African Journal of Biotechnology Vol. 10(51), pp. 10520-10529, 7 September, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.694
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Mutagenic and antimutagenic potentials of fruit juices of five medicinal plants in *Allium cepa* L.: Possible influence of DPPH free radical scavengers

Akinboro Akeem[1], Kamaruzaman Bin Mohamed[1]*, Mohd Zaini Asmawi[2] and Othman Ahmad Sofiman[1]

[1]School of Biological Sciences, Universiti Sains Malaysia, 11800, Pulau Pinang Malaysia.
[2]School of Pharmaceutical Sciences, Universiti Sains Malaysia, 11800, Pulau Pinang, Malaysia.

Accepted 1 July, 2011

Mutagenic and antimutagenic activities of freeze dried fruit juices (FDFJ) of *Morinda elliptica* Ridl. (Rubiaceae), *Morinda citrifolia* L. (Rubiaceae), *Averrhoa bilimbi* L. (Oxalidaceae), *Phyllantus acidus* (L.) Skeels (Phyllantaceae) and *Myristica fragrans* Houtt. (Myristicaceae) in *Allium cepa* L were evaluated. Testing the mutagenic activity, onions were suspended in solution of different concentrations of FDFJ alone in tap water for 48 h. Thereafter, root tips were prepared and observed for dividing cells and chromosomal aberrations using a light microscope. Antimutagenicity screening was similar to the mutagenic evaluation, except that the solution of FDFJ was combined with 0.1% cylophosphamide-CP. Free radicals (2,2-diphenyl-1-picrylhyrazyl) scavenging activity of the FDFJ was tested using butylated hydroxylanisole (BHA) and butylated hydroxytoluene (BHT) as the standards and their phenolic contents were evaluated by comparing with gallic acid equivalents (GAE). The free radicals scavenging power of *M. fragrans* at 1 mg/ml was almost similar to that of BHA and BHT and its phenolic content was 21 ± 6.0 mg GAE/g, the highest among the tested juices. *A. cepa* cell division was inhibited in a dose-dependent manner by the FDFJ of *M. fragrans,* while the induced chromosomal aberrations were non dose dependent. The cytotoxicity and chromosomal aberrations of CP were suppressed throughout the tested concentrations of *M. fragrans*, unlike the effects of other four juices. These results suggest that the observed activities of FDFJ of *M. fragrans* may be due to the quantity and quality of phenolic compounds with antioxidant activity, suggesting its use in preventing the DNA-damaging effects of mutagens.

Key words: *Allium cepa*, antimutagenicity, antioxidants, cyclophosphamide, mutagenicity.

INTRODUCTION

Fruits are often consumed by humans because of their reported dietary and medicinal values, both of which are in the main functions of their chemical constituents. In the recent years, investigations into phytochemicals used as food or to treat illnesses have increased, because juices are often suspected to contain constituents that are harmful or/and protective to the genetic system (nucleic acids) of living organisms. The damaging effects of chemical compounds on the genetic system of organisms have been reported to elicit several human diseases-such as cancer, type II diabetes, Alzheimer's disease, coronary heart disease and aging, through the generation of reactive oxygen species (ROS), free radicals as well

*Corresponding author. E-mail: mkamar_usm@yahoo.com. Tel: +6-019-472-6397.

Abbreviations: FDFJ, Freeze dried fruit juices; **CP,** cyclophosphoamide; **GAE,** gallic acid equivalents; **CA,** chromosomal aberration; **MI,** Mitotic index.

as oxygen anions (O_2) in the cells (Odin, 1997; Ajith and Janardhanan, 2002; Gupta et al., 2008; Rosidah et al., 2008; Zahin et al., 2010). However, antimutagenic compounds prevent and protect the genetic system from any forms of damage that may be caused by either ROS generated through metabolic activity of chemicals in drugs and food substances or through radiation. Consequently, degenerative diseases that occur as a result of assaults done to the macromolecules can therefore be averted. Phenolic compounds are plant metabolites widely spread throughout the plant kingdom (Gülcin et al., 2010). Sulaiman et al. (2011) reported that phenolic compounds have protective role against oxidative damage diseases, perhaps due to their reputed antioxidant activity, made possible by their synergistic effectiveness as hydrogen donors and reducing effects, also as free radical scavengers. Reports have shown that certain phytochemical compounds can induce and prevent DNA damage (Barcelos et al., 2007). Therefore, an investigation into the mutagenicity and antimutagenicity of plant constituents would be beneficial in providing information concerning possible dangers associated with the consumption of plant parts. It could also serve as basis of chemotherapy, especially for cancer, whose occurrence has DNA damage as a principal step to carcinogenesis.

Among the plants that are being used for food and medicinal purposes in Asia and some other countries in the world are; *Morinda elliptica* (L.) Ridl*, Morinda citrifolia* (L.), *Averrhoa bilimbi* (L.), *Phyllantus acidus* (L.), Skeels and *Myristica fragrans* (Houtt.). Often the use of these plants as food or medicines may involve any one or combinations of different parts such as leaves, stem roots and fruit juices. In traditional pharmacopoeia, the fruit of *M. citrifolia*, also known as noni (Hawaiian name) is used to prevent and cure several diseases such as cancers, arthritis, diabetes, high blood pressure, muscle aches and pains, menstrual difficulties, headaches, heart disease, gastric ulcers, mental depression, poor digestion, atherosclerosis, blood vessel difficulties and drug addiction (Chan-Blanco et al., 2006; Liu et al., 2007). These beneficial effects of *M. citrifolia* may result from certain compounds like scopoletin, nitric oxide, alkaloids and sterols, as well as due to the antioxidants potential. *Morinda elliptica* is employed for the treatment of diarrhea, headache and haemorrhoids, as well as to increase appetite. Anthraquinones (AQs), the secondary metabolite compounds isolated from *M. elliptica* had antiviral, antimicrobial, cytotoxic, antitumour-promoting properties and antioxidant activities (Chong et al., 2005). Fruit and leaves extracts of *A. bilimbi* were observed to reduce blood glucose and food intake in diabetic rats (Pushparaj et al., 2000). Semi purified fractions of *A. bilimbi* possessed hypoglycemic and hypolipidemic potencies in Type I diabetic rats that were fed with these fractions orally and intraperitoneally (Tan et al., 2005). Acidic fruit like bilimbi is utilized as a substitute for common sanitizers in Malaysia. The fruit of *A. bilimbi* is used to wash seafood, especially freshwater fish, to remove any foul smell (Norhana et al., 2009). *Myristica fragrans* has been used as an aromatic stimulant, abortifacient, antiflatulent and as a means to induce menses for many years (Donald, 2008). Argenteane, a dilignan and powerful antioxidant like vitamin E, was isolated from the mace of *M. fragrans* (Calliste et al., 2010). Different parts of *Phyllantus acidus* are being used to treat oxidative stress related diseases in Malaysia, South America and Africa (Eldeen et al., 2011). The fruit of *Phyllantus acidus* contains 40 mg/100 g ascorbic acid, the acidic contents of lemon and grapefruits are similar to that of *P. acidus* (Duangpron and Siripong, 2009). *P. acidus* was reported to be rich in adenosine, and lacks cytotoxic effects (Sousa et al., 2007).

However, there are limited reports on the possible toxicity of the juices of these plants in eukaryotic cells, coupled with the fact that there are no known published data on their cyto-geno toxicities in an *in vivo* genetic system as being reported presently. *Allium cepa* (L., 2n = 16) assay is a popular and reliable test for cyto-geno toxicities screening of chemical compounds. It is an established plant bioassay, validated by the international programme on chemical safety (IPCS, WHO), as an efficient and standard test for chemicals screening and *in situ* monitoring of environmental substances genotoxicity (Kumari and Mukherjee, 2009). Its acceptability for testing the effects of both water-soluble and non-soluble substances on cell division and chromosome is due to the similarities in its results compared with those from animal bioassays (Yildiz et al., 2009). *A. cepa* test permits the study of different, but complementary aspects of food contamination and could be helpful to better evaluate cancer hazards related diets (Feretti et al., 2007).

Thus, this study sought to investigate the possible mutagenic and antimutagenic activities of fruit juices of these five plants, as affected by their DPPH free radicals scavenging effectiveness.

MATERIALS AND METHODS

Fruit juice preparation

Fresh fruits, weighing 1800 g each of *M. elliptica* Ridl., *M. citrifolia* L., *A. bilimbi* L., *P. acidus*, (L.) Skeels and *M. fragrans* (Houtt.) were collected from the campus of Universiti Sains Malaysia (USM). They were identified at the herbarium of School of Biological Sciences, USM and voucher specimen numbers; 11077 (*M. elliptica*), 11078 (*P. acidus*), 11079 (*M. citrifolia*), 11080 (*A. bilimbi*), 11081 (*M. fragrans*) were assigned to the plants, thereafter, deposited in the herbarium. The fruits were washed with tap water and cut into pieces. Seeds were removed from *M. elliptica, M. citrifolia* and nuts extracted from *M. fragrans*, after which the fruits were ground separately to fine paste using an electric blender. Juice was squeezed out of the individual fruit paste and filtered through a white muslin cloth. The filtered juice was concentrated at 40°C under rotavapor (R-125) and kept in a deep freezer at -80°C

for 24 h. Frozen juice was freeze-dried at -50°C under vacuum pressure in a Labconco freeze dryer (Lyph-lock 6) and kept at 4°C for screenings.

Antioxidant test

The freeze dried fruit juices (FDFJ) were tested for their ability to scavenge free radicals from 2, 2-diphenyl -1- picryl-hydrazyl (DPPH) following the described method (Rosidah et al., 2008), but with some modifications. Briefly, 2 mg/ml of each of the FDFJ was weighed and dissolved in HPLC grade methanol. To 100 µl solution of the juice prepared in triplicate at each concentration, 200 µl of 0.1 mM DPPH was added in a 96-well microplate. The blank reaction at each concentration contained 100 µl FDFJ + 200 µl HPLC MeOH. The plate was wrapped with aluminium foil and incubated at root temperature for 30 min. Absorbance of the reaction between FDFJ and DPPH solution was measured at 517 nm (wavelength) in a microplate reader (power wave × TM). Butylated hydroxy anisole (BHA) and butylated hydroxy toluene (BHT) prepared at 1 mg/ml served as the reference standards. The percentage free radical scavenging activity of the FDFJ and standards were calculated as shown below (Ak and Gülcin, 2008; Gülcin, 2009).

$$\text{DPPH scavenging activity (\%)} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

Where, $A_{control}$ is the absorbance at 517 nm of the control reaction containing all reagents except the test compound; A_{sample} is the absorbance at 517 nm of reaction containing test compound (Gülcin, 2009; Talaz et al., 2009).

Determination of total phenolic contents

The concentration of phenolic compounds in the FDFJ was determined according to the reported method (Eldeen et al., 2011). Two milligrams of the FDFJ and 1 mg/ml Gallic acid (GA) were separately dissolved in a mixture of HPLC grade methanol:distilled water (5:5 v/v). Different concentrations of the gallic acid solution ranging from 0.004 to 0.5 mg/ml were prepared by two-fold dilution. Each of the FDFJ and GA (20 µl) was mixed with 40 µl Folin-Ciocalteu reagent in a 96 microwell plate and left at room temperature for 10 min. Thereafter, the reaction was terminated by adding 75 µl of 20% sodium carbonate solution, after which 150 µl distilled water was added to the mixture and incubated at 40°C for 30 min. The absorbance was measured at 765 nm using a microplate reader (power wave $_X$ TM). The test was carried out in triplicates. The concentrations of phenolic compounds in the FDFJ was extrapolated from a standard curve drawn using the absorbance at different concentrations of GA and expressed as milligrams of Gallic acid equivalents (GAE) per gram of sample (Gülcin et al., 2010).

***Allium cepa* test**

Preparation of onion bulbs for planting

Onions (*A. cepa* L. 2n = 16) were purchased from Jusco shopping complex, Penang, Malaysia. They were sun dried for one week, after which only the healthy onions (not infected by fungi) were selected for planting.

The dried scales on the onions were removed carefully without damaging the primordial root ring.

Mutagenic evaluation

The FDFJ was dissolved in tap water (TW) at 1, 2, 4 and 8% concentrations (w/v) and then vortexed to obtain better dissolution. Five onions, per concentration, were suspended in the solution of FDFJ + TW in 50 ml beakers and kept in the dark for 24 h for the root growth to occur. Fresh solution of each juice was prepared and further suspension of the onions in the solution continued for another 24 h. Tap water (TW) served as the negative control.

Preparation of root tips for microscopic observation

For slide preparation, root tips from the onions were cut, fixed in ethanol/acetic acid (3:1, v/v) and then kept at 4°C for 24 h. The fixed roots were hydrolyzed in 1N HCL as described (Akinboro and Bakare, 2007). Thereafter, the roots were rinsed with distilled water before slide preparation, 2 to 3 root tips were homogenized on a glass slide and stained in 2 drops of aceto-orcein for 10 min. Excess stain was removed with filter paper and a cover slip was gently placed on the stained smear, then, carefully pressed with the thumb. The cover slip was gently tapped with the index finger to ensure that the stained cells did not overlap. Slides (5 per concentration) were observed for different stages of mitosis and chromosomal aberrations under oil immersion objective lens (100×) of a Nikon Eclipse E400 microscope. Number of dividing cells as well as chromosomal aberrations in 1000 counted cells on a slide was recorded. Mitotic index (MI) and frequency of chromosomal aberration (CA) were calculated using the following formulae:

$$\text{MI (\%)} = \frac{\text{Average number of dividing cells}}{\text{Total number of cells counted}} \times 100$$

$$\text{Frequency of CA (\%)} = \frac{\text{Average number of aberrant cells}}{\text{Total number of cells counted}} \times 100$$

Antimutagenic evaluation

The activity of the FDFJ in suppressing the mutagenic action of cyclophosphamide-CP (CAS: C0768) on the chromosomes of *A. cepa* was tested in similar way to the mutagenic evaluation in this study, except that 0.1% CP (w/v) was added to the solution of the FDFJ at each concentration. The root tips from the onions grown in the mixture of FDFJ + CP were prepared on microscope slides and observed as described earlier. Percentage of mitotic index (MI) and frequency of chromosomal aberrations (CA), recorded at each concentration were calculated. Percentage reduction of CP - induced chromosomal aberrations at different concentrations of FDFJ was calculated as stated below.

$$\text{\% R of CA} = \frac{A - B}{A - C} \times 100$$

Where, '% R' is the percentage reduction of chromosomal aberration (CA); 'A' is the frequency of CA induced by CP alone; 'B' is the frequency of CA induced by the mixture of FDFJ and CP and 'C' is the frequency of CA induced by tap water alone.

Statistical analysis

Data were expressed as percentage of free radicals scavenged, mitotic index, frequency of chromosomal aberration and percentage reduction of CP-induced CA by the FDFJ. Average values of the measured parameters were compared with the mean values of the controls, using Duncan multiple range comparison and Dunnett's tests under One-way ANOVA in SPSS software, 15.0 version. Data with a value of $p \leq 0.05$ were considered significant.

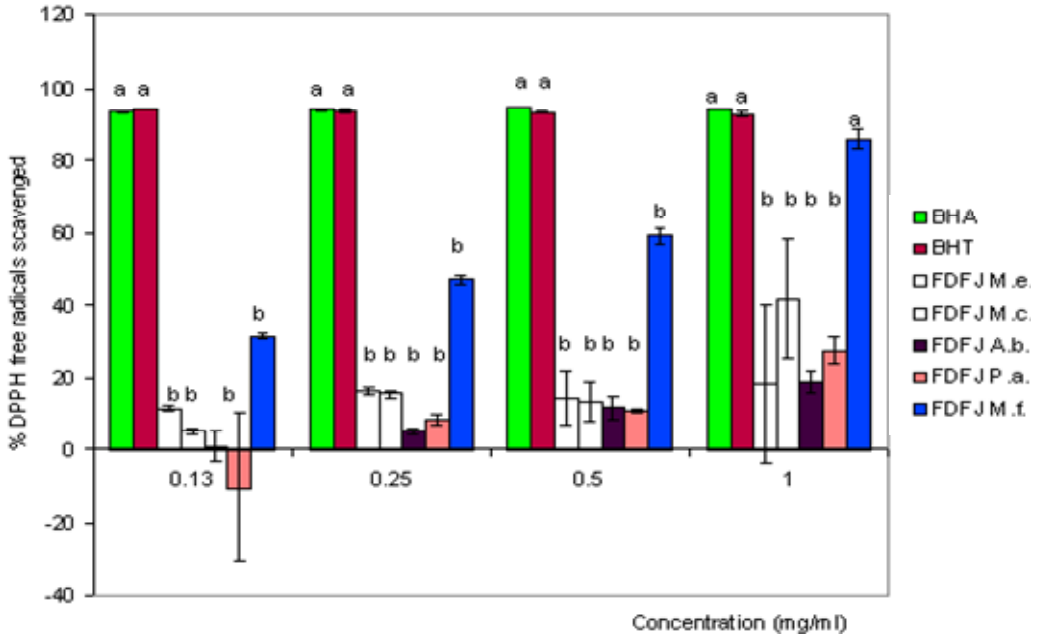

Figure 1. DPPH Scavenging activity of the fruit juices & standards.

The bars represent percentage of DPPH free radicals scavenged at different concentrations of the FDFJ and reference standards calculated from the average (n = 3) absorbance (517 nm) of an experimental reaction (carried out in triplicate) between the juice or standard, and DPPH solution. Error bars represent standard deviation of average percentage DPPH free radical scavenged at different concentrations. 'b' is significantly different from BHA & BHT.

RESULTS

DPPH free radicals scavenging activity of the FDFJ

Figure 1 shows the percentage of DPPH free radicals scavenged by the FDFJ. Among the evaluated fruit juices, FDFJ of *M. fragrans* recorded a concentration dependent DPPH free radical scavenging effect. At 1 mg/ml, the percentage of free radicals scavenged by the FDFJ of *M. fragrans* was 85.7%, amounting to 8.0%, and 7.1% less than the free radicals scavenging activity of BHA and BHT, respectively. Other FDFJ, except the FDFJ of *M. fragrans*, could not cause 50% inhibition of free radicals from DPPH at the tested concentrations. However, BHA and BHT (synthetic antioxidants) demonstrated higher free radicals scavenging activity than any of the evaluated FDFJ.

Total phenolic contents of FDFJ

Table 1 shows phenolic contents of the FDFJ determined by the Folin-Ciocalteau colorimetric method. The least and highest concentrations of phenolics extrapolated from the Gallic acid calibration curve (regression equation $y = 0.5209x + 1.1207$, $r^2 = 0.9315$) were in the range of 9.0 ± 1.5 to 21.0 ± 6.0 mg GAE/g for *A. bilimbi* and *M. fragrans*, respectively. The FDFJ of *M. citrifolia* contained 15.0 ± 0.5 mg GAE/g phenolic content, while it was 12.5 ± 1.0 mg GAE/g and 11.5 ± 2.5 mg GAE/g for *M. elliptica* and *P. acidus*, individually.

Effects of FDFJ on mitosis

The effect of the freeze dried fruit juices alone and its combination with CP, on cell division in the root tips of onion was evaluated (Figure 2). In the mutagenic test, FDFJ of *P. acidus* at 1, 2 and 4% induced higher mitotic indices (MI) than that of the negative control. There was a complete arrest of cell division at 8% concentration of *M. elliptical* and at 4% of *A. bilimbi*. Mitotic indices induced by the FDFJ of *M. elliptica* at 1, 2 and 4% were the least, compared with those of *M. citrifolia, A. bilimbi* and *M. fragrans*. At 1%, mixture of each of the FDFJ with CP, except for the combination of *A. bilimbi* with CP, induced MI value higher than that of CP and FDFJ alone. No dividing cell was observed at 4% of *A. bilimbi* and at 8% of *M. elliptica* and *P. acidus* in the antimutagenic test (Figure 2). At 8%, each of the mixtures of *M. citrifolia* or *M. fragrans* with CP resulted to 49 and 51% mitotic indices of their negative controls, respectively. Interestingly, inhibition of cell division by the combination of FDFJ of *M. fragrans* with CP was dose-dependent (Figure 2).

Table 1. Total phenolic contents of freeze dried fruit juices expressed as milligram gallic acid equivalent (GAE) per gram of dry sample.

Freeze dried fruit juice	Total phenolic content (Mean ± standard deviation, mg GAE/g)
Morinda elliptica	12.5 ± 1.0
Morinda citrifolia	15.0 ± 0.5
Averrhoa bilimbi	9.0 ± 1.5
Phyllantus acidus	11.5 ± 2.5
Myristica fragrans	21.0 ± 6.0

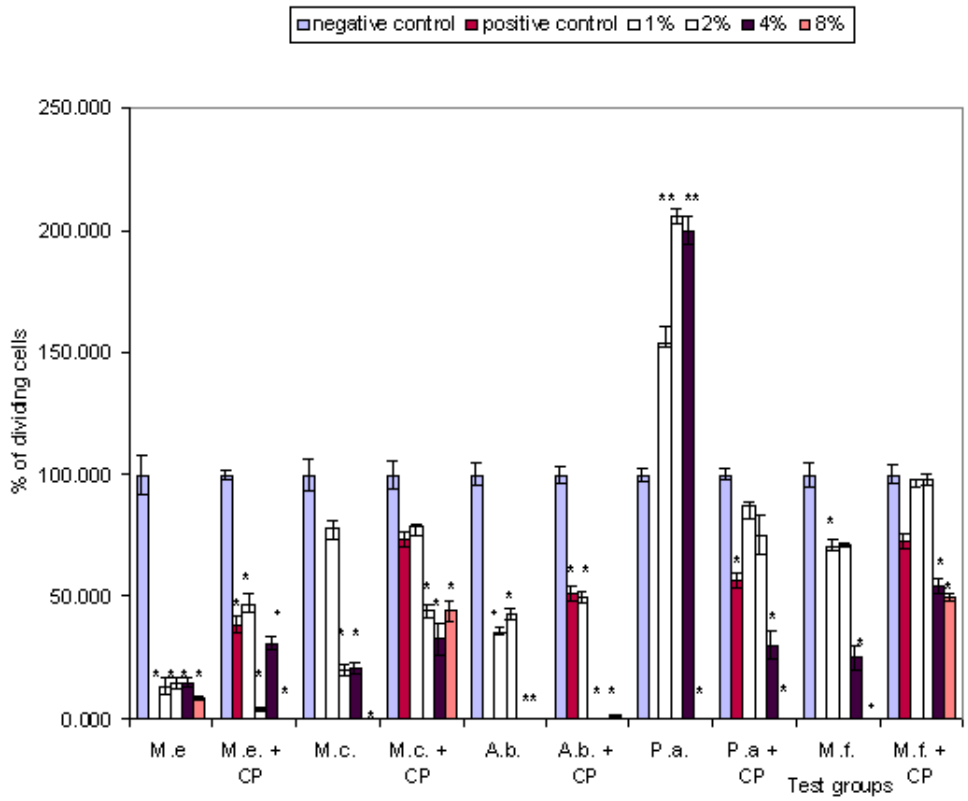

Figure 2. Effects of FDFJ alone, and FDFJ + CP on mitosis in the root tips cells of A. *cepa* L. The bars represent percentage mitotic index (in an average of 1000 counted cells on a slide) of the negative control caused by FDFJ and FDFJ + CP at different concentrations. * values are significantly lower than the negative control ($p \leq 0.05$); ** values are significantly higher than the negative control ($p \leq 0.05$).

Effects of the FDFJ on *A. cepa* chromosomes

Table 2 presents different types of chromosomal aberrations and frequencies induced by the FDFJ alone. Induced chromosomal aberrations were disturbed spindle, bridges, breaks and sticky chromosomes. There were more disturbed spindles aberrations than any other forms. Chromosomal aberrations induced by the juices of *M. citrifolia* (at 4%) and *P. acidus* (at 2 and 4%) were significantly different from the negative control ($p \leq 0.05$) (Table 2). Fruit juices of *M. elliptica*, *A. bilimbi* and *M. fragrans* caused chromosomal aberrations that were insignificantly different ($p \geq 0.05$) from those recorded for their individual negative controls.

The potency of the FDFJ to reduce the mutagenic effect of CP on the chromosomes of *A. cepa* is shown in Table 3. The frequency of chromosomal aberrations (CA) induced by CP alone was the highest, compared with those caused at different concentrations of the FDFJ + CP. At 2% of the FDFJ of *M. citrifolia*, 8% of *A. bilimbi* and *P. acidus*, the percentage reduction of CP-induced CA was negative, suggesting that the mixture of each

Table 2. Types and frequencies of chromosome aberrations induced by FDFJ alone and FDFJ + CP in *A. cepa* cells.

Fruit juice	Concentration (%)	Chromosome aberration				
		Disturbed spindle	Chromosome bridge	Chromosome break	Sticky chromosome	Frequency of chromosome aberration
M. elliptica	Control	1	-	-	-	0.02
	1	1	-	-	-	0.02
	2	-	-	-	-	0.00
	4	1	-	-	-	0.02
	8	1	1	-	-	0.04
M. citrifolia	Control	-	-	-	-	0.00
	1	1	-	-	-	0.02
	2	-	-	-	-	0.00
	4	2	-	-	-	0.04*
	8	-	-	-	-	0.00
A. bilimbi	Control	-	1	1	-	0.04
	1	1	-	-	-	0.02
	2	-	-	3	-	0.06
	4	-	-	-	-	0.00
	8	-	-	-	-	0.00
P. acidus	Control	-	-	-	-	0.00
	1	-	-	-	1	0.02
	2	4	-	1	-	0.10*
	4	-	1	1	1	0.06*
	8	-	-	-	-	0.00
M. fragrans	Control	1	1	1	-	0.06
	1	-	-	-	-	0.00
	2	4	1	2	-	0.14
	4	-	-	1	1	0.04
	8	-	-	-	-	0.00

Five thousand cells, per concentration, were observed for different types of chromosomal aberrations in A. cepa cells. * Frequencies of chromosomal aberrations are significantly different from the negative control ($p \leq 0.05$).

these FDFJ with CP caused appreciable number of aberrant cells. In addition, suppression of themutagenic action of CP by the FDFJ of *M. elliptica*, *M. citrifolia*, *A.bilimbi* and *P. acidus* was observed, but, not at all the tested concentrations. The number of dividing cells at 2% of *M. elliptica* was < 2, while 8% of the juice, 2 and 4% of *A. bilimbi* induced complete arrest of cell division. The FDFJ of *P. acidus* suppressed chromosomal damaging effect of CP most at 1 and least at 4% of the juice, however, there was induction of more aberrant cells at 8% concentration of this juice, resulting to negative percentage reduction of CP-induced CA. Interestingly, the FDFJ of *M. fragrans* suppressed CP-induced CA at all the selected concentrations.

DISCUSSION

Many chronic diseases such as cancers, atherosclerosis, diabetes, aging and other degenerative disorders inhumans can be elicited by overproduction of free radicals causing oxidative damage to biomolecules like lipids, proteins and DNA. Fruits and others plants may contain a vast number of free radical scavenging

Table 3. Effects of FDFJ of *M. elliptica, M. citrifolia, A. bilimbi, P. acidus* and *M. fragrans* on cyclophosphamide – induced chromosomal aberrations in *A. cepa.*

Fruit juice	Concentration (%)	Mean mitotic index (MI) ± SD	Average number of chromosomal aberration (CA) ± SD	Proportion of CA in MI	% reduction of chromosome aberration
	Control	24.00 ± 2.00	1.50 ± 1.29	0.06	-
M. elliptica	CP	9.25 ± 3.78	2.75* ± 0.96	0.30	-
	1	11.25 ± 3.30	1.50 ± 0.58	0.13	69.83
	2	1.00 ± 0.82	0.00 ± 0.00	0.00	MI < 2
	4	7.50 ± 3.00	0.50 ± 0.58	0.07	98.23
	8	0.00 ± 0.00	0.00 ± 0.00	0.00	TA
M. citrifolia	Control	19.75 ± 5.68	1.50 ± 0.58	0.08	-
	CP	14.50 ± 5.45	6.00* ± 3.27	0.41	-
	1	15.50 ± 3.42	2.00 ± 1.41	0.13	84.29
	2	8.75 ± 2.75	5.25* ± 3.59	0.60	-55.12
	4	6.50 ± 6.35	1.50 ± 0.58	0.23	54.17
	8	8.75 ± 4.27	1.25 ± 0.96	0.14	80.20
A. bilimbi	Control	14.50 ± 3.42	0.75 ± 0.96	0.05	-
	CP	7.50 ± 1.73	4.25 *± 2.06	0.57	-
	1	7.25 ± 2.63	1.25 ± 1.50	0.17	76.56
	2	0.00 ± 0.00	0.00 ± 0.00	0.00	TA
	4	0.00 ± 0.00	0.00 ± 0.00	0.00	TA
	8	0.25 ± 0.50	0.25 ± 0.10	1.00	-84.15
P. acidus	Control	18.25 ± 2.22	0.25 ± 0.50	0.01	-
	CP	10.33 ± 1.53	2.33* ± 1.53	0.23	-
	1	16.00 ± 5.89	0.25 ± 0.50	0.02	99.09
	2	13.75 ± 7.63	1.00* ± 1.16	0.07	72.14
	4	5.50 ± 5.80	1.00* ± 1.41	0.18	20.65
	8	3.00 ± 1.50	0.75* ± 0.47	0.25	-11.54
M. fragrans	Control	12.00 ± 3.46	0.33 ± 0.58	0.03	-
	CP	8.75 ± 4.19	3.75* ± 1.71	0.43	-
	1	11.75 ± 3.50	2.50* ± 1.73	0.21	53.81
	2	11.75 ± 2.50	1.75* ± 1.26	0.15	69.72
	4	6.50 ± 2.89	1.25 ± 1.26	0.19	58.91
	8	6.00 ± 1.63	1.50* ± 1.29	0.25	44.52

Five thousand cells, per concentration, were observed for different mitotic stages and chromosomal aberrations. Mitotic index (MI) was calculated as the percentage of dividing cells observed in 1000 counted cells on a slide; and frequency of chromosomal aberration (CA) was calculated as the percentage of aberrant cells in 1000 counted cells per slide. To calculate the % reduction of CP-induced CA, the proportion of CA in the recorded MI at each concentration was determined. Percentage reduction of CP-induced CA was not computed when there was total arrest (TA) of cell division and when MI < 2. * Frequencies of CA are not significantly different from the positive control -CP ($p \geq 0.05$).

molecules like phenolic and nitrogen compounds, vitamins and some other endogenous metabolites, which are rich in antioxidants activity (Cai et al., 2004). The antioxidant activity of the FDFJ in this study could be due to their phenolic compounds. The antioxidant activity of phenolic compounds has been reported (Lopes et al., 2004). Thus, the higher phenolic content recorded for the FDFJ of *M. fragrans* than in the other FDFJ explains its concentration dependent antioxidant activity (Figure 1). The results of free radical scavenging effects of these FDFJ on DPPH solution are in line with the earlier reports (Wang et al., 2010; Dussossoy et al., 2011; Eldeen et al., 2011). Mitotic index is a parameter that reveals cytotoxic effects of chemical substances on the cells, as high and

low mitotic indices may suggest the degree of cytotoxicity that a chemical inflicts on meristematic cells. In this study, the induction of less MI values than the MI of individual negative controls by the FDFJ of *M. elliptica*, *M. citrifolia*, *A. bilimbi* and *M. fragrans* alone suggests cytotoxicty. This agrees with the earlier reports of Chong et al. (2005). Extracts of the plants have been reported to have anti-bacterial activity based on their ability to inhibit microbial cell growth (Norhana et al., 2009; Chatterjee et al., 2007; Chiang and Abdullah, 2007; Ikeda et al., 2009).This kind of activity is therefore expected to be cytotoxic. In addition, the observed cytotoxic effects of the FDFJ on the cell division in *A. cepa* may be due to their reported antioxidant properties (Calliste et al., 2010; Ahmad et al., 2005). Some naturally occurring anthraquinones in *Morinda* species and lignans in *M. fragrans* scavenge reactive oxygen species (ROS) and free radicals, thereby preventing carcinogenesis. It is expected that their anti-cancer effectiveness would be cytotoxic as a result of suppression of mitosis. We are of the opinion that the mitotic inhibitory effect of the FDFJ of *M. elliptica*, *M. citrifolia* and *A. bilimbi* on the cell division in *A. cepa* was cytostatic due to the induction of non-dose dependent inhibition of mitosis in *A. cepa*. This may be connected with the kinds and total contents of free radicals scavenging molecules in form of phenolic compounds in the FDFJ. However, the dose- response inhibition of mitosis by the FDFJ of *M. fragrans* is an indication of cytotoxicity which might have been caused by phenolic compounds acting as antioxidants. This supports the report that polyphenols such as lignans, flavonoids, which are important classes of plant derived compounds, possess a variety of biological activities such as anti-tumor and anti-mitotic activities (Chatterjee et al., 2007). Inhibition of mitosis may occur due to suppression of DNA/ protein (Histones) synthesis or an arrest in the G_2 phase of the cell cycle, preventing the cell from entering mitosis (Turkoglu, 2008). The induction of higher *P. acidus* at 1, 2 and 4% implies lack of cytotoxicity. It had earlier been reported that extract of *P. acidus* was non-cytotoxic (Sousa et al., 2007). This may be in connection with its richness in adenosine, a precursor for DNA synthesis during cell division. Induction of higher MI by the mixture of each of the FDFJ with CP at 1% than the MI obtained for CP alone suggests suppression of CP-induced cytotoxicity. Suppression of CP- induced cytotoxicity was more observed with the FDFJ of *M. fragrans*, perhaps due to the quantity and quality of phenolic compounds which enhance its free radicals scavenging activity (Figure 1 and Table 1). Cyclophosphamide (CP) is a drug usually employed in treating a wide range of neoplastic diseases as well as some non-malignant ones. The therapeutic function of phosphoramide mustard and the major anti-neoplastic metabolite of CP is cytotoxic (Anderson et al., 1995). Complete arrest of cell division, by the FDFJ alone as well as the mixtures of FDFJ of *M. elliptical* and *P. acidus* with CP at 8% suggests occurrence of cell death which may be caused through a reaction between components of the FDFJ or/and CP to generate product(s) capable of; interfering with the normal progression of mitosis, preventing cells from entering the prophase, or blocking the mitotic cycle at interphase or/and causing an increase in G_2 and S phases duration (Yildiz et al., 2009).

The induction of chromosomal aberrations that were not significantly different ($p \leq 0.05$) from those induced by the negative controls and non dose-related, suggests weak mutagenic action of the FDFJ of *M. elliptica*, *A. bilimbi* and *M. fragrans*. Similarly, obtainment of non dose-dependent chromosomal aberrations caused by the FDFJ of *M. citrifolia* and *P. acidus* can also justify their weak mutagenic effects on the chromosomes of *A. cepa*. Nevertheless, the observed mutagenic effects of the FDFJ may be due to some phenolic compounds and vitamin C reported as natural occurring antioxidants in these juices (Chan-Blanco et al., 2006; Chatterjee et al., 2007; Norhana et al., 2009). A reaction between phenolic compounds and vitamin C in the presence of Cu^{2+} and Fe^{3+} may enhance mutagenic activity through generation of reactive oxygen species (ROS) which may interact with DNA directly, facilitate redox cycling or interacting with molecular oxygen to produce oxidative stress and genotoxic ROS, that may cause DNA damage (Frankel et al., 2004).

Mutagens and carcinogens such as CP may act through the generation of ROS that play a major role as endogenous initiators of degenerative processes, such as DNA damage and mutation and its promotion (Negi et al., 2003). The reduced values of CA observed with the FDFJ + CP compared with those induced by CP alone implies antimutagenic effectiveness of the evaluated FDFJ. This activity may be connected to DPPH free radicals scavenging molecules in the FDFJ, counteracting free radicals from the acrolein component of CP. CP is an alkylating agent that causes chromosomal aberrations and DNA damage through generation of carbonium ions, which react with the electron-rich area of the nucleic acids and proteins (Schneider et al., 1997; De et al., 1995). Among the investigated FDFJ, the FDFJ of *M. fragrans* scavenged DPPH free radicals in a dose-dependent manner. It is possible to say that antimutagenic activity of the FDFJ of *M. fragrans* against CP-induced mutagenicity may due to antioxidants.

Myricetin (3, 3, 4, 5, 5, 7-hexahyroxylflavone) is a natural flavonoid, found in many fruits, vegetables, herbs and other plants, capable of scavenging ROS and ensures cytoprotective and antimutagenic effects (Wang et al., 2010). Argenteane, a dilignan antioxidant that is powerful like vitamin E was isolated from *M. fragrans* (Calliste et al., 2010). It is not surprising that the free radicals scavenging activity of the FDJF of *M. fragrans* was almost the same as that of BHA and BHT at 1 mg/ml in this study. Vitamins A, C and E have been reported to protect DNA from the mutagenicity of oxy radicals and products of promutagen activation of free radical origin (Odin, 1997). Also, DNA protecting role of phenolic com-

compounds in *M. fragrans* has been reported (Chatterjee et al., 2007). These phytochemicals may be responsible for the observed antimutagenic action of this FDFJ against the mutagenicity of CP. To some extents, it is therefore, logical to say that there is a direct relationship between phenolic contents, antioxidant and antimutagenic activities. This is in line with the opinions of some investigators who previously used Ames test to evaluate mutagenicity and antimutagenicity of plant extracts and fruit juices (Zahin et al., 2010; Schneider et al., 1997; Cherdshewasart et al., 2008; Ham et al., 2009; Karekar et al., 2000; Oh et al., 2008; Rauscher et al., 1998; Son et al., 2003). In the other of FDFJ except *M. fragrans*, induction of negative percentage reduction of CP-induced CA or total arrest of cell division by the mixture of the FDFJ and CP at certain concentrations may be due to prooxidative activity of phenoilc compounds, causing generation of ROS to cause more DNA damages. The induction of CA by CP in this study was possible due to the metabolism of CP to phosphoramide mustard and acrolein constituents. With the breaking down of CP to these two constituents by the *A. cepa* cellular components, the suitability and reliability of the *A. cepa* test in evaluating the antimutagenicity of chemical compounds against mutagenicity of an indirect mutagen like CP is convincingly supported by our results. Anticarcinogenic and antimutagenic activity of medicinal and food plants may be due to a variety of mechanisms such as inhibition of genotoxic effects, inhibition of cell proliferation, signal transduction modulation, scavenging of free radicals, induction of detoxification enzymes, induction of cell- cycle arrest and apoptosis, modulation of cytoskeletal proteins playing a key role in mitosis and the inhibition of topoisomerase I or II activity (Zahin et al., 2010). Therefore, it can be concluded from our results that the mechanism of antimutagenic effect of the FDFJ on the mutagenic action of CP may be due to prevention of acrolein free radicals-induced DNA damage in *A. cepa* cells.

Conclusions

In this study, it was clearly demonstrated that the FDFJ of *M. fragrans*, among the evaluated fruit juices, possessed consistent cytotoxic and non significant genotoxic effects on the cell division and chromosome structure in *Allium cepa*.

This FDFJ also had significant antimutagenic activity against CP-induced mutagenicity at all tested concentrations. These activities are not unlikely to be connected with the phenolic compounds, acting as antioxidants in the FDFJ of *M. fragrans*. It is therefore, logical to say that the type (s) of free radical scavengers present in this juice may be promising candidate(s) for cancer prevention. Nevertheless, further investigations should be geared towards validation of these findings using the animal bioassays.

ACKNOWLEDGEMENTS

Thanks to the managements of Universiti Sains Malaysia (USM) and Academy of Sciences for the Developing World (TWAS) for providing financial support and facilities to undertake this investigation by sponsoring Mr. Akinboro Akeem's Ph.D. programme under the USM-TWAS postgraduate fellowship scheme. Mr. Peter, C. Boyce is also appreciated for editing this manuscript prior to submission.

REFERENCES

Ahmad R, Abdul Manaf A, Daud AI, Nor Hadiani I, Khozirah S, Nordin HJL (2005). Antioxidants radical scavenging, anti-inflammatory, cytotoxic and antibacterial activities of methanolic extract of some Hedyotis species. Life Sci. 76: 1953-1964.

Ajith TA, Janardhanan KK (2002). Antioxidant and antihepatotoxic activities of *Phellinus rimosus* (Berk) Pilat. J. Ethnopharmacol. 81: 387-391.

Ak T, Gülcin I (2008). Antioxidant and radical scavenging properties of curcumin, Chemico-Biol. Interactions. 174: 27-37.

Akinboro A, Bakare AA (2007). Cytotoxic and genotoxic effects of aqueous extracts of five medicinal plants on *Allium cepa* Linn. J. Ethnopharmacol. 112: 470-475.

Anderson D, Bishop JB, Garner RC, Ostrosky-Wegman P, Selby PB (1995).Cyclophosphamide: Review of its mutagenicity for an assessment of potential germ risks. Mutation Res. 330: 115-181.

Barcelos GRM, Fernanda S, Mateus PM, Maria AMM, Ilce M (2007). Evaluation of mutagenicity and antimutagenicity of cashew stem bark methanolic extract *in vitro*. *Journal of Ethnopharmacology*. 114: 268-273.

Cai Y, Luo Q, Sun M, Corke H (2004). Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. *Life Sciences*. 74: 2157-2184.

Calliste CA, Kozlowski D, Durox JL, Champavier Y, Chulia AJ, Trouillas P(2010). A new antioxidant from wild nutmeg. Food Chem. 118: 489-496.

Chan-Blanco Y, Fabrice V, Ana MP, Max R, Jean-Marc B, Pierre B (2006). The noni fruit (*Morinda citrifolia* L.): A review of agricultural research, nutritional and therapeutic properties. J. Food Composition and Analysis. 19: 645-654.

Chatterjee S, Zareena N, Gautam S, Soumyakanti A, Prasad SV, Arun S (2007). Antioxidant activity of some phenolic constituents from green pepper (*Piper nigrum* L.) and fresh nutmeg mace (*Myristica fragrans*). Food Chem. 101: 515-523.

Cherdshewasart W, Sutjit W, Pulcharoen K, Panriansaen R, Chulasiri M (2008). Antimutagenic potential of the Thai herb, *Mucuna collettii* Lace. J. Ethnopharmacol. 115: 96-103.

Chiang L, Abdullah MA (2007). Enhanced anthraquinones production from adsorbent-treated *Morinda elliptica* cell suspension cultures in production medium strategy. Process Biochem. 42 757-763.

Chong TM, Mohd AA, Oi Ming L, Fadzillah MNA, Nordin HL (2005). Effective elicitation factors in *Morinda elliptica* cell suspension culture. Process Biochem. 40: 3397-3405.

De AK, Agarwal K, Mukherjee A, Sengupta D (1995). Inhibition by capsaicin against cyclophosphamide-induced clastogenicity and DNA damage in mice. Mutation Res. 335: 253-258.

Donald GB, Nutmeg (*Myristica fragrans* Houtt.) (2008). Toxic Plants, and venomous animals, in Med. Toxicol. Natural Substances*:* Foods, Fungi, Med. Herbs,(Barceloux D G, Hoboken N J, (Eds.) John Wiley & Sons), pp.67-70.

Duangpron P, Siripong P (2009). Effect of Auxin and Cytokinin on Phyllantusol A production by Callus Cultures of *Phyllantus acidus* Skeels. American-Eurasian J. Agric & Environ. Sci. 5(2): 258-263.

Dussossoy E, Brat P, Bony E, Boudard F, Poucheret P, Mertz C, Giaimis J, Michel A (2011). Characterization, anti-oxidative and anti-inflammatory effects of Costa Rican noni juice *(Morinda citrifolia L.)*.

J. Ethnopharmacol. 133: 108-115.
Eldeen IMS, Seow E-M, Abdullah R, Sulaiman SF (2011). *In vitro* antibacterial, antioxidant, total phenolic contents and anti-HIV-1 reverse transcriptase activities of extracts of seven *Phyllantus* sp. South Afr. J. Botany. 77: 75-79.
Feretti D, Zerbini I, Zani C, Ceretti E, Moretti M, Monarca S (2007). *Allium cepa* chromosome aberration and micronucleus tests applied to study genotoxicity of extracts from pesticide-treated vegetables and grapes. Food Additives and Contaminants. 24(6): 561-572.
Frankel SIR, Ckless K, Silveira JD, Rubensam G, Brendel M, Erdtmann B, Henriques JAP (2004). Study of antioxidant and mutagenic activity of different orange juices. Food Chem. 88: 45-55.
Gülcin I (2009). Antioxidant activity of L-Adrenaline: Activity-structure insight. Chem. Bio. Interact. 71-80.
Gülcin I, Bursal E, Şehitoglu MH, Bilsel M, Gören AC (2010). Polyphenol contents and antioxidant activity of lyophilized aqueous extract of proplis from Erzurum, Turkey. Food and Chem. Toxicol. 48: 2227-2238.
Gupta RK, Achyut NK, Sandhya D, Ameetabh T, Vibha T, Ramesh C, Geeta W (2008). *In vivo* evaluation of anti-oxidant and anti-lipidimic potential of *Annona squamosa* aqueous extract in Type 2 diabetic models. J. Ethnopharmacol. 118: 21-25.
Ham SS, Kim SH, Moon SY, Chung MJ, Cui CB, Han EK, Chung CK, Choe M (2009). Antimutagenic effects of subfractions of Chaga mushroom (*inonotus obliquus*) extract. Mutation Res. 672: 55-59.
Ikeda R, Wada M, Nishigaki T, Nakashima K (2009). Quantification of coumarin derivatives inNoni (*Morinda citrifolia*) and their contribution of quenching effect on oxygen species.Food Chem. 113: 1169-1172.
Karekar V, Joshi S, Shinde SL (2000). Antimutagenic profile of three antioxidants in the Ames assays and the Drosophila wing spot test. Mutation Res. 468: 183-194.
Kumari MA, Mukherjee NC (2009). Genotoxicity of silver nanoparticles in *Allium cepa*. Sci. Total Environ. 407: 5243-5246.
Liu CH, Xue YR, Ye Yong H, Yuan FF, Liu JY, Shuang JL (2007). Extraction and Characterization of Antioxidant Compositions from Fermented Fruit Juice of *Morinda citrifolia* (Noni). Agr. Sci. China. 6(12): 1494-1501.
Lopes e Lopes MI, Saffi J, Echeverrigaray S, Henriques JAP, Salvador M (2004). Mutagenic and antioxidant activities of *Croton lechleri* sap in biological systems. J. Ethnopharmacol. 95: 437-445.
Negi PS, Jayaprakasha GK, Jena BS (2003). Antioxidant and antimutagenic activities of pomegranate peel extracts. Food Chem. 80: 393-397.
Norhana WNM, Mohd Nor Azman A, Susan EP, Hilton CD, Gary AD (2009). Effects of bilimbi (*Averrhoa bilimbi*) and tamarind (*Tamarindus indica* L.) juice on *Listeria monocytogenes* Scott A and *Salmonella Typhimurium* ATCC 14028 and the sensory properties of raw shrimps. Int. J. Food Microbiol. 136: 88-94.
Odin AP (1997). Vitamins as antimutagens: Advantages and some possible mechanisms of antimutagenic action. Mutation Res. 386: 39-67.
Oh HT, Kim SH, Choi HJ, Chung MJ, Ham SS (2008). Antioxidative and antimutagenic activities of 70% ethanol extract from masou salmon (*Oncorhynchus masou*). Toxicol. *in Vitro*. 22: 1484-1488.
Pushparaj P, Tan CH, Tan BKH (2000). Effects of *Averrhoa bilimbi* leaf extract on blood glucose and lipids in streptozotocin-diabetic rats. J. Ethnopharmacol. 72: 69-76.
Rauscher R, Edenharder R, Platt KL (1998). *In vitro* antimutagenic and *in vivo* anticlastogenic effects of carotenoids and solvent extracts from fruits and vegetables rich in carotenoids. Mutation Res. 413: 129-142.
Rosidah, Yam MF, Amirin S, Mohd Zaini A (2008). Antioxidant potential of Gynura procumbes. Pharmaceutical Biol. 9(46): 616-625.
Schneider EL, Sternberg M, Tice RR (1997). In vivo analysis of cellular replications. *Proc. Natl. Acad. Sci*. (USA) 74: 2041-2044.
Son YO, Kim J, Lim JC, Chung Y, Chung GH, Lee JC (2003). Ripe fruit of *Solanum nigrum* L. inhibits cells growth and induces apoptosis inMCF-7 cells. Food and Chem. Toxicol. 41: 1421-1428.
Sousa M, Jiraporn O, Roswitha S, Supaporn P, Ana R, Andre S, Tiago G, Chaweewan J, Margarida DA, Rainer S, Kari K (2007). An Extract from the Medicinal Plant *Phyllantus acidus* and its isolated Compounds Induce Airway Chloride Secretion: A Potential Treatment for Cystic Fibrosis. Mol. Pharmacol. 71: 366-376.
Sulaiman SF, Sajak AAB, Ooi KL, Supriatno, Seow EM (2011). Effect of solvents in extracting polyphenols and antioxidants of selected raw vegetables. J. Food Composition and Analysis. 24: 506-515.
Talaz O, Gülcin I, Goksu S, Saracoglu N (2009). Antioxidant activity of 5,10-dihydroindeno [1,2-b] indoles containing substituents on dihydroindeno part. Bioorgan. Med. Chem. 17: 6583-6589.
Tan BKH, Chee HT, Peter NP (2005). Anti-diabetic activity of the semi-purified fractions of *Averrhoa bilimbi* in high fat diet fed-streptozotocin-induced diabetic rats. Life Sci. 76: 2827 2839.
Turkoglu S (2008).Evaluation of genotoxic effects of sodium propionate, calcium propionate and potassium propionate on the root meristem cells of *Allium cepa*. Food and Chem. Toxicol. 46: 2035-2041.
Wang ZH, Kang KA, Zhang R, Piao MJ, Jo SH, Kim JS, Kang SS, Lee JS, Park DH, Hyun JW (2010). Myricetin suppresses oxidative stress-induced cell damage via both direct and indirect antioxidant action. Environ. Toxicol. Pharmacol. 29: 12-18.
Yildiz M, Ibrahim HC, Muhsin KA, Fatih F, Hakan T (2009). Determination of genotoxic effects of copper sulphate and cobalt chloride in *Allium cepa* root cells by chromosome aberration and comet assays. *Chemosphere*. 75: 934-938.
Zahin M, Iqbal A, Farrukh A (2010). Antioxidant and antimutagenic activity of *Carum copticum* fruit extracts. Toxicol. *in vitro*. 24: 1243-1249.