	2.55±0.06**	25.30±2.59
12h	5173	1.63±0.05**	44.46±2.93**
5mg mL^{-1}			
3h	5079	2.37±0.05**	20.62±1.37
6h	5053	0.94±0.05**	20.67±2.78
12h	5000	Toxic	Toxic
10mg mL^{-1}			
3h	5137	1.16±0.03**	29.97±4.76**
6h	5180	0.88±0.04**	44.26±6.33**
12h	5000	Toxic	Toxic

**Significant compared to its control at 0.01 level

A. cepa root meristems of control groups displayed the existence of all phases of mitosis. The different concentrations of the *Rs*AAE caused changes in the proportion of mitotic phase's distribution in comparison to the control. The conspicuous effect was the increase of prophase index reaching 76.41% during soaking in 5mg mL^{-1} for 6h, while treatment with 10mg mL^{-1} caused decrease of prophase index although of this retardation, prophase stage was over the other phases. Telophase index was usually the least frequent and it was not completely detected after several doses of *Rs*AAE (Fig. 2).

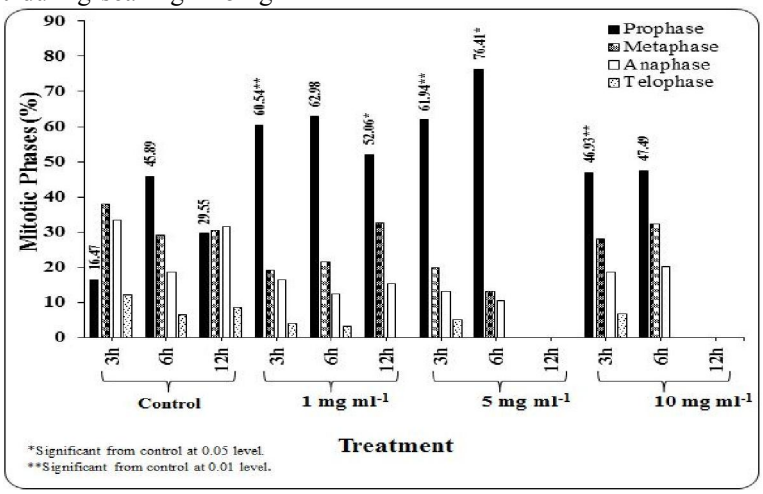
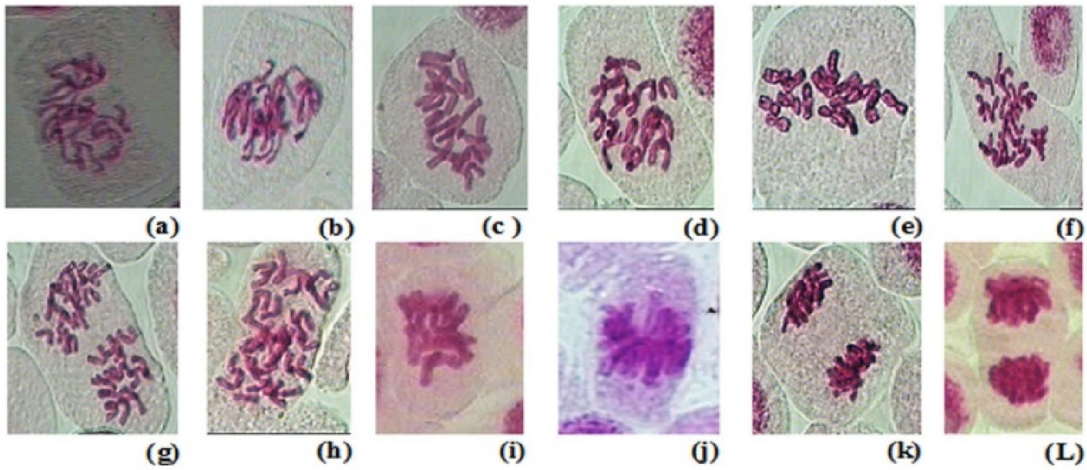


Figure2. Phase index of *Allium cepa* root meristems treated with different concentrations of *Rubus sancatus* aqueous alcoholic extract

*Rs*AAE induces wide range of mitotic abnormalities in *A. cepa* root meristems as compared with the control. The percentage of total abnormalities was significantly increased as compared with control (Table 2). This increment was time and concentration dependent. Mitotic anomalous was manifested by disturbance in the spindle apparatus and formation of irregular prophase and stickiness in some phases. An alteration in chromosome structure was exhibited by strong chromosome's condensation and contraction (Fig. 3). The mitotic observations elucidated that both of c-mitosis and irregular prophase were dominant abnormalities resulting from all treatments.

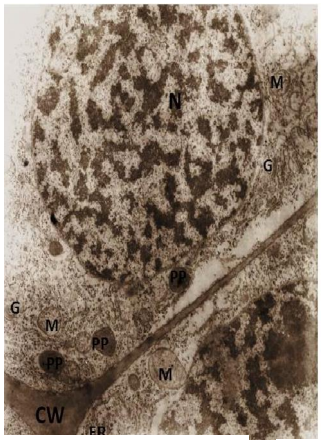


(a-b): Roots treated with 1mg $mL^{-1}$of *Rs*AAE for 12h shows irregular prophase, (c): Roots treated with 10mg $mL^{-1}$of *Rs*AAE for 3h shows disturbed metaphase, (d): Roots treated with 5mg $mL^{-1}$of *Rs*AAE for 6h shows disturbed metaphase, (e): Roots treated with 10mg $mL^{-1}$of *Rs*AAE for 6h show c-metaphase in contracted chromosome, (f-g): Roots treated with 10mg $ml^{-1}$of *Rs*AAE for 6h shows disturbed anaphase in contracted chromosome, (h): Roots treated with 10 mg $mL^{-1}$of *Rs*AAE for 3h shows c-anaphase, (i): Roots treated with 1mg $mL^{-1}$of *Rs*AAE for 12h shows sticky disturbed metaphase, (j): Roots treated with 1mg $mL^{-1}$of *Rs*AAE for 3h shows sticky metaphase,(k): Roots treated with 5 mg $mL^{-1}$of *Rs*AAE for 3h shows sticky anaphase, (L): Roots treated with 1mg $mL^{-1}$of *Rs*AAE for 6h shows sticky anaphase

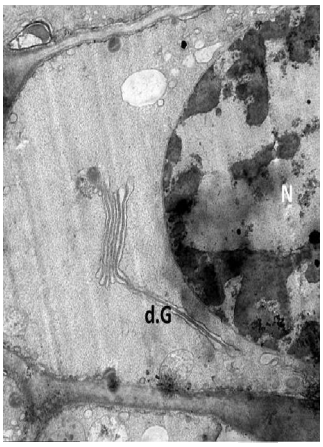
Figure 3. Types of mitotic anomalies induced in *Allium cepa* root meristems after different treatments with *Rubus sancatus* aqueous alcoholic extract

3.3 Ultrastructural analysis

The cytoplasm of control cells showed normal distribution of cellular organelles; it contains a large nucleus, dense cytoplasm containing several ribosomes or polyribosomal complexes and well organized organelles such as mitochondria and proplastids. Moreover, it encloses many elements of Golgi apparatus and endoplasmic reticulum (Fig. 4a). The cytotoxic effects of *Rs*AAE on *A. cepa* root meristems sub-cellular organelles were elucidated in the electron micrographs. There were gradual changes in the structure in the cell's organelles; these changes were influenced by the concentration and time of treatment. The intracellular changes showed stimulation of lytic processes and presence of different deformed organelles. The first sign of cellular toxicity was dilation of the cisternae of Golgi apparatus dictyosomes and ER lumen (Fig 4b-c),while cisternae of Golgi dictyosomes in control cells were found to be parallel, stacked, and straight. The ultrastructural results of the median dose (5mg mL^{-1} of *Rs*AAE for 6h) showed vacuolization of the mitochondria and cell' s proplastids (Fig. 4d). Exposure to high dose (10mg mL^{-1} of *Rs*AAE for 12h) altered the endomembrane system of the cells and induces concentric dilated Golgi dictyosomes attached to multivesicular bodies (MVBs). The accumulation of vesicles around the dictyosomes was not seen; most of the dictyosomes were crescent (Fig 4e). It is also showed the disruption of the mitochondrial membrane and cristae were poorly present. (Fig.4f). Finally, the chromatin material was highly condensed and there was completely disintegration of cell organelles. The vacuoles of treated cells were increased in comparison of control cells then the cytoplasm became structure-less (Fig 4g).



TEM Mag = 6000x (a)



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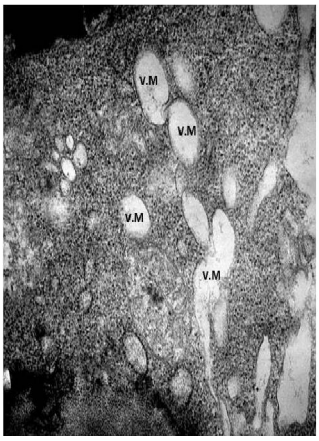
TEM Mag = 40000x (c)



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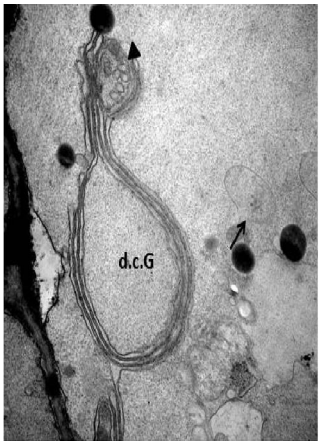

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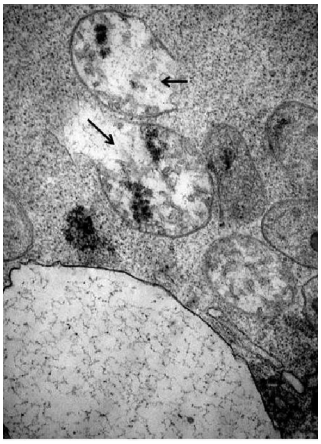
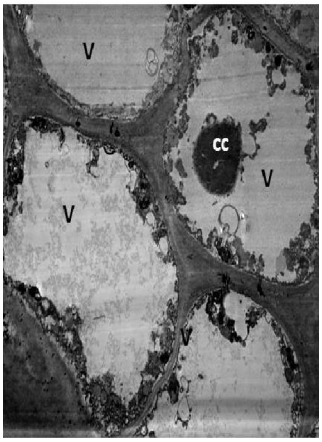


TEM Mag = 30000x (f)



TEM Mag = 5000x (g)

(a): Control root cell, (b-c): Roots treated with 1mg ml^{-1}of *Rs*AAE for 3h shows dilated Golgi cisterna and ER lumen, (d): Roots treated with 5mg ml^{-1}of *Rs*AAE for 6h shows vacuolization in mitochondria.(e-g): Roots treated with 10mg ml^{-1}of *Rs*AAE for 12h show concentric dilated Golgi cisterna, rupture of mitochondria' s membrane and vacuolization of the cytoplasm, N: nucleus, M: mitochondrion, CW: cell wall, PP: proplastids, ER: endoplasmic reticulum, d.G: dilated Golgi apparatus, line: dilated endoplasmic reticulum, v.M: vacuolated mitochondrion, d.c.G: dilated concentric Golgi apparatus, V: vacuole, cc: condensed chromatin, arrow: rupture mitochondrion, arrow head: multivesicular bodies

Figure 4. Electron micrographs of control and treated root meristems of *Allium cepa*

3.4 *Biochemical analysis*

A gradual decrease in protein content of *A. cepa* root cells was obvious after most treatments (Fig. 5). The application of 10mg mL^{-1} of *Rs*AAE for 12h induced the least protein content reaching 0.55mg $100g^{-1}$, whereas 2.53mg $100g^{-1}$was the highest protein content which was induced by 1mg $mL^{-1}$of *Rs*AAE for 3h. The decreased frequencies of protein content were statistically significant.

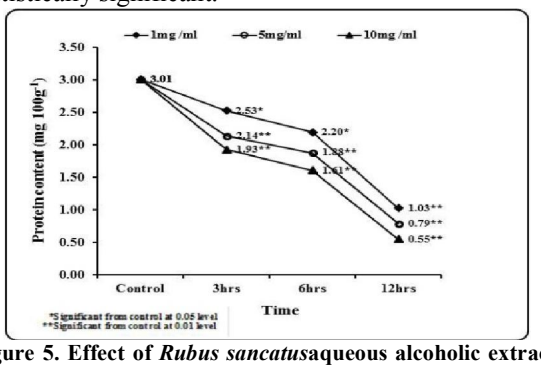
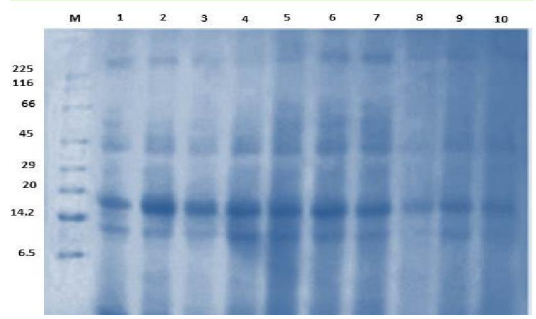


Figure 5. Effect of *Rubus sancatus*aqueous alcoholic extract on protein content of *Allium cepa* roots

Modification in gene expression of *A. cepa* roots was represented in the protein banding pattern (Fig. 6). The data analysis reveals that the number of *de novo* induced band was 11 bands with molecular weights of (225, 116, 76.1, 71.5, 59.8, 40.3, 27.9, 20, 9.4, 7.6 &6.5kDa). Some bands were disappeared in specific treatment, while 6 bands with molecular weight of 245, 66, 55.7, 50.6, 45, 19.2 & 12.3kDa were common bands. Moreover, treatment with *Rs*AAE changed the intensity of induced bands as compared with their control (Table 3).The different concentrations of *Rs*AAE altered the electrophoretic pattern of *Allium cepa* root proteins but didn't show dose response relationship.



M: Wide range marker, 1: Control, 2: Roots treated with 1mg ml^{-1} for 3h, 3: Roots treated with 1mg ml^{-1} for 6h, 4: Roots treated with 1mg ml^{-1} for 12h, 5: Roots treated with 5mg ml^{-1} for 3h, 6: Roots treated with 5mg ml^{-1} for 6h, 7: Roots treated with 5mg ml^{-1} for 12h, 8: Roots treated with 10mg ml^{-1} for 3h, 9: Roots treated with 10mg ml^{-1} for 6h, 10: Roots treated with 10mg ml^{-1} for 10h

Figure 6. SDS-PAGE electrophoretic profile of *Allium cepa* roots treated with different concentrations of *Rubus sancatus* aqueous alcoholic extract

4. Discussion

Recent studies reveled that several plant extracts have different biological activities as antimicrobial, antitumor, anti-inflammatory and antidiuretic activities, from this point of view, it is important to study the mode of action of the whole extract and its biologically active compounds on different biological systems. The present study evaluates the cellular effects of *Rs*AAE using Allium test. The results of HPLC analysis of *Rs*AAE is concurring with the former phytochemical studies on different Rubus species, which had isolated several flavonoids compounds (quercetin, kaemperol and myricetin), phenolic acids (ellagic acid, caffeic acid and chlorogenic acid), tannins (catechin and ellagitannins), anthocyanins and anthocyanin derivatives (cyanidin-3-*O*-rutinoside, cyanidin-3-*O*-glucoside and pelargonidin-3-*O*-(2G)-glucosyl rutinoside) and saturated and unsaturated fatty acids **(Gudej and Tomczyk, 2004; Coates *et al.*, 2007; Gevrenova *et al.*, 2013)**. Caffeoyl sugar esters and ellagitannin were previously isolated from *Rs*AAE by **Hussein *et al.* (2003),** while **Badr *et al.* (2009)** have successfully isolated quercetin and myricetin derivatives from *Rs*AAE. Phenolic compounds are known to have several biological activities as anticancer, antimicrobial and radical scavenging **(Falleh *et al.*, 2008; Oueslati *et al.*, 2012; Velićanski *et al.*, 2012)**. Phenolic compounds have the ability to act as antioxidant agents, but their cytotoxic effect through generating free radical (pro-oxidant agents) is proved by **Galati and OBbrien (2004). Cao *et al.* (1997)** proved that the same phenolic compound could act as both antioxidant and pro-oxidant agents depending on its concentration**.** Quercetin has the ability to enhance the generation of hydroxyl radical, which can cause damage in DNA and other biological molecules **(Laughton *et al.*, 1989).** Cytotoxic effect of phenolic compound plays an important role in their anticancer and antimitotic properties **(Hadi *et al.*, 2000)**.

This work explores the cytostatic properties of *Rs*AAE on cell division of *A. cepa*. This reduction of mitotic activity may be due to blocking of cells in G_2 phase as a result of damage in DNA which activates a series of protein kinases like Chk1 and Chk2 that phosphorylate the Cdc25 protein phosphatase which in turn blocks the dephosphorylation and activation of M phase cyclin dependent kinases (M-Cdk), thereby blocking entry into mitosis **(Harrison and Haber, 2006)**. Delay in *DNA damage checkpoints* provides time for the damaged DNA to be repaired, after which the cell-cycle brakes are released and progress resumes **(Hartwell and Weinert, 1989).** Besides, the inhibition of DNA synthesis could also inhibit the mitotic activity **(Schneiderman *et al.*, 1971)**.

Table 3. Effect of *Rubus sancatus* aqueous alcoholic extract on the protein profile and bands intensities of *Allium cepa* roots

Mol.wt.Kilo Daltons	Control	1mg mL^{-1}			5mg mL^{-1}			10mg mL^{-1}		
		3h	6h	12h	3h	6h	12h	3h	6h	12h
245.0	1.35	1.08	0.52	0.32	0.96	1.2	1.57	0.41	0.94	0.72
225.0	-	-	-	-	-	-	-	-	0.21	-
116.0	-	-	-	-	-	-	0.18	-	0.23	-
76.1	-	-	-	0.34	0.86	1.09	1.09	-	1.52	-
71.5	-	-	-	-	2.15	2.08	1.19	-	1.12	-
66.0	0.16	1.26	1.59	1.21	1.75	2.24	2.64	2.35	1.88	0.13
59.8	-	1.14	1.82	0.89	0.84	1.35	1.72	1.03	1.20	-
55.7	2.60	1.97	1.91	1.29	1.49	2.11	1.32	1.98	0.82	1.60
50.6	2.89	3.25	2.68	2.57	3.32	4.79	3.40	3.65	3.46	2.60
45.0	2.73	2.22	1.42	2.82	3.11	3.83	3.28	1.74	2.42	2.72
40.3	-	2.35	-	2.02	2.89	2.67	2.08	-	2.67	1.58
29.0	1.02	3.43	2.67	3.04	2.23	2.48	2.89	2.01	3.10	-
27.9	-	3.17	3.67	3.79	2.52	3.45	2.82	3.74	3.33	-
25.1	1.47	2.35	2.46	2.39	2.53	1.95	1.41	1.40	3.46	-
20.0	-	-	-	0.49	0.54	0.36	0.30	0.14	1.06	-
19.2	5.04	5.34	4.57	5.88	5.65	5.35	5.84	3.33	5.85	5.28
14.2	0.40	0.55	-	0.68	0.90	0.51	0.53	-	0.23	0.29
12.3	1.93	1.54	1.21	2.52	2.59	2.36	2.67	0.53	2.99	0.70
9.4	-	-	-	-	2.76	1.64	1.01	-	0.56	0.21
7.60	-	-	-	-	3.82	1.85	1.62	-	-	-
6.50	-	-	-	-	2.79	2.03	1.54	-	0.69	-
5.20	1.65	0.43	1.17	1.49	1.67	2.42	1.31	2.70	1.71	-
4.60	0.86	-	-	-	1.51	1.20	1.23	-	-	-
3.70	1.56	1.24	-	0.87	1.66	1.53	1.58	-	0.94	0.96
Bands Sum	**13**	**15**	**12**	**17**	**22**	**22**	**23**	**13**	**22**	**11**

- Absent band

Formerly, *Rubus coreanum* alcoholic extract caused reduction in cell proliferation and mitotic activity causing apoptosis in HT-29 human colon cancer cells by causing inhibition in DNA synthesis and fragmentation in DNA **(Kim *et al.*, 2005).** After treatment with several plant extracts, the inhibition of mitotic activity was previously reported by **Çelik and Aslantürk (2010); Neelamkavil and Thoppil, (2014).** Moreover, the *Rs*AAE showed its influence on the mitotic phases and caused prophase accumulation. This highly prophase accumulation could be due to the blockage of the dividing cells at Chfr point which prevents prophase-metaphase transition as proved by **Scolnick and Halazonetis (2000)** who explained that Chfr protein delays chromosome condensation and nuclear envelope breakdown in response to drug such as taxol and nocodazole that disrupt microtubule structure. According to the present study the spindle disturbance especially c-mitosis was the most common type of abnormalities which indicates that *Rs*AAE not only caused disruption in the spindle structure, but also caused inhibition of spindle formation and prevented its polymerization **(Salmon *et al.*, 1984).** The formation of c-mitosis is usually related to spindle poisons and/or as a result of bending of spindle polypeptides **(Shahin and El-Amoodi, 1991; El-Ghamery *et al.*, 2000).** Accordingly, the spindle disturbance observed herein may be attributed to one or both of the previous causes. The formation of chromosome stickiness could be due to the change in the physiological properties of DNA or its structural protein. So, *Rs*AAE may react with DNA resulting in DNA linking or causing inter or intra chromosomal DNA-protein crosslinking **(Patil and Bhat, 1992).** Chromosome stickiness reflects genotoxic effect of *Rs*AAE usually that leads to cell death. The thickened and shortened chromosome noted through this study in the different phases indicates change in chromatin organization that may regard to disturbance in the balance of the quantity of histones proteins as suggested by **Stryer (1997).**Such previous types of abnormalities were also reported to be induced by other plant extracts **(Adegbite *et al.*, 2009; Asthana and Kumar, 2014)**.

The induced mitotic stress was concomitant with significant ultrastructural alterations as previously proved by **Tawab *et al.*, (2004); Kharshiing (2012).**The ultrastructural investigation has proven to be an informative technique to explain the inhibitory effects of *Rs*AAE. Exposure to *Rs*AAE appears to predominantly affect the endomembrane system of *A. cepa* root meristems. The formation of dilated Golgi dictyosomes is astonishing, as no plant extract has been shown to cause such alteration in dictyosomes. Plant extracts that disrupt mitotic activity by causing inhibition of the microtubule function or formation

always affect the structural integrity of plasma membrane and cell wall but cause no alterations in Golgi dictyosomes **(Majewska *et al.*, 2003)**. This is due to the fact that structural integrity of dictyosomes depends on the intercisternal elements rather than microtubules as observed in animals **(Mollenhauer and Morre, 1980; Rogalski and Singer, 1984)**. Structural alterations in mitochondria like swelling and loss of cristae indicate the effect of *Rs*AAE on mitochondrial respiration. Such changes occur when an ADP level in mitochondria exceeds that of ATP. Flavonoids compounds have the ability to reduce respiratory activity of mitochondria **(Ravanel *et al.*, 1982). Takahashi *et al.* (1998)** proved that quercetin impairs the respiratory activity of soybean hypocotyl mitochondria by an inhibition of phosphate uptake. Such ultrastructural changes have often been observed in other investigations as a result of treatment with toxic factors **(Lemasters *et al.*, 1997). Kolb *et al.* (2004)** suggested that reorganization of the ER lumen occurred when the level of respiratory product was limited or lacking. The occurrence of concentric cisternae of Golgi dictyosomes attached to multivesicular bodies may indicate their autolysis. Such process of intracellular digestion results from the appearance of strange substances in the cell **(Locke and Sykes 1975).**Changes in Golgi dictyosomes associated with formation of dilated of ER lumen may lead to loss in the function of such important organelles. In plant cells, the Golgi apparatus receives substrate from ER for glycosylation of proteins and transports the polysaccharide products, proteins and lipids to different sites, particularly to plasma membrane and cell wall **(Mellman and Simons, 1992).** Hence, the structural and functional integrity of these organelles depend on the products delivered by dictyosomes **(Mollenhauer and Morre, 1980).** Effects of *Rs*AAE on Golgi dictyosomes may block the production of cell wall precursors, which led to the loss of microfibrillar array of cell wall and also inhibited the formation of cell wall in the phragmoplast region of telophase stage. Induction of alteration in the structure of Golgi apparatus accompanied with various mitotic aberrations, disturbance of parallel arrangement of microtubules at the different mitotic stages, verify the anti-mitotic potential of *Rs*AAE as earlier proved about BHT **(Bakeeva *et al.*, 2001).** Furthermore, there was an increase in the number and size of the vacuolar system. Several of these vacuoles contained cellular debris in various stages of degeneration, perhaps indicating an abnormal induction of autophagy, which may be triggered after treatment with *Rs*AAE **(Ambrose and Easty, 1979).** MVBs belong to the late endosomal system in plants **(Šamaj *et al.*, 2005; Lam *et al.*, 2007)**; therefore their existence in treated roots indicated intensified process of endocytosis.

Endoplasmic reticulum and mitochondria are organelles known as a device for protein synthesis and refer to active phase of protein synthesis that in turn reflects activation of genes. The formation of dilated endoplasmic reticulum and the presence of numerous undifferentiated mitochondria may explain the gradual decrease in protein synthesis as the deleterious effect on them may block RNA synthesis or translation process as previously reported by **Lord *et al.* (1991).** Furthermore, the inhibition of protein synthesis may be explained on the basis that *Rs*AAE may block specific step of the three steps of protein synthesis process **(Soll, 1990; Tawab *et al*., 2004).**

Callis (1995) found that protein degradation depends on the activity of the peptide hydrolases present in the cell rather than the quantity of them. Moreover, **Hosseini and Mulligan (2002)** supported this explanation and proved that the decrease in intracellular protein content is caused by increasing in proteolytic activities.

The occurrence of new bands and absence of others would indicate the alteration of gene expression which in turn alter the protein products in response to treatment with *Rs*AAE either on the transcription or post- transcription levels of gene expression. Change in gene expression might intensify or diminish signal-transduction pathway and ultimately exhibited as a simulative or depressive metabolic and growth responses. Similar results were obtained by other investigators following different treatments **(Shehata *et al.*, 2000; Mekki, 2008; Abdel-Hameid *et al.*, 2011)**. Appearance of novel bands could be explained on the basis of mutational event at the regulatory system of some gene(s) that activate its expression **(Shehab *et al.*, 2004; Tawab *et al*., 2004). Ericson and Alfinito (1984)** observed the expression of some protein band under different stresses, while **Telma *et al*. (2008)** found that the expression level of various genes encoding heat shock proteins increased after a short term of oxidative stress treatment. Changing in protein patterns also have been attributed to induction of cytological aberrations by **Mohamed (2005); Abdel-Hamied *et al.* (2008).** The disappearance of some bands in treated *A. cepa* roots was explained on the basis of the induction of mutational events **(Soliman, 2003; Shehab *et al.*, 2004)** that prevent or reduce transcription process **(Muller and Gottschalk, 1973**). **Abdelsalam *et al.* (1993)** reported thatsome electrophoretic bands were disappeared due to the loss of their corresponding genes. They also reported that the reduction of chromosome complement in *Vicia sativa* lead to the complete disappearance of the convicilin like band as a result of the loss of some genetic material due to changes in gene sequences. In

addition, alterations in bands intensity could be attributed to change in the structure or performance of genes and thus they produce changes in the gene expression of the regulatory genes used in the regulatory system of the structural genes **(Hassan, 1996).** This leads to the production of faint or over expressed protein bands **(Barakat and Hassan, 1997).** Alterations in bands intensities in the present study are in agreements with **Mekki (2008); Atef*et al.* (2011).**

Conclusion

Generally, it would appear that exposure to *Rs*AAE initiates a cascade of deleterious changes within *A. cepa* L. root cells. These events manifested the cytotoxic and genotoxic effect of *Rs*AAE. In addition, the proven cytotoxic effect of several medicinal plants on normal cell enhanced more restriction roles in usage of herbal medicine, so it is a must to be careful when we use *Rs*AAE in any biological activity.

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References

1. Abdel-Hamied NR, Atef AAH, Abdel-Hady EA, EL-Ansary AMF (2008). Protective role of vitamin C against genotoxicity of aluminium sulphate in *Viciafaba*.The Journal of Genetic Engineering and Biotech 6 (1): 10-19.
2. Abdel-Hamied NR, Elanany MAM, Atef AAH, Abdel-Hady EAA (2011).Relative mutagenecity of some food preservatives on plant cells. Australian Journal of Basic and Applied Sciences 5(12): 2817-2826.
3. Abdelsalam AZE, Hassan HZ, El-Domyati M, Eweda MA, Bahieldin A, Ibrahim SA (1993). Comparative mutagenic effects of some aromatic compounds using different eukaryotic systems. Egypt. J. Genet. Cytol. 22(1):129-153.
4. Adegbite AE, Ayodele MS, Odunbaku KR, Idehen EO (2009). Mutagenic effect of neem leaf extract used in traditional medicine on *Allium cepa* (L.). Scientific Research and Essays 4(11): 1315-1321.
5. Akcos Y, Yes_ilada E, Ezer N (1998). Anti-inflammatory activity of some Turkish Rubus species. Hacettepe Univ. J. Fac. Pharm 18:33–38.
6. Ambrose EJ, Easty DM (1979). Cell Biology.2nd edn, Thoms Neslon and Sons Ltd.
7. Asthana M, Kumar A (2014). Dose response of *Viola odorata*on meiotic and mitotic chromosomes of *Viciafaba*.British Journal of Pharmaceutical Research. 4(4): 520-530.
8. Atef AAH, Abdel-Hamied NR, Abdel-Hady EA, AL-Ansary AMF (2011).Cytogenetic effect of insecticide telliton and fungicide dithane m-45 on meiotic cells and seed storage proteins of *Viciafaba*. Journal of American Science. 7(1): 19-25.
9. Badr A, El-Demerdash E, Khalifa A, Ghoneim A, Ayoub N, Abdel-Naim A (2009). *Rubus sanctus* protects against carbon tetrachloride-induced toxicity in rat isolated hepatocytes: isolation and characterization of its galloylated flavonoids. J Pharm Pharmacol. 61(11):1511-1520.
10. Bakeeva LE, Zamyatnina VE, ShorningVYu, Aleksandrushkina NI, Vanyushin BF (2001). Effect of the antioxidant ionol (BHT) on growth and development of etiolated wheat seedlings: control of apoptosis, cell division, organelle ultrastructure and plastid differentiation. Biochemistry (Moscow) 66(8): 850-859.
11. Barakat HM, Hassan HZ (1997). Mutagenic effects of pendimethalin herbicide on *Vida faba*and *Allium cepa* plants. Egypt. J. Bot. 37: 13-29.
12. Boulos L. 1999. Flora of Egypt, 1st edn. Vol. 1. Al Hadara Publishing, Cairo, Egypt, p. 245.
13. Bradford MM (1976). A rapid and simple method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analyt. Biochem. 72:248-254.
14. Callis J (1995). Regulation of protein degradation. Plant Cell 7:845-857.
15. Cai Y, Hu X, Huang M, Sun F, Yang B, He J, Wang X, Xia P, Chen J (2012). Characterization of the antibacterial activity and the chemical components of the volatile oil of the leaves of *Rubus parvifolius* L. Molecules 17: 7758-7768.
16. Cao G, Sofic E, Prior RL (1997). Antioxidant and prooxidant behavior of flavonoids: Structure–activity relationships. Free Radical Biology and Medicine 22: 749–760.
17. Çelik TA, Aslantürk ÖS (2010).Evaluation of cytotoxicity and genotoxicity of *Inulaviscosa* leaf extracts with Allium test. Journal of Biomedicine and Biotechnology 1: 1-8.
18. Coates EM, Popa G, Gill Ch, McCann MJ, McDougall GJ, Stewart D, Rowland I (2007). Colon-available raspberry polyphenols exhibit anti-cancer effects on *in vitro* models of colon cancer. Journal of Carcinogenesis 6:4 doi:10.1186/1477-3163-6-4.
19. Cuyacot AR, Mahilum JJM, Madamba MR (2014). Cytotoxicity potentials of some medicinal plants in Mindanao, Philippines. Asian Journal of Plant Science and Research 4(1):81-89.
20. Dall' Acqua S, Cervellati R, Cecilia M, Innocenti G (2008). Evaluation of *in vitro* antioxidant properties of some traditional Sardinian medicinal plants: Investigation of the high antioxidant capacity of *Rubus ulmifolius*. Food Chemistry 106:745–749.
21. El-Ghamery AA, El-Nahas AI, Mansour MM (2000).The action of atrazine herbicide as an inhibitor of cell division on chromosomes and nucleic acids content in root meristems of *Allium cepa* and *Vicia faba*. Cytologia 65:277–287.
22. Erdemoglu N, Kupeli E, Yesilada E (2003).Anti-inflammatory and antinociceptive activity assessment of plants used as remedy in Turkish folk medicine.J Ethnopharmacol. 89:123–129.
23. Ericson MC, Alfinito SH (1984). Proteins produced during salt stress in tobacco cell cultivars. Plant Physiol. 74: 506-509.

24. Falleh H, Ksouri R, Chaieb K, Karray-Bouraoui N, Trabelsi N, Boulaaba M, Abdelly C (2008). Phenolic composition of *Cynaracardunculus*L. organs and their biological activities. C. R. Biologies 331: 372–379.
25. Fiskesjö G (1985). The *Allium* test as a standard in environmental monitoring. Hereditas 102:99–112.
26. Fiskesjö G (1997). *Allium* test for screening chemicals; evaluation of cytologic parameters. In: Wang W, Gorsuch JW, Hughes JS eds. *Plants for environmental studies*. CRC Lewis Publishers, Boca Raton, New York, pp. 308–333.
27. Gabara B, Kalwinek J, Koziróg Z, Żakowska Z, Brycki B (2006). Influence of N, N-bis(3-amninopropylo) dodecyloamine on the ultrastructure of nuclei in *Aspergillus niger* mycelium and on cell proliferation and mitotic disturbances in *Allium cepa* L. root meristem. Acta Biol. Cracov. Ser. Bot. 48:45–52.
28. Galati G, O'Bbrien PJ (2004). Potential toxicity of flavonoids and other dietryphenolics: Significance for their chemopreventive and anticancer properties. Free Radical Biology and Medicine 37:287–303.
29. Gali-Muhtasib H, Bakkar N (2002). Modulating cell cycle: current applications and prospects for future drug development. Curr. Cancer Drug Targets 2: 309-336.
30. Gallagher S, Smith JA (1995). One dimensional gel electrophoresis of protein from Ausubel FM, Brent R, Kingston RE *et al.,* eds. *current protocols in molecular biology*. Green Publishing and Wiley-Interscience, New York, Unit 10.2.1-10.2.21.
31. Gevrenova R, Badjakov I, Nikolova M, Doichinova I (2013). Phenolic derivatives in raspberry (Rubus L.) germplasm collection in Bulgaria. Biochemical Systematics and Ecology 50: 419–427.
32. Glińska S, Gabara B (2000). Localization of selenium deposits in meristematic cells of *Allium sativum* L. roots treated with selenium salts. Folia Histochem.Cytobiol. 38:143–147.
33. Gudej J, Tomczyk M (2004). Determination of flavonoids, tannins and ellagic acid in leaves from Rubus species. Arch. Pharm. Res. 27: 1114–1119.
34. Hadi SM, Asad SF, Singh S, Ahmad A (2000).Putative mechanism for anticancer and apoptosis inducing properties of plant-derived polyphenolic compounds. International Union of Biochemistry and Molecular Biology Life 50: 167-171.
35. Harrison JC, Haber JE. 2006. Surviving the breakup: the DNA damage checkpoint. Ann. Rev. Genet. 40: 209-235.
36. Hartwell LH, Weinert TA (1989). Checkpoints: control that ensure the order of cell cycle events. Sci. 246:629–634.
37. Hassan HZ (1996). Evaluation of mutagenic effects of the two insecticides basudin and decis on *Viciafaba*plants. Egypt. J. Genet Cytol. 25: 27-38.
38. Hosseini R, Mulligan BJ (2002). Application of rice (*Oryza sativa* L.) suspension culture in studying senescence *in vitro* (I). Single strand preferring nuclease activity. Electronic Journal of Biotechnology 5(1):42-54.
39. Hussein AM, Ayoub NA, Nawwar MAM (2003).Caffeoyl sugar esters and an ellagitannin from *Rubus sanctus*. Phytochemistry 63:905–911.
40. Kim EJ, Lee Y, Shin H., Jung Han Park JH (2005). Induction of apoptosis by the aqueous extract of *Rubus coreanum*in HT-29 human colon cancer cells. Nutrition 21:1141–1148.
41. Kharshiing EV (2012). Aqueous extracts of dried fruits of *Zanthoxylum armatum* DC., (Rutaceae) induce cellular and nuclear damage coupled with inhibition of mitotic activity *in vivo*. American Journal of Plant Sciences 3:1646-1653.
42. Kolb RM, Dolder H, Cortelazzo AL (2004). Effect of anoxia on root ultrastructure of four neotropical trees. Protoplasma 224: 99–105.
43. Komal A (2013). Mitotic aberrations induced by *cassia occidentalis* L. in *Allium cepa* L. root tip cells. Indian Journal of Fundamental and Applied Life Sciences 3 (4): 1-4.
44. Laemmli UK (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680-685.
45. Lam SK, Siu CL, Hillmer S (2007). Rice SCAMP1 defines clathrin-coated, trans-Golgi-located tubular-vesicular structures as an early endosome in tobacco BY-2 cells. Plant Cell 19: 296–319.
46. Laughton MJ, Halliwell B, Evans PJ, Robin J, Hoult S (1989).Antioxidant and pro-oxidant actions of the plant phenolicsquercetin, gossypol and myricetin.Effects on lipid peroxidation, hydroxyl radical generation and bleomycin-dependent damage to DNA. Biochemical Pharmacology 38: 2859–2865.
47. Lemasters J, Nieminen A, Qian T, Trost L, Herman B. 1997.The mitochondrial permeability transition in toxic, hypoxic and reperfusion injury. Mol. Cell. Biochem.174: 159-165.
48. Locke M, Sykes AK (1975).The role of the Golgi complex in the isolation and digestion of organelles. Tissue Cell 7: 143-158.
49. Lord JM, Hartley MR, Roberts LM (1991). Ribosome inactivating proteins of plants. Semin. Cell Biol. 2(1): 15-22.
50. Majewska AE, Wolska E, Śliwińska M, Furmanowa N, Urbanska A, Pietrosiuk A, Zobel A, Kura M (2003). Antimitotic effect, G_2/M accumulation, chromosomal and ultrastructure changes in meristematic cells of *Allium cepa* L. root tips treated with the extract from *Rhodiolarosea*roots. *Caryologia*56(3): 337-351.
51. Mekki L (2008).The effect of three agricultural chemicals on mitotic division and total seed protein banding profiles of alfalfa (*Viciafaba*). Int. J. Agri. Biol 10(5): 499–504.
52. Mellman I, Simons K (1992). The Golgi complex: *in vitro*veritas? Cell 68: 829.
53. Mohamed ThR (2005). A possible role for jasmonic acid hormone and mycorrhizal fungi (Glomus spp.) in adaptation of *Zea mays* plants to heavy metals stress. Egypt. J. Biotech., 20: 75- 90.
54. Mollenhauer HH, Morre DJ (1980). The Golgi apparatus, In: Stumpf PK, Conn EE eds, Biochemistry of Plants: a comprehensive treatise, vol. (1) Academic Press, New York, pp. 437–488.
55. Mollinedo F, Gajate C (2003).Microtubules, microtubule-interfering agents and apoptosis. *Apoptosis*8: 413-450.
56. Muller HP, Gottschelk W (1973). Quantitative and qualitative situation of *Pisum* Quantitative and qualitative situation of *Pisumsativum*from Nuclear Techniques for Seed Protein Improvement. IAEA. Vienna. P: 235-253.

57. Neelamkavil SV, Thoppil JE (2014). Toxicological evaluation of polar and nonpolar components of *Isodoncoetsa* (Lamiaceae). Turk J Bot 38: 252-257.
58. OueslatiS,Trabelsia N, Boulaabaa M, Legaultb J, Abdellya Ch, Ksouria R (2012). Evaluation of antioxidant activities of the edible and medicinal Suaeda species and related phenolic compounds. Industrial Crops and Products. 36: 513–518.
59. Patil BC, Bhat GI (1992). A comparative study of MHG and EMS in the induction of chromosomal aberrations on lateral root meristems in *Clitoria ternate* L. *Cytologia* 57:259-264.
60. Peşin I, Koca U, Keleş H, Akkol EK (2009). Wound healing activity of *Rubus sanctus* Schreber (Rosaceae): Preclinical study in animal models. eCAM Page 1 of 7 doi:10.1093/ecam/nep137.
61. Rank J, Nielsen MH (1993).A modified *Allium* test as a tool in the screening of the genotoxicity of complex mixtures. Hereditas 118: 49–53.
62. Ravanel P, Tissut M, Douce R (1982). Effects of kaempferol on the oxidative properties of intact plant mitochondria. Plant Physiol. 69: 375–378.
63. Reynolds E (1963). The use of lead citrate at a high pH as an electron-opaque stain in electron microscopy. J. Cell Biol. 17: 208-212.
64. Rogalski AA, Singer SJ (1984). Association of Golgi complex with microtubules, J. Cell Biol. 99: 1092.
65. Salmon ED, Mickseel M, Hays T (1984).Rapid rate of tubulin dissociation from microtubule in the mitotic spindle *in vivo* measured by blocking polymerization with colchicine. J. Cell Biolog. 99:1066–1075.
66. Šamaj J, Read ND, Volkmann D, Menzel D, Baluška, F (2005). The endocytic network in plants. Trends Cell Biol. 15: 425–433.
67. Schneiderman MH, Dewey WC, Highfield DP (1971). Inhibition of DNA synthesis in synchronized Chinise hamster cell treated in G1 with cycloheleximide, Exp. Cell Res. 67:147–155.
68. Scolnick D, Halazonetis T (2000). Chfr defines a mitotic stress checkpoint that delays entry into metaphase. Nature 406:430–435.
69. Serteser A, Kargioğlu M, GokV, Bağci Y, Ozcan MM, Arslan D (2008).Determination of antioxidant effects of some plant species wild growing in Turkey.Int J Food Sci Nutr.12:1-9.
70. Shahin SA, El-Amoodi KhH (1991). Induction of numerical chromosomal aberrations during DNA synthesis using the fungicides nimrod and rubigan-4 in root tips of *Viciafaba* L. Mutat. Res. 261: 169–176.
71. Sharma CBSR (1983). Plant meristems as monitors of genetic toxicity of environmental chemicals. Curr Sci. 52: 1000-1002.
72. Shehab AS, Tawab SAF, Morci MM (2004). Stimulation of cell division and gene expression in *Viciafaba*L. using leaf powder of *AzadirachteIndica*. Egypt. J. Biotech., 17: 499- 514.
73. Shehata MM, Habib A, Khalifa NS, Salama MS (2000). Cytological and biochemical effects of 5-florouracil and colchicine on *Vida faba*plants. Egypt. J. Biotech. 7: 218-233.
74. Soliman MH (2002). Allelopathic potential of sunflower residues in controlling wild oat associated with wheat. PhD Thesis, Faculty of Science, Cairo University, Egypt.
75. Soliman MSA (2003). Environmental impact of mobile communication tower on barley and broad bean plants. Bull. Fac. Sci. Assiut Univ., 32(2-D): 79- 89.
76. Soll D (1990). The accuracy of amino acylation-ensuring the fidelity of the genetic code. Experientia 46(11-12): 1089-1096.
77. Stryer L (1997). Biochemia. Wydawnictwo Naukowe PWN, Warszawa, 1032–1069.
78. Takahashi L, Sert MA, Kelmer-Bracht AM, Bracht A, Ishii-Iwamoto EL (1998). Effect of rutin and quercetin on mitochondrial metabolism and on ATP levels in germinating tissues of Glycinae max. Plant Physiol. Biochem. 36: 495–501.
79. Tawab SAF, Adam ZM, Sobieh Sh (2004). Suppression of mitotic process associated with metaphase arrest of *Allium cepa* L. roots using. I. *Rosmarinusofficinalis*L. water extract. Int. J. Agri. Biol. 6(4):690-698.
80. Telma ES, ZanorML, Valle EM (2008). Investigating the role of plant heat shock proteins during oxidative stress. Plant Signal Behav. 3(10): 856-857.
81. Velićanski AS, Cvetković DD, Markov SL (2012). Screening of antibacterial activity of raspberry (*Rubus idaeus*L.) Fruit and pomace extracts. Apteff 43:305-313.
82. Venskutonis PR, Dvaranauskaite A, Labokas J (2007). Radical scavenging activity and composition of raspberry (*Rubus idaeus*) leaves from different locations in Lithuania. Fitoterapia 78: 162–165.
83. Wahid F, Junga H, Khanb T, Hwang K, Park JS, Chang S, Khan MA, Kim YY(2011). Effects of *Rubus coreanus* extract on visual processes in bullfrog's eye. Journal of Ethnopharmacology 138: 333– 339.
84. Wilson CM (1983).Staining of proteins on gels: comparison of dyes and procedures.Methods in Enzymol. 91: 236–247.
85. Wong WS, McLean AEM (1999). Effects of phenolic antioxidants and flavonoids on DNA synthesis in rat liver, spleen, and testis *in vitro*.Toxicology 139: 243–253.
86. Zheng ZX, Zhang LJ, Huang QL, Huang CX, Wei XD, Ying HZ (2007). Antitumor effect of total saponins of *Rubus parvifolius* on malignant melanoma. China journal of Chinesemateriamedica 32(19):2055-2058.

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