Anti-oxidant andanti-hemolytic activity of Buchananialanzan Sprengleaf and stem bark extract

Bhushan Muley*[1], Pankaj Masih[1], Neeraj Sharma[1]

[1] Patel College of Pharmacy, MPU, Bhopal.

*Corresponding Author: -

Bhushan Muley

Research Scholar

Patel College of Pharmacy

Madhyanchal Professional University, Bhopal, M.P.

E-mail : - muley.b@gmail.com

Abstract:

Reactive oxygen species (ROS) have been found to play an important role in the initiation and/or progression of various diseases, such as atherosclerosis, inflammatory injury, cancer, and cardiovascular disease. Antioxidants can protect the human body from damage caused by ROS and the concomitant lipid peroxidation, protein damage, and DNA strand breaking. Antioxidant and antihemolytic activities of various extract of BuchananialanzanSpreng leaf, and stem bark extract were investigated employing different in vitro assay systems. Ethanol extracts showed good antioxidant activity. Extracts exhibited good antioxidant activity in the haemoglobin-induced and also they were capable of scavenging hydrogen peroxide in a concentration dependent manner. Ethanol extracts showed good antihemolytic activity than other extract against hydrogen peroxide-induced hemolysis.

Keywords: Antioxidant, anti-hemolytic, BuchananialanzanSpreng bark and BuchananialanzanSpreng leaf and Petroleum ether, Ethyl acetate, Ethanol and Aqueous extract

Introduction:

Medicinal plants are integral and indispensable part of the traditional system of medicine practiced. Worldwide because of their economical viability, easy accessibility and centuries old experience. As Natures gift, these are considered to be biocompatible, environment friendly, non-toxic, much cheaper and quite freely available in comparison to synthetic substances [1].Reactive oxygen species (ROS) have been found to play an important role in the initiation and/or progression of various diseases, such as atherosclerosis, inflammatory injury, cancer, and cardiovascular disease.[2] Antioxidants can protect the human body from damage caused by ROS and the concomitant lipid peroxidation, protein damage, and DNA strand breaking[3] and, thus, can prevent the above-mentioned diseases. The human body has multiple mechanism antioxidant systems to protect against ROS-induced damage [4].It is not enough for protection against oxidative stress induced by ROS. Therefore, certain amounts of exogenous antioxidants are constantly required to maintain an adequate level of

antioxidants in order to balance the ROS in the human body. On the other hand, many side effects of synthetic antioxidants, such as butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT), have been reported [5]. BuchananialanzanSpreng, commonly known as char, achar and chironji, belongs to family Anacardiaceae. It was first described by Francis Hamilton in 1798. The tree is natural wild growth in the tropical deciduous forests of Northern, Western and Central India, mostly in the States of Chhattisgarh, Jharkhand, Madhya Pradesh and in Varanasi and Mirzapur districts of Uttar Pradesh. Besides India, the plant is also found in other tropical Asian countries, Australia and Pacific islands [6-10].Traditional indigenous knowledge reveals the immense value of almost all parts of the plant like roots, leaves, fruits, seeds and gum for various medicinal uses. The roots are acrid, astringent, cooling, depurative and constipating and are useful in treatment of diarrhoea. Extract of the root is also used as an expectorant and for curing biliousness and blood diseases. The leaf juice is used as expectorant, aphrodisiac, purgative, blood purifier, thirst-quencher and cures digestive disorders. It contains 2.64% tannins (0.35% gallo-tannins), triterpenoids, saponins, flavonoids and reducing sugars. Powdered or crushed leaves are applied to wounds. The chironji seeds/kernels are nutritional, palatable and used as a substitute for almonds in confectionery [11, 12].Tribal people of Jharkhand and Chhattisgarh are using BuchananialanzanSpreng. (Family:-Anacardiaceae) mainly for wound healing, antidiarrheal, analgesic and antiulcer. Buchananialanzanis a popular herb used for curing many diseases. Anti-intrinsic haemorrhage, blood-borne diarrhoea and tonic are established [13, 14]. The proposed work was to evaluate Anti-oxidant and anti-hemolytic activity of BuchananialanzanSpreng leaf, and stem bark extract.

Material and methods:

The leaves of BuchananialanzanSpreng were collected from the Chhattisgarh in the month of April. The plant was identified and authenticated from Saifia Science College, Bhopal (S. No. 166/sfi/bpl). Fresh leaves and stems bark of BuchananialanzanSpreng were used for Pharmacognostical studies. The leaves and stems were separated and dried under shade. Leaves and stems bark were powdered to 60# separately and stored in airtight containers and used for phytochemical and pharmacological studies.

Extraction process of plant: BuchananialanzanSpreng leaf, and stem bark extracted sequentially with petroleum ether, ethyl acetate and ethanol using continuous hot extraction method i.e. Soxhlet extraction. The completely dried bark of BuchananialanzanSpreng was coarsely powdered and then defatted with non-polar solvent petroleum ether, then extracted with ethyl acetate and ethanol. The marc was finally macerated with water for 24h to obtain the aqueous extract. The same successive solvent extraction procedure was followed for stem bark of BuchananialanzanSpreng to get petroleum ether, ethyl acetate, ethanol and aqueous extract.

Anti-oxidant Activity

Chemicals and plant extract: 1,1-diphenyl-2-picryl hydrazyl (DPPH), DMSO, Ascorbic acid Sodium nitro prusside, Griess reagent (1%w/v sulphanilamide, 2% w/v H3PO4 and N-(1-naphthyl) ethylene diamine di hydrochloride), Hydrogen peroxide

(H_2O_2) Thiobarbituric acid Sodium phosphate buffer, 1mM ferric chloride Nitro blue tetrazolium, Phenazenemethosulphate, Nicotinamide adenine phosphate di nucleotide

EABB: Ethyl acetate extract of Buchananialanzan bark (100-500µg/ml)
EOBB: Ethanol extract of Buchananialanzan bark (100-500µg/ml)
AQBB: Aqueous extract of Buchananialanzan bark (100-500µg/ml)
EABL: Ethyl acetate extract of Buchananialanzan leaves (100-500µg/ml)
EOBL: Ethanol extract Buchananialanzan leaves (100-500µg/ml)
AQBL: Aqueous extract of Buchananialanzan leaves (100-500µg/ml)

Procedure: For the assessment of free radical scavenging activity, the extracts of selected plants Ethyl acetate and Ethanol and aqueous extract extracts of Buchananialanzan bark and leaves were dissolved in 5% DMSO. DPPH, Nitric oxide, hydroxyl radical, superoxide radical methods were carried in the present study.

Determination of DPPH radical scavenging activity of Buchananialanzan bark and leaves extracts: The free radical scavenging activity of the Ethyl acetate and Ethanol and aqueous extracts of Buchananialanzan bark and leaves extracts were evaluated using 1,1 diphenyl-2- picrylhydrazyl (DPPH), by the method**.** In its radical form, DPPH absorbs at 517nm, but upon reduction by an antioxidant or a radical species, the absorption decreases.1ml of 0.25mM solution of DPPH in DMSO was added to the different concentrations of selected plant extracts which were dissolved in DMSO (100- 500µg/ml). After 30 min, the absorbance was measured at 517nm by UV-Visible spectrophotometer (shimadzu UV-Vis1800). All the test analysis were run in triplicate and averaged. Lower absorbance of reaction mixture indicates higher free radical scavenging activity. Ascorbic was used as a positive control. Ascorbic acid was used as a standard. The percentage DPPH decolonization of the sample was calculated by the equation:

Percentage of DPPH scavenging = [(A0–A1)/A0] X 100,
A0 = Absorbance of the control, and
A1=Absorbance of the extract/ standard.

Determination of Nitric Oxide (NO) radical scavenging activity Buchananialanzan bark and leaves extracts: Sodium nitro prusside (SNP) (1ml of mM) was mixed with 1ml of selected plants extracts in different concentrations (100µg/ml-500µg/ml) in (Di methyl sulphoxide) DMSO. The mixture was incubated at 25oc for 180 minutes. To 1ml of the incubated solution, 1ml of griess reagent was added. The absorbance of the chromophores formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with N-(1-naphtyl) ethylene diaminedihydrochloride was read at 546nm by UV-Visible spectrophotometer (Shimadzu UV-Vis1800). All the test analysis were run in triplicate and averaged. Lower absorbance of reaction mixture indicates higher free radical scavenging activity. Ascorbic was used as a positive control. The percentage inhibition was calculated by

Percentage of Nitric oxide scavenged = [(A0–A1)/A0] X 100,
Where A0 = Absorbance of the control, and
A1 =Absorbance of the extract/ standard.

Determination of hydroxyl radical scavenging activity Buchananialanzan bark and leaves extracts: Hydroxyl radical scavenging activity was determined based on the ability to compete with deoxyribose for hydroxyl radical. Hydroxyl radical produced by the reduction of H2O2 by iron in the presence of ascorbic acid degrade deoxyribose to form products, which on heating with 2-thiobarbuturic acid (TBA) for a pink colouredchromogen. The reaction mixture, of a final volume of 1ml, containing 0.4ml of 20mM sodium phosphate buffer (pH 7.4), 0.1ml of 100-500 µg/ml of selected plant extracts in DMSO, 0.1ml of 60mM deoxyribose, 0.1 ml of 10mM H2O2, 0.1 ml of 1mM ferric chloride, 0.1 ml of 1Mm Ethylene diamine tetra acetic acid (EDTA) and 0.1ml of 2mM 1-ascorbic acid, was incubated at 370 C for 1 h. The reaction was terminated by the addition of 1 ml of 17mM TBA and 1ml of 17Mm trichloro acetic acid (TCA) .The mixture was boiled for 15 min, cooled in ice and the absorbance was measured at 532nm by UV-Visible spectrophotometer (Shimadzu UV-Vis 1800). All the test analysis were run in triplicate and averaged. L-Ascorbic acid was used as a positive control. The hydroxyl radical scavenging of the extract was reported as the percentage of inhibition of the deoxyribose degradation and was calculated according to the following equation

Calculation of % Inhibition:

% Inhibition = [(A0-AT)/A0]*100

Where A0 = Absorbance of the control, and

AT =Absorbance of the extract/ standard.

Determination of super oxide radical scavenging activity of Buchananialanzan bark and leaves extracts: Super oxide anion derived from dissolved oxygen by a PMS-NADH coupling reaction reduces nitrobluetetrazolium (NBT), which forms a violet coloured complex. A decrease in colour after addition of the antioxidant is a measure of its super oxide scavenging agent14. The experiment, the super oxide radicals were generated in 3 ml of Tris-HCL buffer(16Mm pH 8) containing 1 ml of NBT (50 µM), 1 ml NADH (78 µM) and test solution of selected plant extracts ,(100-500 µg/ml) to the above reaction mixture, 1 ml PMS solution(10 µ M) was added and incubated at 250c for 5 min . The absorbance was read at 560nm (Shimadzu UV-Vis 1800) against blank sample. Decrease in absorbance of the reaction mixture incubated increases super oxide anion scavenging activity. All the test analysis were run in triplicate and averaged. L-ascorbic acid was used as a positive control.

The % inhibition of super oxide anion generated was calculated using the following equation

% inhibition = [(A0-AT)/A0]*100

Where A0 = Absorbance of the control, and

AT =Absorbance of test extract/ standard.

Determination of Anti-hemolytic effects of Buchananialanzan bark and leaves extracts:

Red blood cell suspension Anti hemolytic activity of the Buchananialanzan bark and leaves extracts were assessed as described by Sumathi et al., (2013) with a slight modification. Erythrocytes from male wistar rat blood were separated by centrifugation

and washed with phosphate buffer (pH 7.4), and diluted with phosphate buffered saline to give 4% suspension. 1 g of extract/ml of saline buffer was added to 2 ml of the erythrocyte suspension and the volume made up to 5 ml with saline buffer. After incubation the mixture at 370C for 5min and then 0.5 ml of H_2O_2 solution in saline buffer added to induce oxidative degradation of the membrane lipids.

Assay for hemolysis: The concentration of H_2O_2 in the reaction mixture was adjusted to bring about 90% hemolysis of the blood cells after 240 min. After incubation the reaction mixture was centrifuged at 1500 rpm for 10 min and the extent of hemolysis was determined by measuring the absorbance, corresponding to haemoglobin liberation, at 540 nm.The reaction without extract was used as control sample. % Inhibition = 100 × (1 – ODsample) / ODcontrol

Result and discussion:

Anti-oxidant Activity: Analysis of the free radical scavenging activities of the selected Buchananialanzan bark and leaves extractsrevealed a concentration dependent free radical scavenging activity resulting from reduction of DPPH, NO, Hydroxyl radical and superoxide radical to non-radical form. The scavenging activity of Ascorbic acid, a known antioxidant used as positive control, was however higher.

In case of Buchananialanzan bark the order of reduction potential was: Ascorbic acid> EOBB> EABB > AQBB. Even in case of Buchananialanzan bark and leaves extracts the reduction potential was in the same order as: Ascorbic acid> EOBL> EABL > AQBL

The ethanol extract of Buchananialanzan bark at varied concentrations showed remarkable inhibitory effect of nitric oxide radical scavenging activity compared to other extract. Results revealed that all the tested extracts showed the percentage of inhibition in a dose dependent manner. The ethanol extract of Buchananialanzan leaves at varied concentrations showed remarkable inhibitory effect of nitric oxide radical scavenging activity compared to other extract. The ethanol extract of Buchananialanzan bark at varied concentrations showed remarkable inhibitory effect of nitric oxide radical scavenging activity compared to other extract. The ethanol extract of Buchananialanzan bark showed more activity than ethanol extract of Buchananialanzan leaves

The hydroxyl radical is an extremely reactive free radical formed in biological systems and has been implicated as a highly damaging species in free radical pathology, capable of damaging almost every molecule found in living cells. The ethanol extract of Buchananialanzan bark at varied concentrations showed remarkable inhibitory effect of Hydroxyl radical scavenging activity compared to ethyl acetate and aqueous extract. The ethanol extract of Buchananialanzan leaves at varied concentrations showed remarkable inhibitory effect of Hydroxyl radical scavenging activity compared to ethyl acetate and aqueous extract.. The ethanol extract of Buchananialanzan bark showed more inhibitory effect of Hydroxyl radical scavenging activity compared ethanol extract of Buchananialanzan bark.

Superoxide is a reactive oxygen species, which can cause damage to the cells and DNA leading to various diseases. The reduction of NBT in presence of antioxidants was measured. The decrease of absorbance at 560 nm with antioxidants thus indicates the

consumption of superoxide anion in the reaction mixture.The ethanol extract of Buchananialanzan bark at varied concentrations showed remarkable inhibitory effect of superoxide radical activity scavenging compared to ethyl acetate and aqueous extract. The ethanol extract of Buchananialanzan leaves at varied concentrations showed remarkable inhibitory effect of superoxide radical scavenging activity compared to ethyl acetate and aqueous extract. The ethanol extract of Buchananialanzan bark showed more inhibitory effect of superoxide radical scavenging activity compared ethanol extract of Buchananialanzan leaves.

Antihaemolytic activity of Buchananialanzan bark and leaves extracts: Anti Haemolytic activity of Buchananialanzan bark and leaves extracts was screened against male Wistar rat erythrocytes. The haemolysis induced by extracts in red blood cells was concentration dependent. Extract exhibited differential pattern haemolytic effect towards male Wistar rat erythrocytes.

Result indicated that the EABB 50mg/ml of extract showed 61.54 % haemolytic activity. Whereas Buchananialanzan leaves ethanol extract showed more haemolyticactivity than ethyl acetate and aqueous extract. Same pattern were observed in case of Buchananialanzan bark extract. In case of Buchananialanzan bark ethanol extract of showed the highest haemolytic activity at 50mg/ml of 75.87% lysis of erythrocytes was found to be increased with an increase of extract concentration. The ethanol extract of bark and leaves showed higher haemolytic effect on wistar rat red blood cell in all concentrations

The percentage haemolysis and IC50 values were calculated. The extracts were potent against haemolysis of the erythrocyte in concentration dependent manner. The ethanol extract of bark exhibited the highest antihaemolytic effect with IC50 of 38 mg/ml followed by ethyl acetate and the least was aqueous extract haemolysis of the erythrocyte. The IC50 value of ascorbic acid was 19.12mg/ml. Ethanol extract of bark was found more effective against the lower the IC50 the more protection offered against haemolysis by the extracts.

Conclusion:

Analysis of the free radical scavenging activities (Anti-oxidant Activity)of the selected Buchananialanzan bark and leaves extractsrevealed a concentration dependent free radical scavenging activity resulting from reduction of DPPH, NO, Hydroxyl radical and superoxide radical to non-radical form. In case of Buchananialanzan bark the order of reduction potential of ethanol extract of Buchananialanzan bark and leaves extracts the reduction potential was found higher than other extract. The ethanol extract of Buchananialanzan bark at varied concentrations showed remarkable inhibitory effect of nitric oxide radical scavenging activity compared to other extract. Results revealed that all the tested extracts showed the percentage of inhibition in a dose dependent manner. The ethanol extract of Buchananialanzan leaves at varied concentrations showed remarkable inhibitory effect of nitric oxide radical scavenging activity compared to other extract. The haemolysis induced by extracts in red blood cells was concentration dependent. Extract exhibited differential pattern haemolytic effect towards male Wistar rat erythrocytes. Result indicated that the EABB 50mg/ml of extract showed 61.54 %

haemolytic activity. Whereas Buchananialanzan leaves ethanol extract showed more haemolytic activity than ethyl acetate and aqueous extract. Same pattern were observed in case of Buchananialanzan bark extract. In case of Buchananialanzan bark ethanol extract of showed the highest haemolytic activity at 50mg/ml of 75.87% lysis of erythrocytes was found to be increased with an increase of extract concentration. The ethanol extract of bark and leaves showed higher haemolytic effect on wistar rat red blood cell in all concentrations. The percentage haemolysis and IC50 values were calculated. The extracts were potent against haemolysis of the erythrocyte in concentration dependent manner. The ethanol extract of bark exhibited the highest antihaemolytic effect with IC50 of 38 mg/ml followed by ethyl acetate and the least was aqueous extract haemolysis of the erythrocyte. The IC50 value of ascorbic acid was 19.12mg/ml. Ethanol extract of bark was found more effective against the lower the IC50 the more protection offered against haemolysis by the extracts.

Polyphenolic compounds, such as flavonoids, are widely found in food products derived from plant sources, and they have been shown potent antioxidant and antihemolytic activities. DPPH is a stable nitrogen-centered free radical the color of which changes from violet to yellow upon reduction by either the process of hydrogen- or electron-donation. Substances that are able to perform this reaction can be considered as free radical scavengers and, therefore, antioxidants. Phenolic compounds of this plant seem to have direct roles for its good free radical scavenging activity.

Acknowledgement: -

The Author would like to thank HOD, Pharmacognosy department, Dean and Professor of MPU, Bhopal , M.P. India, for providing Laboratory facilities and permitting to carry out my research work.

References:

1. Kirtikar KR, Basu BD. Indian Medicinal Plants: Lalit Mohan Basu, 2nd ed., Vol. III, Allahabad, 1935.
2. Halliwell, B. 1994. Free radicals, antioxidants and human diseases; curiosity, cause or consequence?. Lancet, 334: 721–724.
3. Ghosal, S., Tripathi, V.K. and Chauhan, S. 1996. Active constituents of Emblicaofficinalis. Part I. The chemistry and antioxidant effects of two new hydrolysable tannins, emblicanin A and B. Indian Journal of Chemistry, 35B: 941–948.
4. Anderson, D. 1999. Antioxidant defences against reactive oxygen species causing genetic and other damage. Mutation Research, 350: 103–108.
5. Nabavi, S.F., Ebrahimzadeh, M.A., Nabavi, S.M. and Eslami, B. 2010. Antioxidant activity of flower, stem and leaf extracts of Ferula gummosaBoiss. Grasas Y Aceites, 61(3): 244–250.
6. Anon. Wealth of India, A dictionary of Indian raw materials and Indian products, B revised ed., Vol. 2: Publication and Information Directorate CSIR: New Delhi, 1952.

7. Pandey GP. Effects of gaseous hydrogen fluoride on the leaves of Terminaliatomentosa and Buchananialanzan trees. J Environ Pollution (Series A) 1985; 37:323-334.
8. Singh P et al. Preservation of BuchananialanzanSpreng. Seeds by Ocimumcanum Sims essential oil. Annals Plant ProtecSci 2011; 19(2):407-410.
9. 5. Sharma VK et al. Phosphate uptake and distribution of the seedling of Buchananialanzan. Tropical Ecol 1970; 11(2):231-232.
10. Choubey A et al. Some aspects of germination studies in BuchananialanzanSpreng. seeds. J Tropical Forestry 1997; 13(11):65- 73.
11. Shukla SK, Solanki KR. Studies on seed germination, plant survival and growth of chironjee (BuchananialanzanSpreng.). J Tropical Forestry 2000;16:44-49.
12. Sharma A. Scientific harvesting for quality seed collection of BuchananialanzanSpreng. for its conservation and sustainable management---- case study of Chhindwara, Madhya Pradesh, India. Int J Bio-Sci Bio-Technol 2012; 4(1):65-74.
13. Kumar J et al. Chironji nut (Buchananialanzan) processing, present practices and scope. Ind J Trad Knowledge 2012; 11(1):202- 204.
14. Malik SK et al. Genetic resources of chironji (BuchananialanzanSpreng.): a socio-economic important tree species of central Indian population. Genetic Resource Crop Evaluation 2012; 59:615-623.

Table 1: *In vitro* 50% inhibition concentration (IC_{50}) of Buchananialanzan bark and leavesextracts on DPPH, Nitric oxide, Hydroxyl and superoxide radical scavenging model

Extract /compound	50% inhibition concentration (IC_{50}) (µg/ml)			
	DPPH model	NO model	Hydroxyl radical model	superoxide model
Ascorbic acid	68	70	84	150
EABB	200	170	280	280
EOBB	79	95	170	210
AQBB	291	280	355	360
EABL	230	185	295	295
EOBL	85	100	180	225
AQBL	300	298	370	380

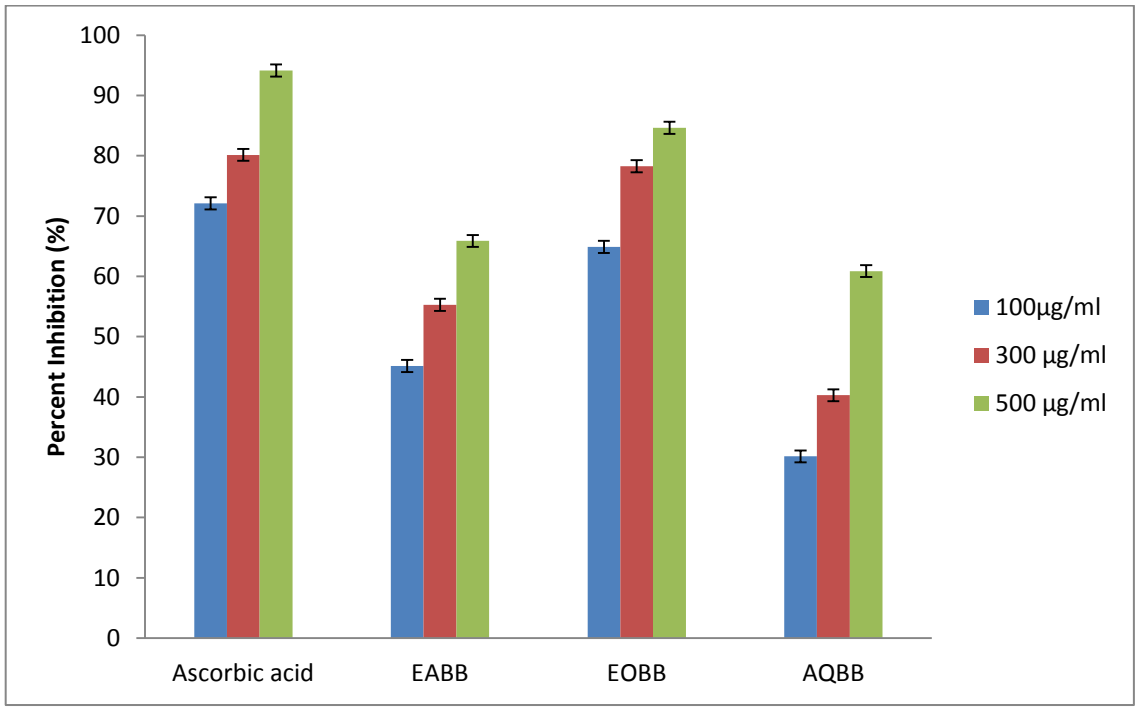

Figure 1: Graphical representation ofBuchananialanzan bark extracts on DPPH radical scavenging model

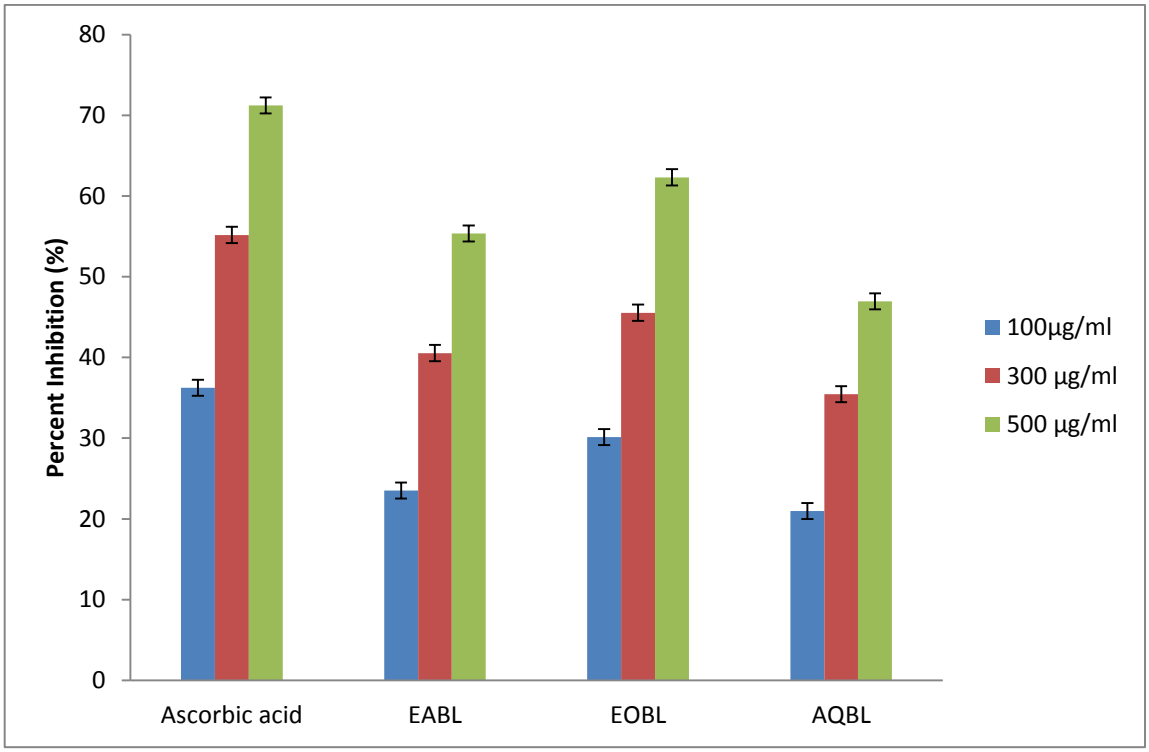

Figure 2: Graphical representation ofBuchananialanzan leavesextracts on DPPH radical scavenging model

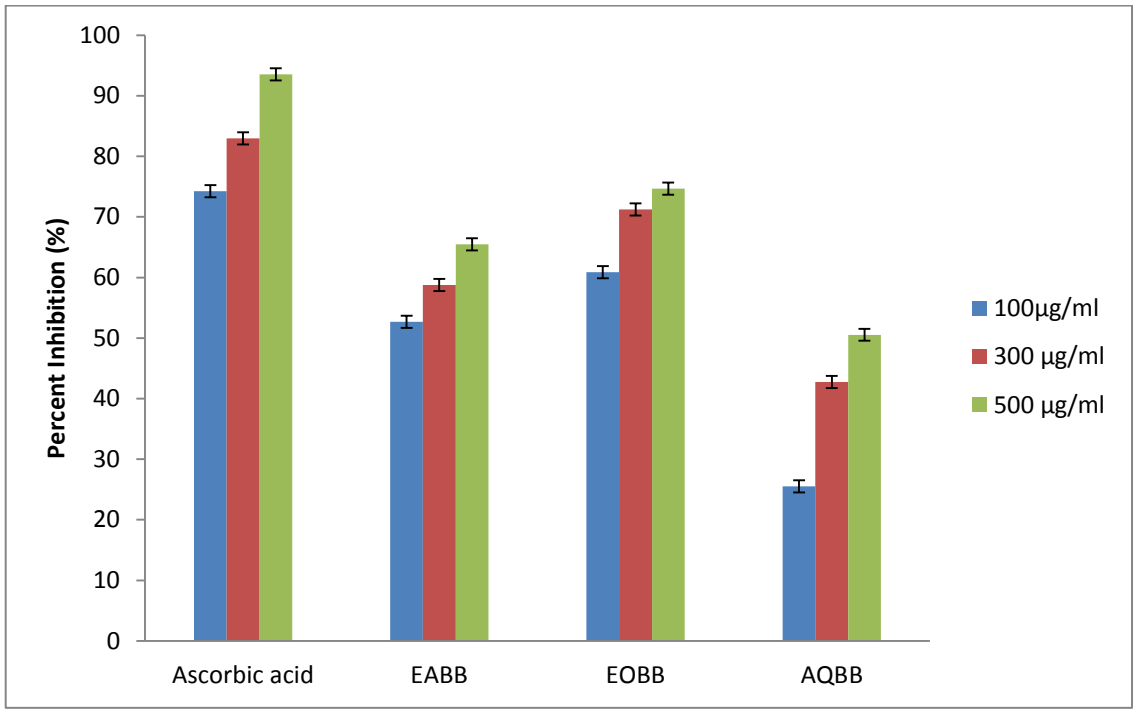

Figure 3: Results of Buchananialanzan bark extracts on Nitric oxide radical scavenging model

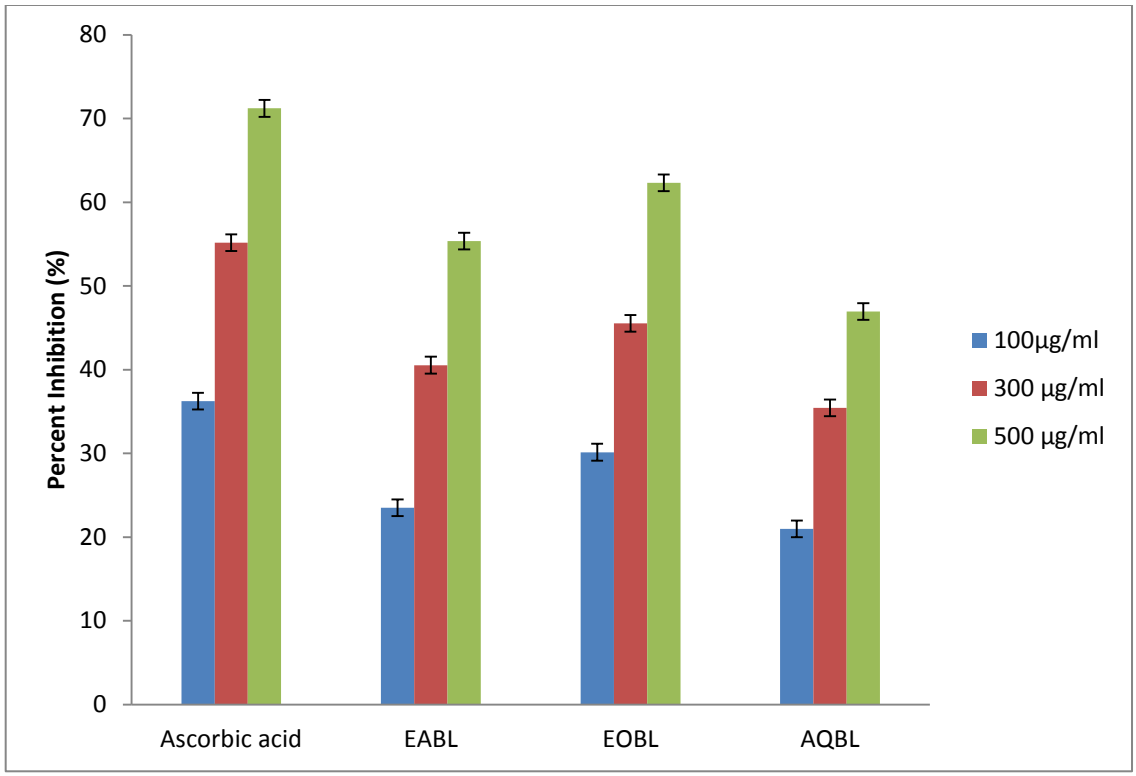

Figure 4: Results of Buchananialanzan leaves extracts on Nitric oxide radical scavenging model

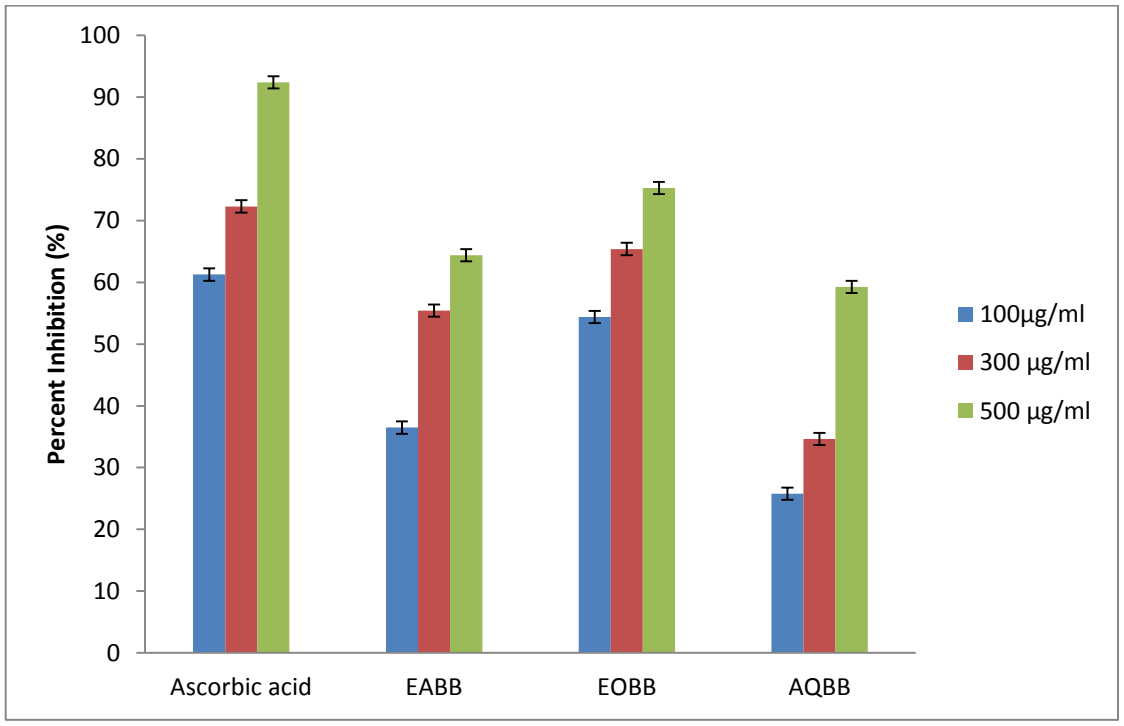

Figure 5: Results of Buchananialanzanbarkextracts on Hydroxyl radical scavenging model

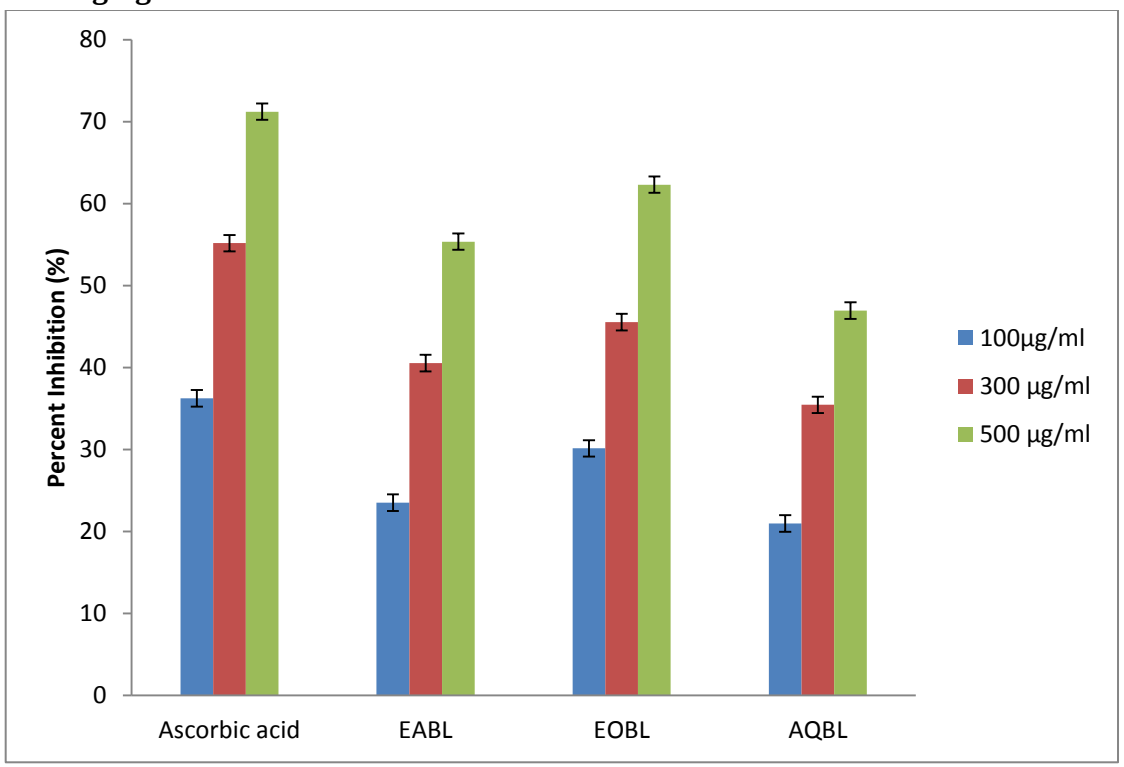

Figure 6: Results of Buchananialanzan leaves extracts on Hydroxyl radical scavenging model

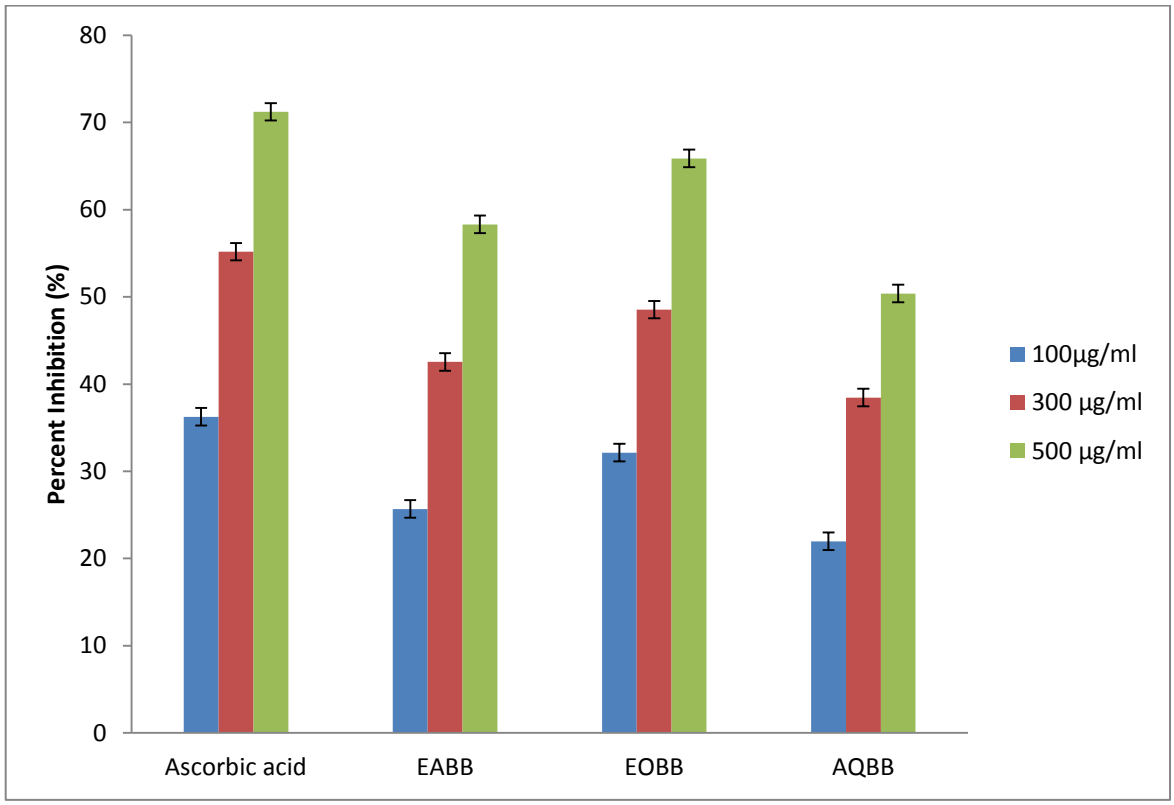

Figure 7: Results of Buchananialanzan bark extracts on Super oxide radical scavenging activity

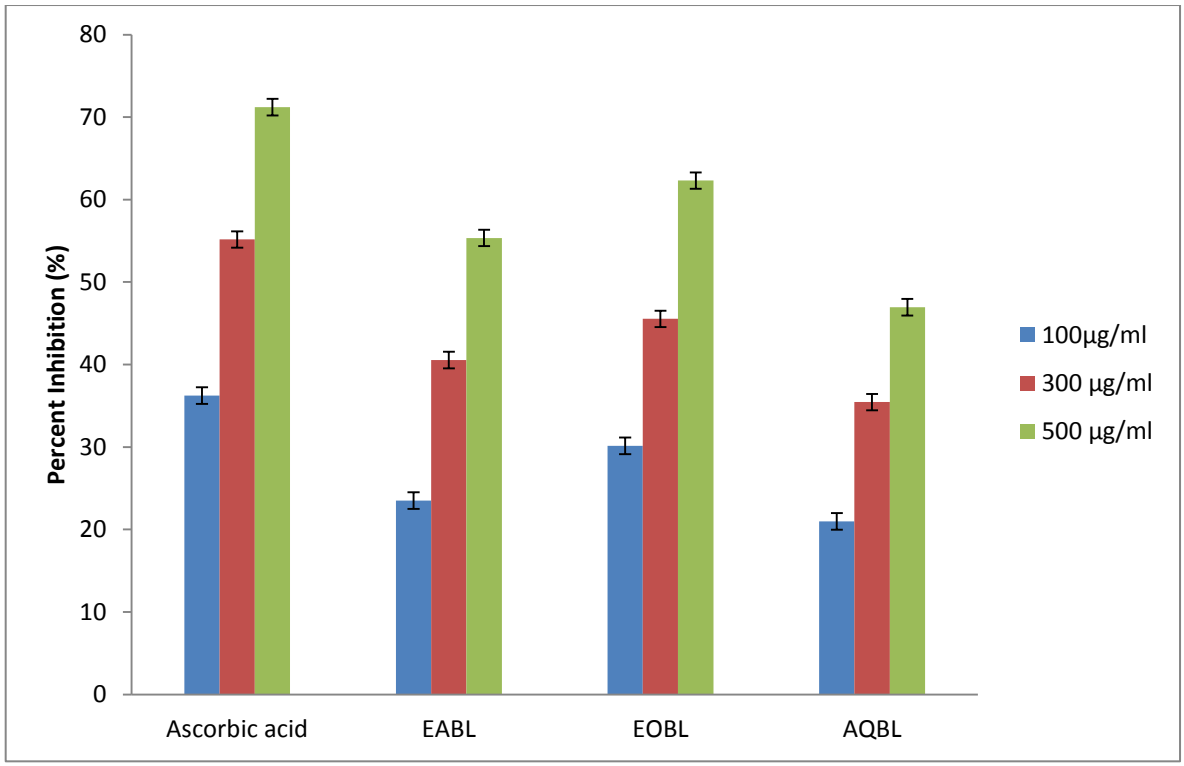

Figure 8: Results of Buchananialanzan leaves extracts on Super oxide radical scavenging activity

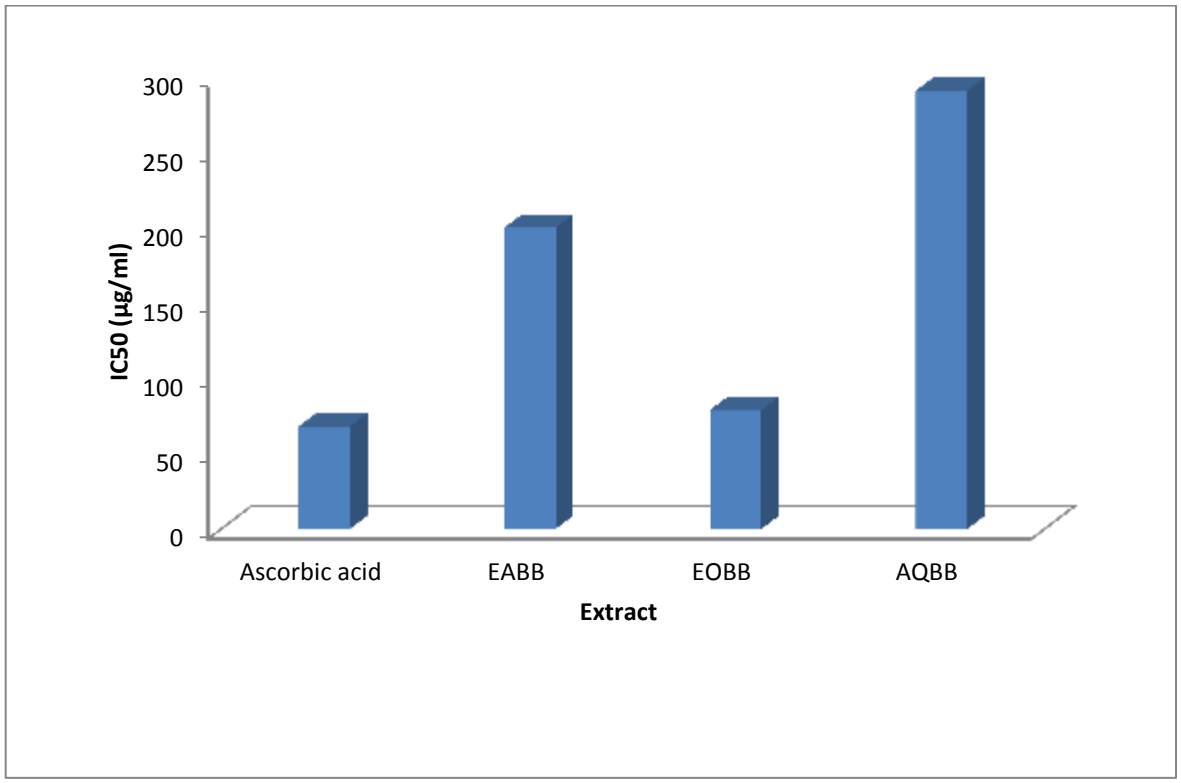

Figure 9:*In vitro* 50% inhibition concentration (IC_{50}) of Buchananialanzan bark extracts on DPPH radical scavenging model

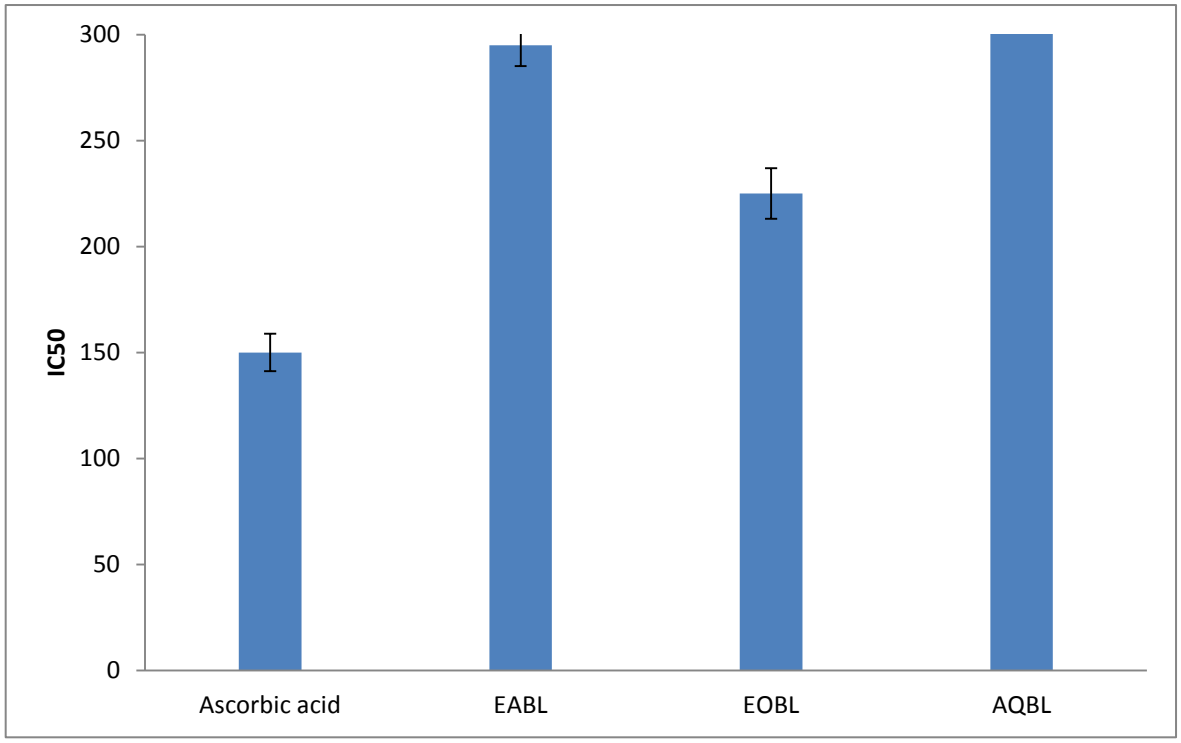

Figure 10:*In vitro* 50% inhibition concentration (IC_{50}) of Buchananialanzan leaves extracts on DPPH radical scavenging model

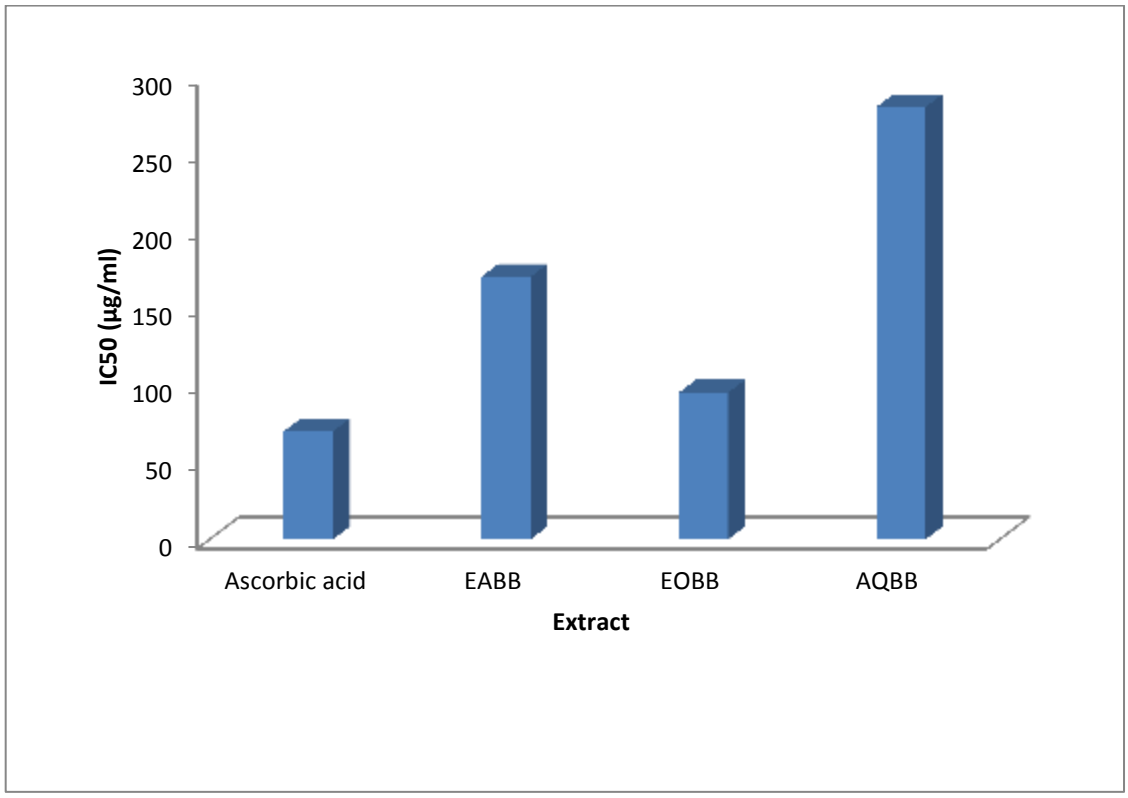

Figure 11:*In vitro* 50% inhibition concentration (IC_{50}) of Buchananialanzan bark extracts on Nitric oxide radical scavenging model

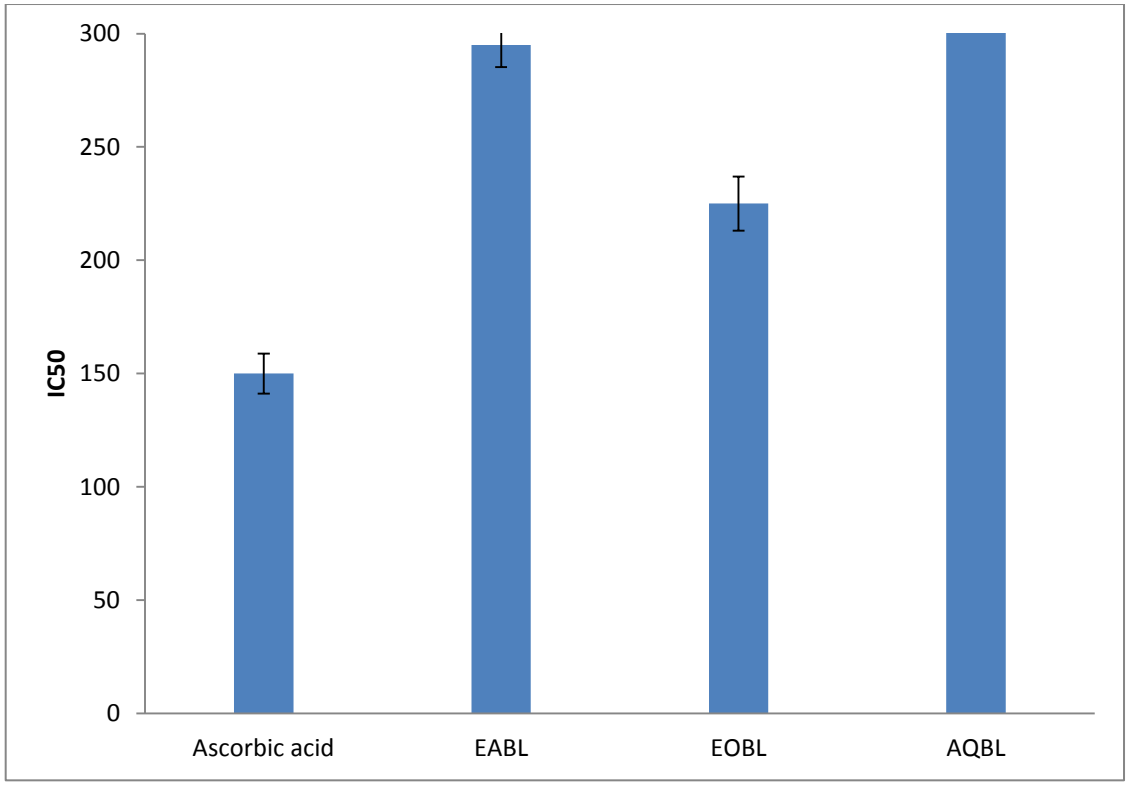

Figure 12: *In vitro* 50% inhibition concentration (IC_{50}) of Buchananialanzanleavesextracts on Nitric oxide radical scavenging model

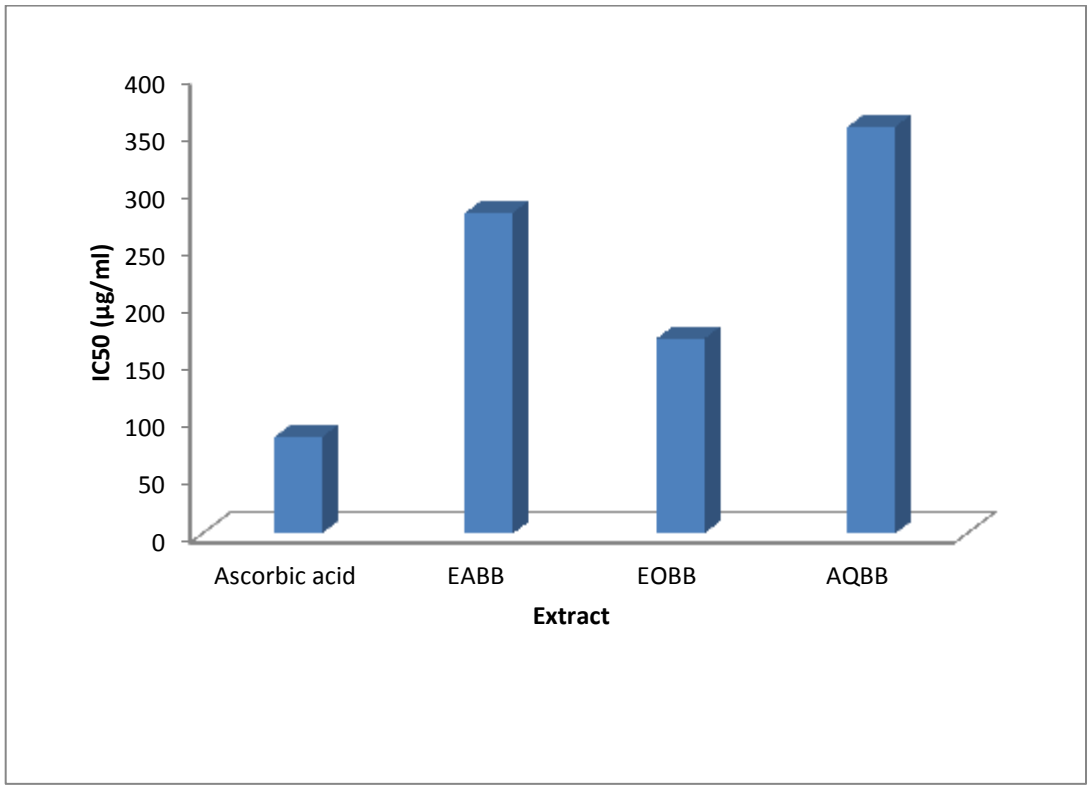

Figure 13: *In vitro* 50% inhibition concentration (IC_{50}) of Buchananialanzan bark extracts on hydroxyl radical scavenging model

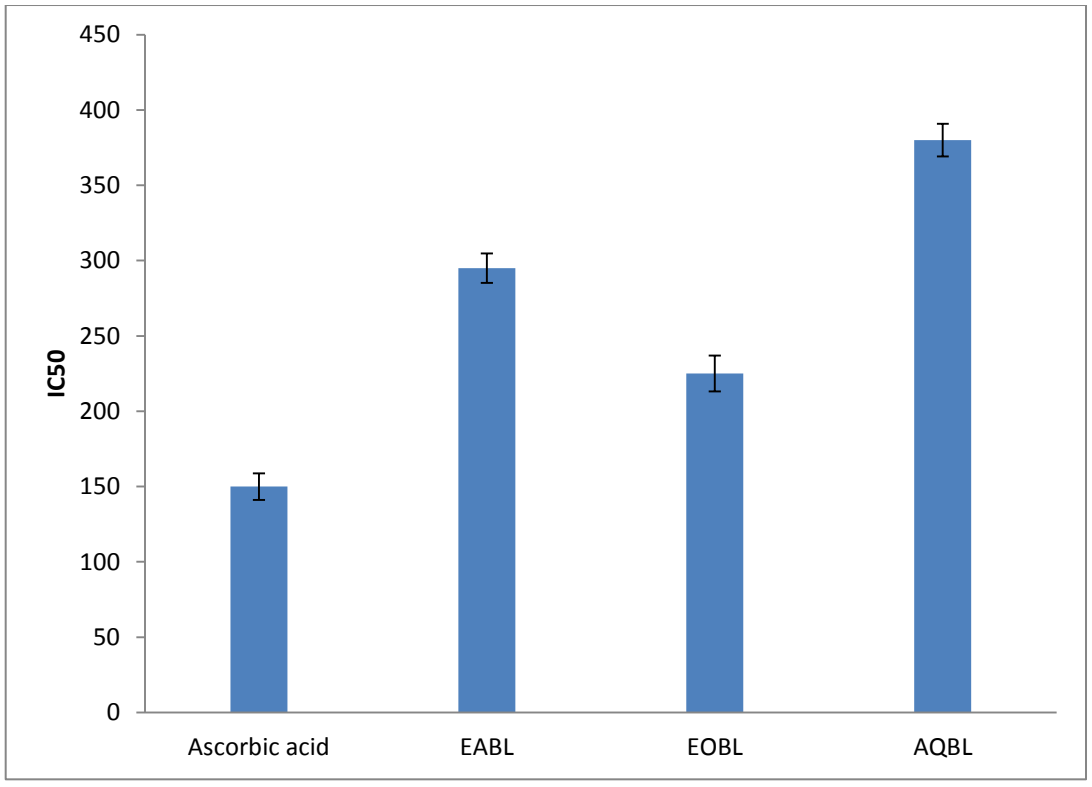

Figure 14: *In vitro* 50% inhibition concentration (IC_{50}) of Buchananialanzan leaves extracts on hydroxyl radical scavenging model

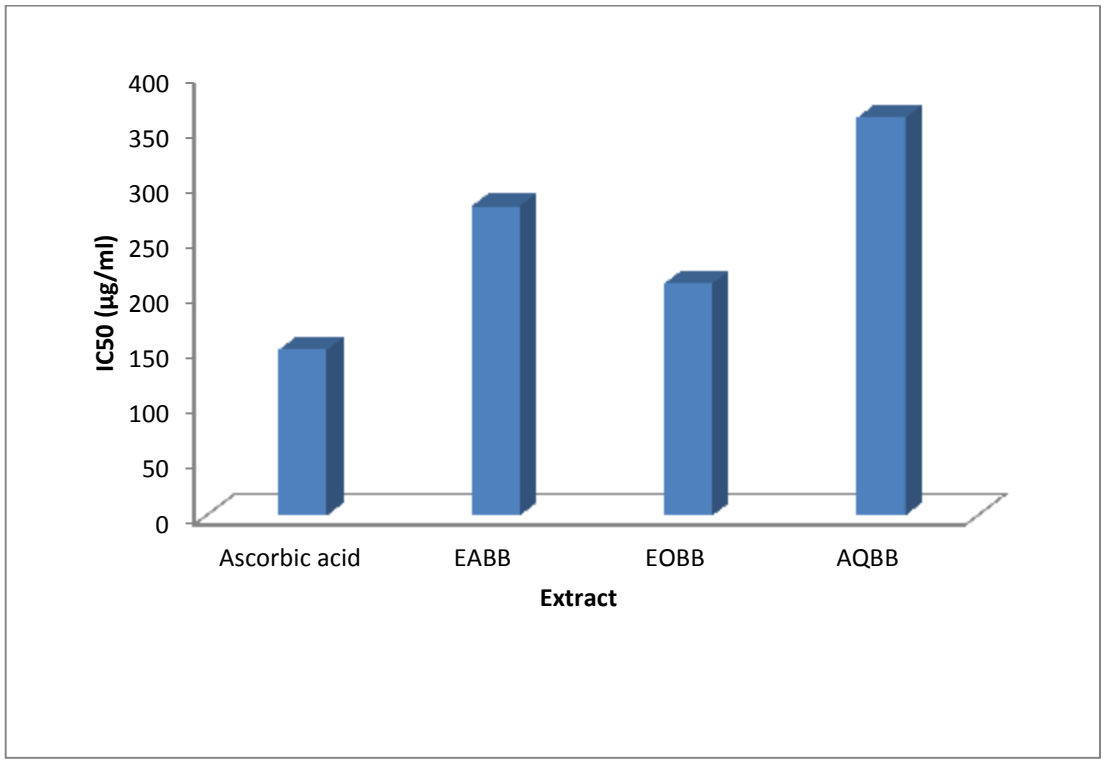

Figure 15: *In vitro* 50% inhibition concentration (IC_{50}) of Buchananialanzan bark extracts on Superoxide radical scavenging model

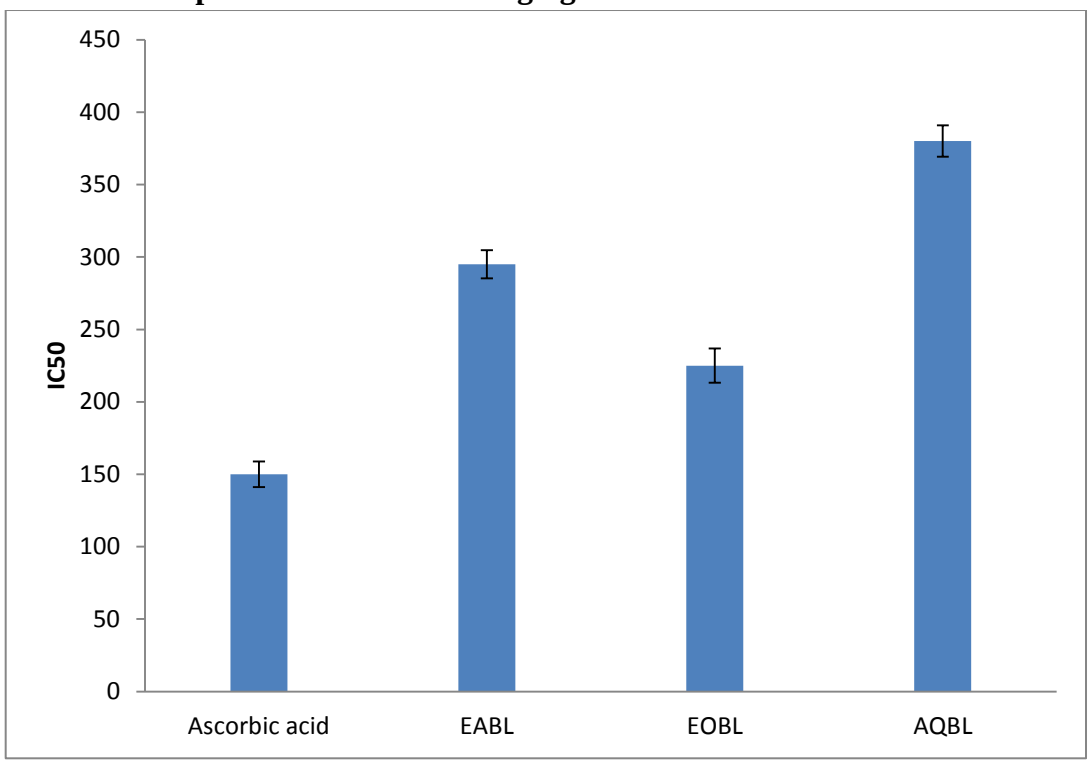

Figure 16: *In vitro* 50% inhibition concentration (IC_{50}) of Buchananialanzan leaves extracts on Superoxide radical scavenging model

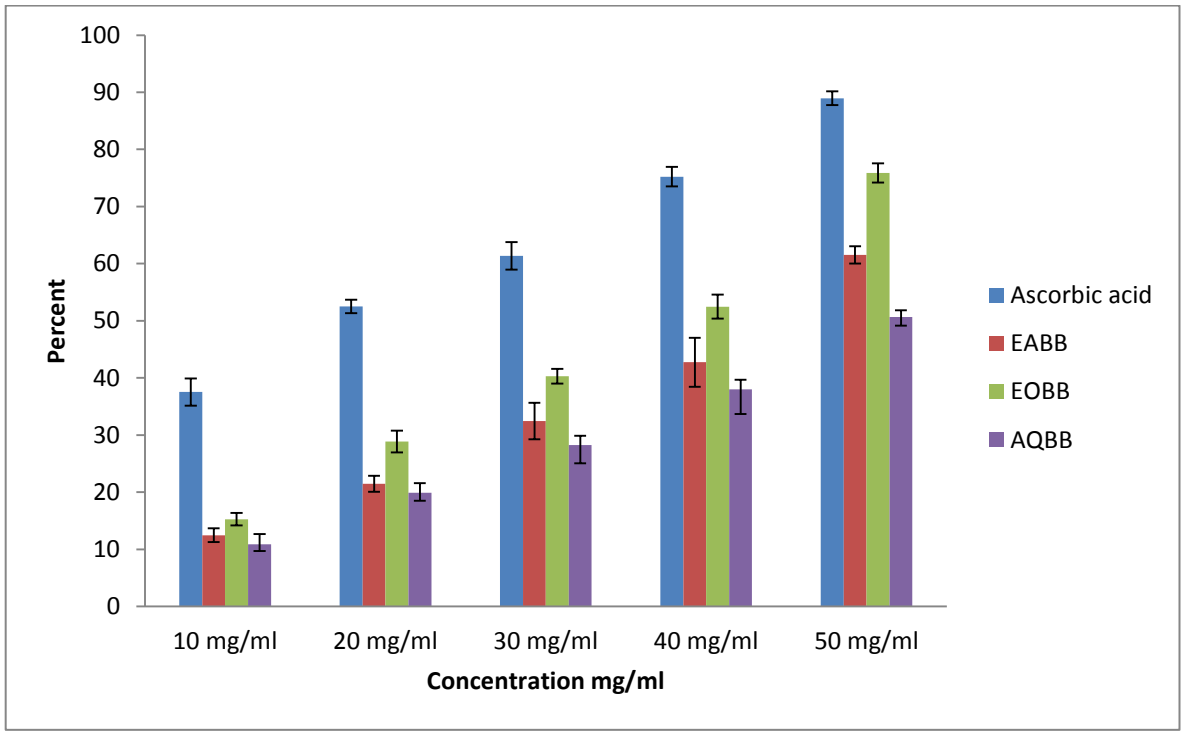

Figure 17: Percent Anti-haemolytic activity of Buchananialanzan bark extracts using male wistar blood cell

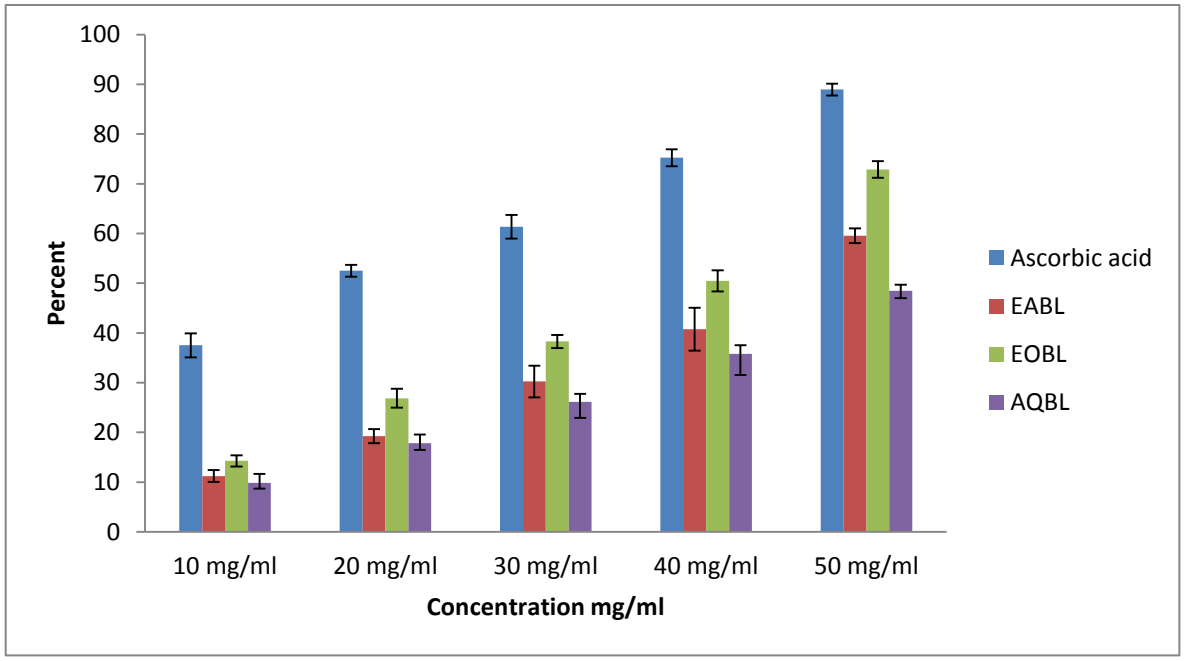

Figure 18: Percent Anti-haemolytic activityof Buchananialanzan leaves extracts using male wistar blood cell

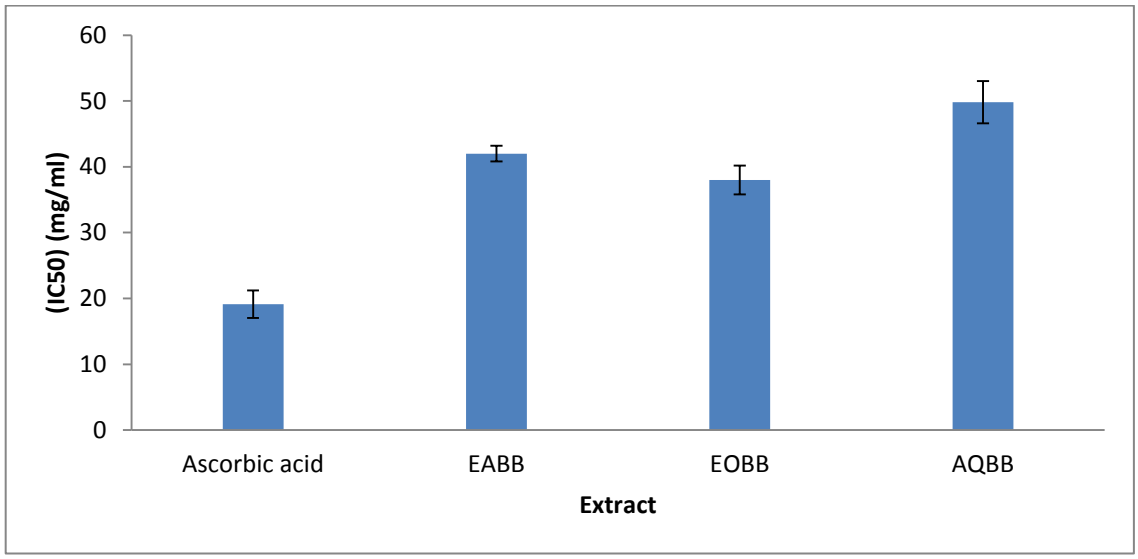

Figure 19: IC 50 of Buchananialanzan bark extractsfor Anti-haemolytic activity

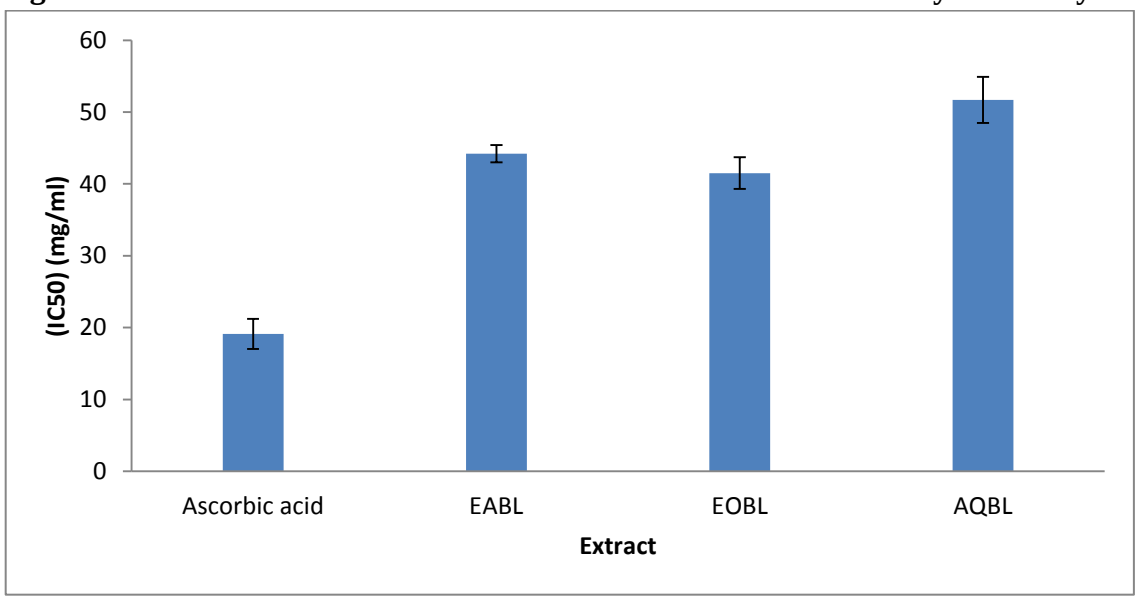

Figure 20: IC 50 of Buchananialanzan leaves extractsfor Anti-haemolytic activity