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Antioxidant and Antimutagenic (Anticlastogenic) Activity of Alcoholic Extract of *Bauhinia variegata* (Linn.) Root

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Antioxidant and Antimutagenic (Anticlastogenic) activity of alcoholic extract of *Bauhinia variegata* (Linn.) root.
Place: C. U. Shah College of Pharmacy and Research, Wadhwan, Surendranagar, Gujarat, India.
Methodology: Shade dried *Bauhinia variegata* (Linn.) root, extraction was carryout by isolation extract were subjected to primary and secondary Phytochemical investigation. Then *In-vitro* antioxidant properties were estimated by reducing power and nitric oxide free radical scavenging method. Based on Phytochemical constituent and antioxidant properties In-vivo Antimutagenic (Anticlastogenic) activity was performed.
Results: Preliminary phytochemical investigation revealed the presence of carbohydrates, free

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amino acids, and secondary metabolites like tannins, phenolic compounds and flavonoids, then polyphenol estimation found ALBV contains 86.38% phenolic compounds. In antioxidant properties determination IC50 respectively found 55.27±2.57 µg/ml, 125.52±8.15 µg/ml against of Ascorbic acid and Curcumin. Then *In-vivo* Antimutagenic (Anticlastogenic) activity ALBV shows significant reeducation in % MNPCE, % MNNCE and P/N ratio at 24 h, 48 h and 72 h against the cyclophosphamide-induced mutagenicity.

Conclusion: Therefore, from the present study, it is concluded that alcoholic extract of *Bauhinia variegata* root (ALBV) can prove to be a very good antioxidant and effective chemopreventive against cyclophosphamide-induced mutagenesis.

Keywords: Bauhinia variegata root; anticlastogenic; polychromatic erythrocytes; micronucleated polychromatic erythrocytes; micronucleated normochromatic erythrocytes.

1. INTRODUCTION

Free radicals are the highly reactive species capable of widespread, indiscriminate oxidation and peroxidation of proteins, lipids and DNA which can lead to significant cellular damage, the involvement of free radicals in the pathological process such as ageing, behavioural and psychiatric disorders, cancer, atherosclerosis and rheumatoid arthritis is well recognized [1].

The human body has several enzymatic and non-enzymatic antioxidant mechanisms to combat oxidative stress. The non-enzymatic antioxidants are either produced naturally in the body or supplied through foods and/or supplements [2].

Antioxidant reacts with reactive oxygen species (ROS) to quench the radicals and to produce less aggressive chemicals species likely to cause tissue damage. Much attention has been focused on the use of antioxidants because of their protective effect against damage from reactive oxygen species, on this basis the beneficial effect of antioxidants are being increased [3]. It is now widely recognized that the antioxidants are also useful in the treatment of cancer. The changes in the base pair sequence of genetic material (either DNA or RNA) is called mutations, mutations can be caused by copying errors in the genetic material during cell division and/or by exposure to ultraviolet and/or ionizing radiation, chemical mutagens, viruses or it can also occur deliberately under cellular control during processes such as meiosis or DNA replication [4,5]. One of the best ways to minimize the effect of a mutagen is by the use of anticlastogens; they act by interfering with DNA repair and/or with mutagen metabolites and/or with free radicals.

Oxidative stress induces a cellular redox imbalance which has been observed in various cancer cells. Polyphenols have been shown to inhibit cancer-associated enzyme telomerase, cell cycle and induce apoptosis [6]. Many important anticancer drugs are derived from plant sources, e.g., taxol from *Taxus brevifolia* and camptothecin from *Cascuta reflexa*. Compounds having low side effects, inducing apoptosis and target specific cytotoxicity to the cancer cells are drugs of choice [7].

Bauhinia variegata Linn (Caesalpiniaceae) grows as a medium-sized, deciduous tree found throughout India and is commonly called Kanchanara in Sanskrit. It is traditionally used in bronchitis, leprosy, tumours and ulcer [8] and its extracts have been found to have antibacterial and antifungal activity [9]. Phytochemical studies revealed the presence of 5,7-dimethoxy and-dihydroxy flavanone-4-O-α-L –rhamnopyronosyl-β- D-glucopyranosides, naringenin 5, 7-dimethyl ether 4'-rhamnoglucoside, a novel flavonol glycoside 5,7,3´,4´-tetrahydroxy-3-methoxy-7-O-α-L-rhamnopyranosyl (1→3)-O-*β*-D-galacto pyranoside, lupeol, β -sitosterol and quercetin [10-13]. The present study is focused to find out the antioxidant and antimutagenic (Anticlastogenic) effect of an ethanolic root extract of *Bauhinia variegata,* against cyclo-phosphamide-induced micronucleus test in mice.

2. MATERIALS AND METHODS

2.1 Extraction

Air-dried & coarsely powdered (350 gm.) of *Bauhinia variegata* root was taken. Extraction was carried out in a soxhlet extractor using ethanol. The extract was concentrated to dryness under reduced pressure and it was preserved in a refrigerator [14,15].

2.2 Phytochemical Investigation

The alcoholic extract of *Bauhinia variegata* root (ALBV) was subjected to various phytochemical tests for identification of secondary metabolites present in them [16].

2.3 Determination of Total Polyphenols

The total polyphenol content was determined by UV method, using tannic acid as standard, according to the method described by the International Organization for Standardization 14502-1. Briefly, 1.0 ml of the diluted sample ALBV was transferred in duplicate to separate tubes containing 5.0 ml of a 1/10 dilution of Folin - Ciocalteu's reagent in water. Then, 4.0 ml of a sodium carbonate solution (7.5% w/v) was added. The tubes were then allowed to stand at room temperature for 60 min before absorbance at 765 nm was measured against water. The concentration of polyphenols in samples were derived from a standard curve of tannic acid ranging from 10 to 50 µg/ml and expressed in terms percentage [17].

2.4 Determination of Antioxidant Properties Reducing Power Assay

Various concentrations of the extracts in 1.0 ml of deionized water were mixed with phosphate buffer (2.5 ml) and potassium ferricyanide (2.5 ml) and incubated at 50°C for 20 min. Aliquots of trichloroacetic acid (2.5 ml) were added to the mixture, which was then centrifuged at 3000 rpm for 10 min whenever necessary. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and a freshly prepared ferric chloride solution (0.5 ml). The absorbance was measured at 700 nm. A blank was prepared without adding extract. Ascorbic acid at various concentrations was used as standard. Increased absorbance of the reaction mixture indicates an increase in reducing power [18].

2.5 Nitric Oxide Free Radical Scavenging Activity

Various concentrations of the extracts in 1.0 ml of methanol were mixed with Sodium nitroprusside (10 mM) in phosphate buffer and made up to 200 µl with methanol mixture of solution Incubate at room temperature for 150 minutes. After the incubation period, 5 ml of Griess reagent was added then absorbance was taken at 546 nm. A blank was prepared without adding extract. Curcumin at various concentrations was used as standard the % reduction and IC50 were calculated [19].

2.6 Experimental Animals

Adult Swiss albino mice of either sex weighing (18-22 g) obtain from Zydus Research Centre, Ahmedabad, for the experimental purpose were all acclimatized for 7 days under standard husbandry conditions i.e.; room temperature of (25±1)°C; relative humidity of 45%-55% and a 12:12 h light/ dark cycle.

2.7 Acute Toxicity Studies

The oral acute toxicity study of ALBV was carried out in Swiss albino mice, using the Organization for Economic Co-operation and Development (OECD) guidelines (OECD 423). The animals received a single dose of 2000 mg/kg orally by gavages and were observed for toxic symptoms and mortality, continuously for first 4 h after dosing. Finally, the number of survivors was noted after 24 hrs and these animals were then maintained for further 14 days with observations made daily.

2.8 Micronucleus Test in Mice

Swiss albino mice of weight range 18-22 g were divided into thirteen groups (G1 to G13), each group consisting of six animals; the G1 normal control group without any treatment, G2 vehicle control received 2% acacia; G3 and G4 are extract control, received extract ALBV 200 and 400 mg/kg p.o. for 7 consecutive days, the normal control, vehicle (2% acacia) and extract Control is sacrificed on 7^{th} day after last dosing, all control is same for 24 h, 48 h and 72 h. G5, G6 and G7 were challenged with cyclophosphamide (75 mg/kg, i.p.) and bone marrow samples were collected from these animals 24, 48 and 72 h after cyclophosphamide injection respectively.

The animals in the groups G8 to G10 and G11 to G13 received ALBV 200 mg/kg and 400 mg/kg, p.o. respectively for 7 consecutive days. On 7^{th} day after the ALBV treatment, all the animals in the group G8 to G13 were treated with cyclophosphamide (CP) and the bone marrow samples from G6, G7 and G8 were collected after 24, 48 and 72 h of cyclophosphamide administration, similarly from G9, G10 and G11 group animals, the bone marrow samples were

collected after 24, 48 and 72 h after the cyclophosphamide administration respectively; in brief, bone marrow was aspirated from femur into 1 ml of 5% bovine albumin in phosphate-buffered saline (pH 7.2) [20,21].

The cell suspension was centrifuged (1000 rpm for 5 min) and the smears were prepared from the pellet on chemically clean glass slides and stained with may-Grunewald and Giemsa stain. The smears were analyzed under the oil immersion objective (100X) for the presence of micronuclei (MN) in polychromatic erythrocytes (PCE) and normochromic erythrocytes (NCE). P/N (polychromatic erythrocytes/ normochromic erythrocytes) ration was determined by counting a total of 500 erythrocytes per animal and total of 2000 erythrocytes were examined for the presence of micronuclei per each animal sample [22,23].

2.9 Statistical Analysis

Values were expressed as mean ± SEM from 6 animals. Statistical difference in mean will be analyzed using one way ANOVA followed by Turkey's multiple comparison tests $P < 0.05$ were considered statically significant.

3. RESULTS AND DISCUSSION

3.1 Phytochemical

The Phytochemical studies of alcoholic extracts of *Bauhinia variegata* root (ALBV) revealed the presence of carbohydrates, glycosides, saponins, tannins, phenolic compounds, proteins & free amino acids and flavonoids.

3.2 Determination of Total Polyphenols

The estimation of polyphenolic content was performed by based on a primary phytochemical investigation by Folin- Ciocalteu's reagent, the extracts exhibit that, the ALBV found to contain 91.38%.

Table 1. Quantitative determination Polyphenols at 760 nm

Extract	Polyphenols (%)
Alcoholic Extracts of *Bauhinia variegata root*(ALBV)	86.38%

3.3 Antioxidant Activity

The extracts containing varying quantities of total polyphenols were comparatively studied for their antioxidant potentialities. Two different *in vitro* methods namely Reducing Power by $FeCl_3$ and Nitric Oxide Free Radical Scavenging Activity were employed, ascorbic acid was used as a standard in Reducing Power by $FeCl_3$, the ALBV significantly decreased the absorbance and the IC50 value 65.27±2.43 µg/ml was found, to possess more significant antioxidant activity, however, the IC50 value is lesser then ascorbic acid. In Nitric Oxide Free Radical Scavenging Activity, the Curcumin was used as a standard, ALBV has offered good free radical scavenging activity by decreasing the absorbance and the IC50 value 325.452±13.05 µg/ml the IC50 value is lesser then Curcumin. The IC50 value of the different antioxidant activity of *Bauhinia variegata* root extract (ALBV) was shown in Table 2.

Based on the percentage of polyphenolic content and antioxidant potential, of the ALBV was selected for evaluation of the antimutagenic potential.

3.4 Acute Oral Toxicity Studies

The acute oral toxicity (AOT) study of ALBV was observed that it was safe up to 2000 mg/kg body weight and it was not showing any mortality, based on AOT selected dose for Antimutagenic activity are 200 mg/kg body weight and 400 mg/kg body weight.

3.5 Antimutagenic Activity

The probability of spontaneous mutation was evaluated for untreated normal control group, vehicle control group treated with 2% acacia and ALBV treated groups, by collecting the bone marrow samples of the respective groups without cyclophosphamide treatment and the samples were evaluated for frequency of Polychromatic Erythrocytes (PCE's), Normochromatic Erythrocytes (NCE's) and concurrently looked for frequency of Micronucleated Polychromatic Erythrocytes (MNPCE's) and Micronucleated Normochromatic Erythrocytes (MNNCE's) respectively and the P/N ratio was calculated.

Similar to untreated group and vehicle-treated groups, the positive control group animals treated with cyclophosphamide (75 mg/kg, i.p.) were evaluated for the frequency of MNPCE's, MNNCE's and P/N ratio after the 24 h, 48 h and 72 h of cyclophosphamide administration; the observations showed that there was a significant increase in the frequency of MNPCE's and

MNNCE's, also there is increase in P/N ratio, at 24 h, 48 h and 72 h after cyclophosphamide administration compare to normal control group.

The antimutagenic activity of ALBV was evaluated by its inhibitory effect on cyclophosphamide-induced mutagenesis. The ALBV at 200 and 400 mg/kg, p.o., has significantly decreased the frequency of MNPCEs and MNNCEs, also there is a significant decrease in P/N ratio, compared to the corresponding positive control group treated with cyclophosphamide. The results are given respectively in Tables 3, 4 and 5.

The chemicals which are damage the genetic material either by inducing chromosomal abnormalities (like numerical and structural abnormalities) and/or by damaging the DNA sequencing, leading to mutation are called clastogens. The mutation in somatic cells leads to pathological conditions such as carcinogenesis [24,25]. The clastogenic potential of a drug like cyclophosphamide and another anticancer drug can be studied by using animal models like in vivo micronucleus test and chromosomal aberration test for the evaluation of the antimutagenic (clastogenic) effect of test drugs.

Reactive oxygen spices (ROS) are the byproducts of normal cell metabolism during electron-transport processes, such as mitochondrial respiration and metabolism of xenobiotics by the microsomal system. ROS can also be generated as the main products of membrane-bound NADPH oxidase in phagocytes. Under normal physiological conditions, when the functioning of antioxidant systems is adequate, ROS is probably of low hazard for an organism. However, there is an excessive increase in ROS generation under the influence of some exogenous or endogenous factors, and also when there is an insufficiency of antioxidant systems, which can result in development of oxidative stress, Oxidative stress is responsible for disturbance of the stable equilibrium between pro-oxidant and antioxidant processes in a direction where pro-oxidant processes prevail. This disturbance leads to various types of damage at the molecular and cellular level in such cases antioxidants can act as stabilizers of homeostasis [26,27]. It is well known that antioxidants are almost universal antimutagenic agents. A reason for this effect is the genotoxicity of reactive oxygen species [28,29].

In the present study, ALBV showed significant antioxidant activity in all the experimental antioxidant models. However, the ALBV was found to possess more potent antioxidant activity maybe because it's high polyphenolic content. Based on the percentage of polyphenolic content and antioxidant potential, the ALBV was selected for evaluating the antimutagenic potential.

Table 2. IC50 value of different antioxidant activity µg/ml

Antioxidant determining method	IC50 value of ascorbic acid (µg/ml) ± S.D	IC50 Value of curcumin (µg/ml) ± S.D	IC50 value of extract ALBV (µg/ml) ± S.D
Reducing Power by $FeCl_3$	15.54±1.28	----------	55.27±2.57
Nitric Oxide scavenging	----------	45.37±5.05	125.52±8.15

Value are mean ± S.D.; n=3

Table 3. Antimutagenic effect of ALBV after 24 h of the clastogenic challenge

Sr. no.	Group	% MNPCE	% MNNCE	P/N ratio
1	Normal control	1.321±0.028	1.921±0.042	0.791±0.005
2	Vehicle control (acacia 2% in water)	1.474±0.035	1.879±0.038	0.784±0.024
3	ALBV (200 mg/kg, p.o.)	1.437±0.023	1.864±0.041	0.771±0.023
4	ALBV (400 mg/kg, p.o.)	1.395±0.030	1.723±0.039	0.809±0.028
5	CP (75 mg/kg, i.p.)	5.536±0.024*	11.527±0.059*	0.480±0.138*
6	ALBV (200 mg/kg, p.o.) + CP (75 mg/kg, i.p.)	2.613±0.020[a]	5.826±0.029[a]	0.448±0.125[b]
7	ALBV (400 mg/kg, p.o.) + CP (75 mg/kg, i.p.)	2.328±0.031[a]	6.871±0.032[a]	0.338±0.136[b]

*All the values are expressed as Mean ± SEM, n = 6. *P< 0.001 vs normal control group, [a]P< 0.001, [b]P<0.05 vs cyclophosphamide after 24 hours of clastogenic challenge*

Table 4. Antimutagenic effect of ALBV after 48 h of the clastogenic challenge

Sr. no.	Group	% MNPCE	% MNNCE	P/N Ratio
1	Normal control	1.521±0.028	1.921±0.042	0.791±0.005
2	Vehicle control (acacia 2% in water)	1.474±0.035	1.879±0.038	0.784±0.024
3	ALBV (200 mg/kg, p.o.)	1.437±0.023	1.864±0.041	0.771±0.023
4	ALBV (400 mg/kg, p.o.)	1.395±0.030	1.723±0.039	0.809±0.028
5	CP (75 mg/kg, i.p.)	9.354±0.170*	12.216±0.064*	0.765±0.029ns
6	ALBV (200 mg/kg, p.o.) + CP (75 mg/kg, i.p.)	3.293±0.137a	6.815±0.045a	0.483±0.053a
7	ALBV (400 mg/kg, p.o.) + CP (75 mg/kg, i.p.)	2.672±0.032a	5.624±0.173a	0.475±0.125a

*All the values are expressed as Mean ± SEM, n = 6. *P< 0.001 vs normal control group, aP< 0.001, bP<0.05 vs cyclophosphamide after 48 hours of clastogenic challenge. n-Non Significant*

Table 5. Antimutagenic effect of ALBV after 72 h of the clastogenic challenge

Sr. no.	Group	% MNPCE	% MNNCE	P/N Ratio
1	Normal control	1.521±0.028	1.921±0.042	0.791±0.005
2	Vehicle control (acacia 2% in water)	1.474±0.035	1.879±0.038	0.784±0.024
3	ALBV (200 mg/kg, p.o.)	1.437±0.023	1.864±0.041	0.771±0.023
4	ALBV (400 mg/kg, p.o.)	1.395±0.030	1.723±0.129	0.809±0.095
5	CP (75 mg/kg, i.p.)	10.863±0.095*	12.979±0.087*	0.836±0.072ns
6	ALBV (200 mg/kg, p.o.) + CP (75 mg/kg, i.p.)	4.152±0.038a	5.748±0.022a	0.722±0.034b
7	ALBV (400 mg/kg, p.o.) + CP (75 mg/kg, i.p.)	3.175±0.024a	4.863±0.027a	0.652±0.048a

*All the values are expressed as Mean ± SEM, n = 6. *P< 0.001 vs normal control group, aP< 0.001, bP<0.05 vs cyclophosphamide after 72 hours of clastogenic challenge. Ns-Non Significant*

The antimutagenic potential of ALBV was determined by cyclophosphamide-induced mutagenicity. Cyclophosphamide is a well known anti-cancer agent belongs to the class of alkylating agents, it undergoes biotransformation to give active metabolites namely aldophosphamide and phosphoramide mustard; the initial metabolic step is activated by CYP2B6 (and, to a much lower extent by CYP3A4) and involves hydroxylation of the oxazaphosphorine ring to generate carbinolamine [30]. The carbinolamine undergoes nonenzymatic hydrolysis to give aldophosphamide either in the bloodstream or inside the cell, inside the cell, the aldophosphamide will undergo decomposition into well-known mutagens namely acrolein and phosphoramide mustard [31]. Lethal adverse effects associated with cyclophosphamide usage include immunosuppression, nephrotoxicity, carcinogenicity and mutagenicity, through its metabolites either by depleting the antioxidant levels, acting as pro-oxidant or/and by directly damaging the DNA of the host.

The mutagenicity of cyclophosphamide is associated with the formation of the ultimate cytotoxic metabolite phosphoramide mustard through the intermediate agents hydroxycyclophosphamide and des-chloroethyl cyclophosphamide, which is capable of inducing DNA crosslinks and strand lesions [31].

The antimutagenic effect of ALBV observed on micronucleus test was highly significant at 200 and 400 mg/kg, p.o. but there was no significant difference between 200 and 400 mg/kg in their antimutagenic activity. The possible mechanism of action might be associated with detoxification of the toxic metabolites and/or scavenging of superoxide free radicals and/ or by altering the activation and detoxification of xenobiotics.

4. CONCLUSION

Therefore, from the present study, it is concluded that alcoholic extract of *Bauhinia variegata* root (ALBV) can prove to be a very good antioxidant and effective chemopreventive against cyclophosphamide-induced mutagenesis.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The experimental protocols were approved by the Institutional Animal Ethical Committee (IAEC)

of C.U. Shah College of Pharmacy and Research, Surendranagar (Gujarat) and were conducted in strict compliance according to ethical principles and guidelines provided by the Committee for Control and Supervision of Experiments on Animals (CPCSEA).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Viswanatha GLS, Vaidya SK, Ramesh C, Krishnadas N, Rangappa S. Antioxidant and antimutagenic activities of bark extract of *Terminalia arjuna*. Asian Pacific J. of Tropical Med. 2010;12:965-970.
2. Mishra A, Kumar S, Bhargava A, Sharma B, Pandey AK. Studies on *in vitro* antioxidant and antistaphylococcal activities of some important medicinal plants. Cell Mol. Biol. 2011;57:16–25.
3. Vaidya SK, Bothara SB. Total Polyphenolic content and *in-vitro* antioxidant potential of extracts of creeping herb *Ipomoea reniformis* (Roxb.) Choisy. American J. of Phytomed and Clin. Therapeutics. 2014; 12:1462-1469.
4. Aminetzach YT, McPherson JM, Petrov DA. Pesticide resistance via transposition-mediated adaptive gene truncation in Drosophila. Science. 2005;309:764-7.
5. Burrus V, Waldor MK. Shaping bacterial genomes with integrative and conjugative elements. Res. Microbiol. 2004;155:376-86.
6. Naasani I, Seimiya H, Tsuruo T. Telomerase inhibition, telomere shortening, and senescence of cancer cells by tea catechins. Biochem. Biophys. Res. Commun. 1998;249:391-396.
7. Kumar S, Chashoo G, Saxena AK, Pandey AK. *Parthenium hysterophorus*: A probable source of anticancer, antioxidant and anti-HIV agents. Bio. Med. Res. Int. 2013;8: 107-34.
8. Golwala DK, Patel LD. Pharmacognostical studies of *Bauhinia variegata* root. Journal of Young Pharmacist 2009;1:36-41.
9. Ali MS, Azhar I, Amtul Z, Ahmada K, Usmanghani B. Antimicrobial screening of Caesalpiniaceae. Fitoterapia. 1999;70: 299-304.
10. Gupta AK, Vidyapati TJ, Chauhan JS. 5, 7-dihydroxyflavonone-4-O-α-L-rhanmopyranosyl-β–D–glucopyranoside from the stem of *Bauhinia variegata*. Indian J of Chem. 1979;18:85-86.
11. Gupta AK, Vidyapati TJ, Chauhan JS. Chemical examination of stem of *Bauhinia variegata*. Planta Medica. 1980;38:174-176.
12. Yadava RN, Reddy VMS. A new flavone glycoside 5,7,3´,4´-tetrahydroxy-3-methoxy-7-O-α-L-rhamnopyranosyl(1→3)-O-β-D-galactopyranoside from *Bauhinia variegate* Linn. J of Asian Nat Product Res. 2001;3:341-346.
13. Duret S, Paris RR. The flavonoid of several species of Bauhinia. Plant Medicinal Phytotherapy. 1977;11:213-216.
14. Rangari VD. Pharmacognosy & Phytochemistry. 8th Ed. Nashik: Career Publication. 2008; 130-134.
15. Harbone JR. Phytochemical Methods: A guide to modern techniques of plant analysis. Science Paperbacks. 1998;4:4-8.
16. Golwala DK, Patel LD. Pharmacognostical studies of *Bauhinia variegata* Linn. Stem. International J. of Pharmaceutical Res. 2012;3:127-130.
17. Vaidya SK, Bothara SB. Total polyphenolic content and *in-vitro* antioxidant potential of extracts of creeping herb *Ipomoea reniformis (Roxb.*) Choisy. American J. of Phytomed and Clin. Therapeutics. 2014; 12:1462-1469.
18. Nayan R, Bhalodia, Pankaj B, Nariya R, Acharya N, Shukla VJ. *In vitro* antioxidant activity of hydro alcoholic extract from the fruit pulp of *Cassia fistula* Linn. Ayu. 2013;34:209–214.
19. Boora F, Chirisa E, Mukanganyama S. Evaluation of nitrite radical scavenging properties of selected Zimbabwean plant extracts and their phytoconstituents. J of Food Processing. 2014;1-7.
20. Makoto H, James TM, David GG, David HB. *In vivo* rodent erythrocyte micronucleus assay. Mut. Res. Genet. Toxicol. Environ. Mutagen. 2007;1:10-30.
21. Borroto JIG, Creus A, Marcos R, Molla R, Zepatero J. The mutagenic potential of the furylethylene derivative 2-furyl-1-nitroethene in the mouse bone marrow micronucleus test. Toxic Sci. 2003;72: 359-362.
22. Gerald W. Free radicals can kill you: Lavoisier's oxygen revolution. FASEB. 2010;24:649-652.
23. Predrag L, Omar AS, Arieh B. Ursodeoxycholic acid and superoxide

anion. World J Gastroenterol. 2005;31: 4875-4878.
24. Olinski R, Gackowski D, Foksinski M, Rozalski R. Oxidative DNA damage: assessment of the role in carcinogenesis, atherosclerosis and acquired immune-deficiency syndrome. Free Radical Bio. Med. 2002;9:192-200.
25. Smith MA, Perry G, Pryor WA. Causes and consequences of oxidative stress in Alzheimer's disease. Free Radic. Biol. Med. 2002;32:1049-1056.
26. Tetsuya M, Hiroshi K, Tatsuhiko K, Shoji T. Leanness, smoking, and enhanced oxidative DNA damage. Cancer Epidemiol. Biomarkers Prev. 2006;3:582-585.
27. Jostein D, Egil K, Trond S. Bystander effects in UV-induced genomic instability: Antioxidants inhibit delayed mutagenesis induced by ultraviolet A and B radiation. J Carcinogenesis. 2005;11:1-9.
28. Khan PK, Sinha SP. Antimutagenic profile of antioxidant vitamins in drosophila mutation test. Biomed Env Sci. 2008;21: 163-6.
29. Demetrios S, Spyridon O, Demetrios K. Plant phenolics protect from bleomycin-induced oxidative stress and mutagenicity in *Salmonella typhimurium* TA102. Anticancer Res. 2004;2:743-746.
30. Huang A, Roy P, Waxman DJ. Role of human liver microsomal CYP3A4 and CYP2B6 in catalyzing N-dechloroethylation of cyclophosphamide and ifosfamide. Biochem Pharmacol. 2000;59:961-972.
31. Victoria FR Thoma LL, David AW. Cancer and chemotherapy. Foye's Principles of Medicinal Chemistry. 6th Ed. Wolters Kluwer Pvt Ltd., New Delhi. 2008;1157-1159.

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