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Antioxidant and Lipid Profile of Ethanolic Root Extract and Fractions of *Agave Sasilana* in Albino Rats

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Abstract
The present study investigates the antioxidant and lipid profile of the ethanolic root extract and fractions of *Agave sasilana* in albino rats. Forty-eight male albino rats of mean weight 180 g were used for the study. The animals for the study were grouped into eight groups of six rats each. There were seven test groups and a control group. Regular doses of 25, 50, 75, 100, 125, 150 and 175 mg/kg body weight were administered intraperitoneally twice daily for 7 days to the rats in the treatment groups 1, 2, 3, 4, 5, 6 and 7 respectively. Animals in the control group were given 3 ml/kg body weight of normal saline. At the end of the experiment, the animals were sacrificed under mild ether anesthesia The blood samples were collected with EDTA sample bottle for the analysis of the antioxidant and lipid profile. From the result obtained, there was significant ($p< 0.05$) increase in the antioxidant activity (vitamin C, DPPH radical scavenging activity, anti-lipid peroxidative properties and nitric oxide radical scavenging activity) in the test groups when compared to the control group, there was significant ($p< 0.05$) reduction in the plasma lipid profile (total cholesterol, triacylglycerol, low density lipoprotein) in the test groups when compared to the control group, while there was significant ($p< 0.05$) increase in high density lipoprotein in the test groups when compared to the control group. The study indicates that ethanolic root extract and fractions of *Agave sasilana* may have exerted antioxidant and hypolipidemic effects in the albino rats, and may also be used pharmacologically in the management of hyperlipidemia and diseases implicated by free radicals.
Keywords*: Agave sasilana; antioxidants; lipid profile; Reduced glutathione; High density lipoprotein; Low density lipoprotein; Superoxide dismutase; Total cholesterol.*

1. Introduction

Oxidative stress (OS) results from an imbalance between the production of reactive species and their degradation, which is associated with different physiological disorders.[1] In addition, oxidative stress causes cumulative damage in molecules (proteins, lipids, DNA, RNA, carbohydrates) in the human body, which accelerates aging.[2] Therefore, antioxidant biomolecules can act against the process involving oxidative stress by fighting or preventing its damage due to their antioxidant activity. These antioxidant biomolecules can act as an intracellular modulator of

oxidative stress, acting either directly with reactive species, through the transfer of hydrogen atoms, electron transfer and/or chelation of transition metals[3], or indirectly through the activation of the phase II detoxifiers, which are the major line of defense against oxidative stress and its damage.[4]

Antioxidants also play a major role in the protection against molecular oxidative damage. Disturbances of antioxidant defence systems have been demonstrated, including alteration in the activities of antioxidant enzymes and impaired glutathione (GSH) metabolism.[5] Plant-derived herbal remedies are apparently effective, produce minimal or no side effects in clinical experience and are of relatively low costs as compared with oral synthetic hypoglycemic agents.[6] The protective role of antioxidant enzymes is well known and has been investigated extensively in oxidative stress patients and experimental animals.[7]

Arteriosclerosis or coronary artery disease is a condition characterized by deposits of lipids, mainly cholesterol on the inner walls of the arteries. These deposits narrow the arterial channels and partly block the normal flow of blood through them.[8] *Agave* belongs to the family Agavaceae and is widely distributed in tropical and subtropical regions of the world, and, due to their ability to grow in dry lands and their several potential applications, plants of this genus have been called "plants of the century".[9] The species *Agave sisalana*, commonly known as sisal, is a species that originates from Mexico and is widely cultivated in the Northeast region of Brazil.[10] In several countries, the juice of Agave sisalana leaves presents great ethnopharmacological importance because it is used as an antiseptic in the topical treatment of skin diseases as well as a poultice on wounds.[11] Orally, it is used to treat indigestion, flatulence, jaundice, constipation, and dysentery.[12] The present study was undertaken to study the antioxidant and lipid profile of ethanolic root extract and fractions of *Agave sasilana* in albino rats.

2. Materials and Method

Plant material

Fresh root part of the plant *Agave sasilana* were locally sourced in Umudike farm, Abia State, Nigeria and was identified by Prof. Garuba Omosun, a taxonomist, of the Plant Science and Biotechnology Department, Michael Okpara University of Agriculture, Umudike, Nigeria. The fresh roots were washed and dried under shade at room temperature, using a blender; the roots were blended into powder.

Extraction

The powdered roots of *Agave sasilana* (100 g) were soaked in ethanol for 48 hours and the extract filtered using a Whatman no. 1 filter paper, the filtrate was allowed to evaporate to dryness, under a water bath with a temperature set at 40°C.

Animals

Healthy looking male albino rats of mean weight of 180 g were used for the study. All animals were kept in metabolic cages in the animal house of the Department of Biochemistry, Michael Okpara University of Agriculture, Umudike, Nigeria), under normal room conditions and acclimatized for two (2) weeks. Commercial pellet diet (Vital growers mash by Grand Cereals and Oil Mills, Nigeria) and water were given to the animals *ad libitum*.

Experimental design

Forty-eight (48) male albino rats of mean weight 180 g were used for the study. The animals for the study were grouped into eight groups of six rats each. There were seven test groups and a control group. Regular doses of 25, 50, 75, 100, 125, 150 and 175 mg/kg body weight were administered intraperitoneally twice daily for 7 days to the rats in the treatment groups 1, 2, 3, 4, 5, 6 and 7 respectively. Animals in the normal control group were given 3 ml/kg body weight of normal saline. The rats were examined physically for signs of good health and toxicity.

At the end of the experiment, all the animals in the various groups were anaesthetized, dissected and blood collected via cardiac puncture. The blood samples were collected with EDTA sample bottle for the analysis of the antioxidant and lipid profile of the ethanolic root extract and fractions of *Agave sasilana* in albino rats.

Biochemical estimations

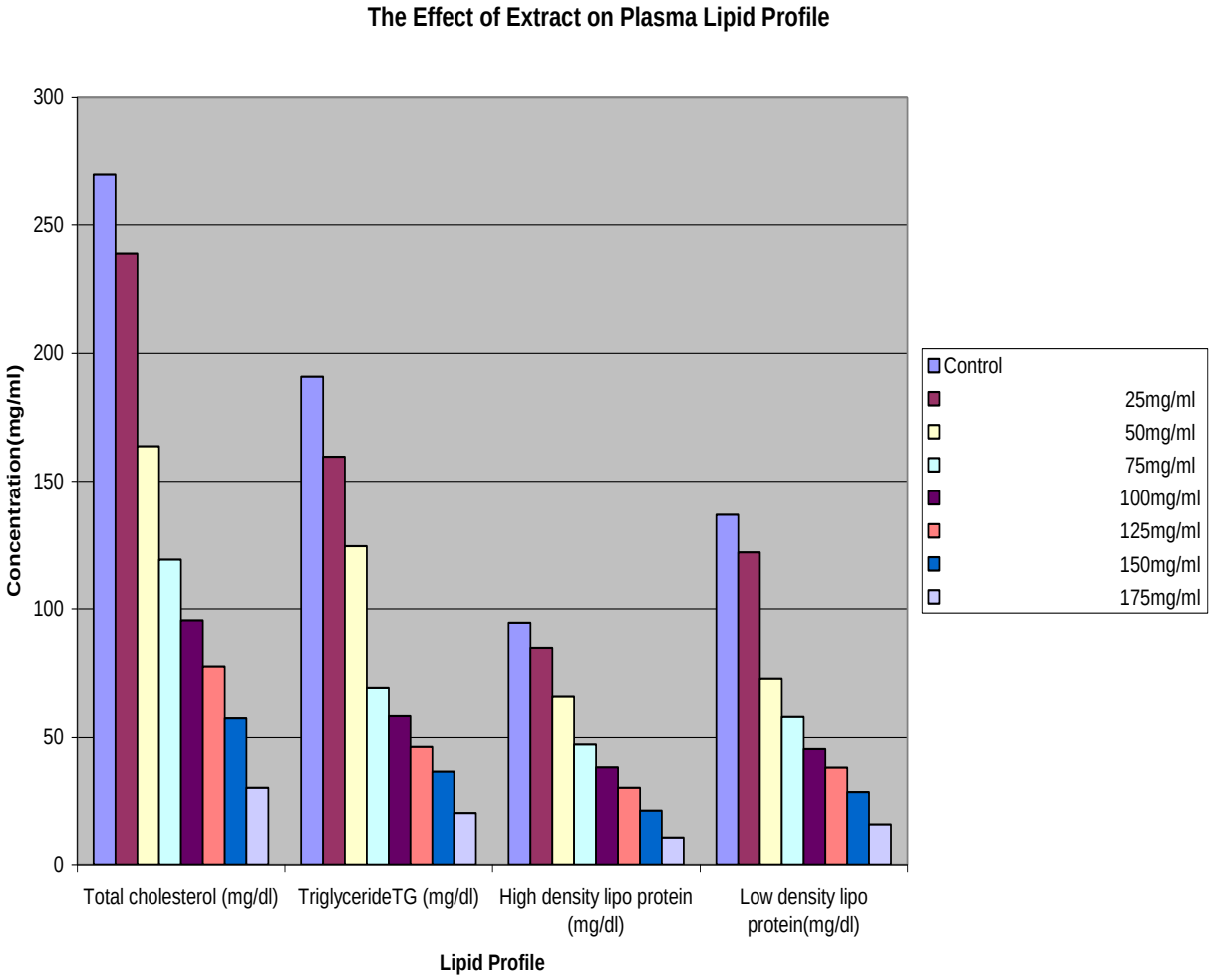
Lipid peroxidation (MDA) was estimated according to the method of [13] Vitamin C concentration were measured by method of [14]. DPPH Free radical scavenging activity was determined using the modified method of [15]. The anti-lipid peroxidative property of the extract was determined using a modified thiobarbituvic acid reactive species (TBARS) essay of [16]. The total plasma cholesterol was determined by the method described by[17]. Total plasma triglyceride was determined following the method described by [18]. The method of [19] was adopted in the determination of plasma HDL-cholesterol. LDL-cholesterol was estimated by indirect method using the method of [20].

3. Results

Effect of the Extract on Plasma Lipid Profile

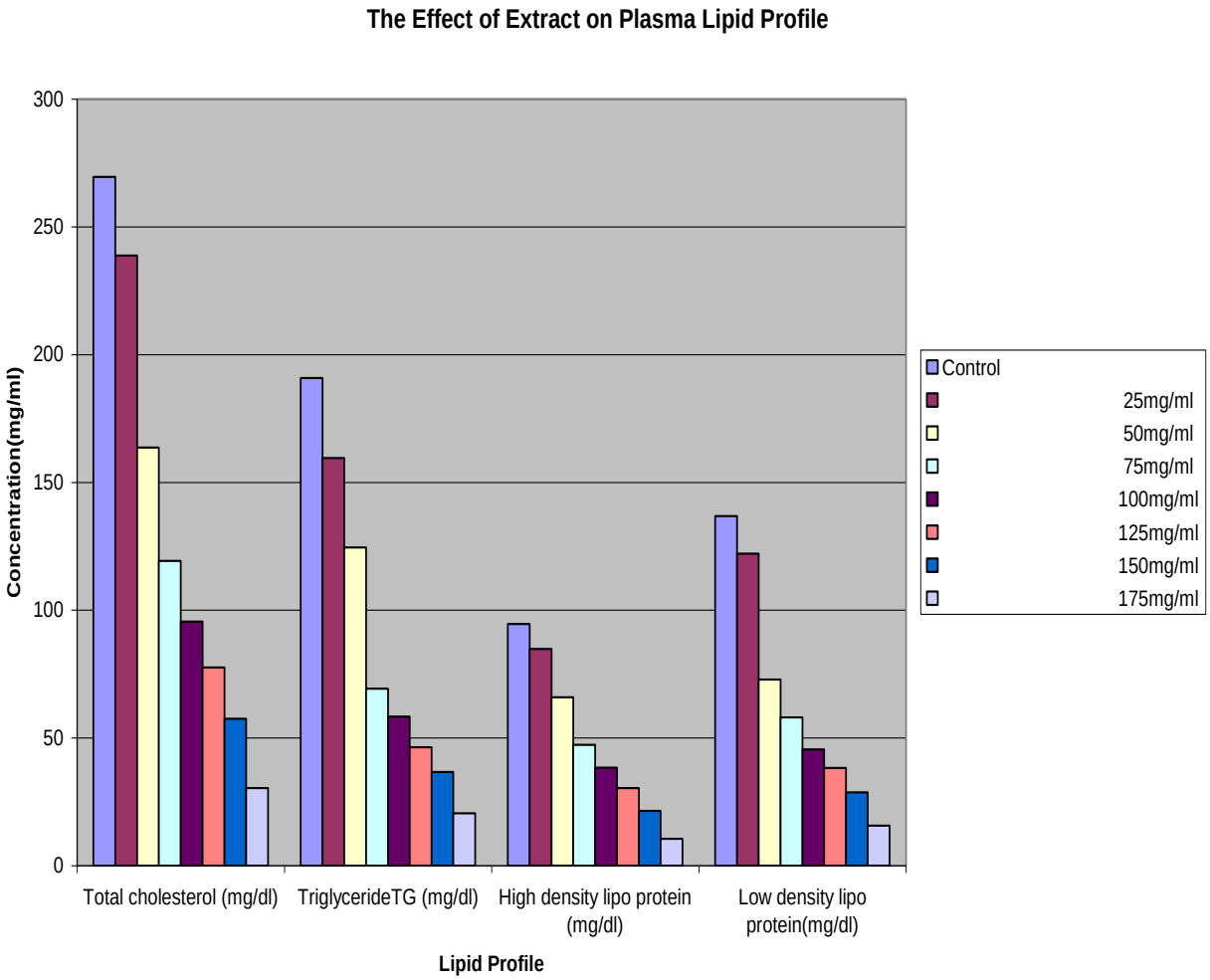
The result of the effect of the root extract of *Agave sasilana* on plasma lipid is as presented in figure 3.1 below. The result shows that the cholesterol level decreased with increase in the dosage of the extract. Group 7 for instance which received the highest dosage of the extract had 30.350 ± 0.166 mg/dL cholesterol level. This is lower when compared with group I (239.48±0.743) mg/dL and the control group (269.48±0.347). The decrease in the mean cholesterol level when compared with the control group is quite significant ($p < 0.05$). Similarly, such decrease was observed with the lipoproteins (HDL and LDL).

Figure 3.1: Result of the Effect of The Extract on Plasma Lipid Profile



Antioxidant Activity of Ethanolic Root Extract of *Agave Sasilana*

The ethanolic extract of the root of *Agave sasilana* was found to possess free radical scavenging activity at different concentrations (25-175 mg/dL). The radical scavenging activities were concentration dependent as seen in figure 3.2. Figure 3.3 shows the DPPH radical scavenging activity of Vitamin C. The scavenging activity of the Vitamin C increases with increase in the concentration. The root extract of *Agave sasilana* shows anti-lipid peroxidative characteristic which is dose dependent (Figure 3.4). The result of the nitric oxide radical scavenging activity of the ethanolic root extract of *Agave sasilana* is presented in figure 3.5. The plant extract possessed antioxidant activity.



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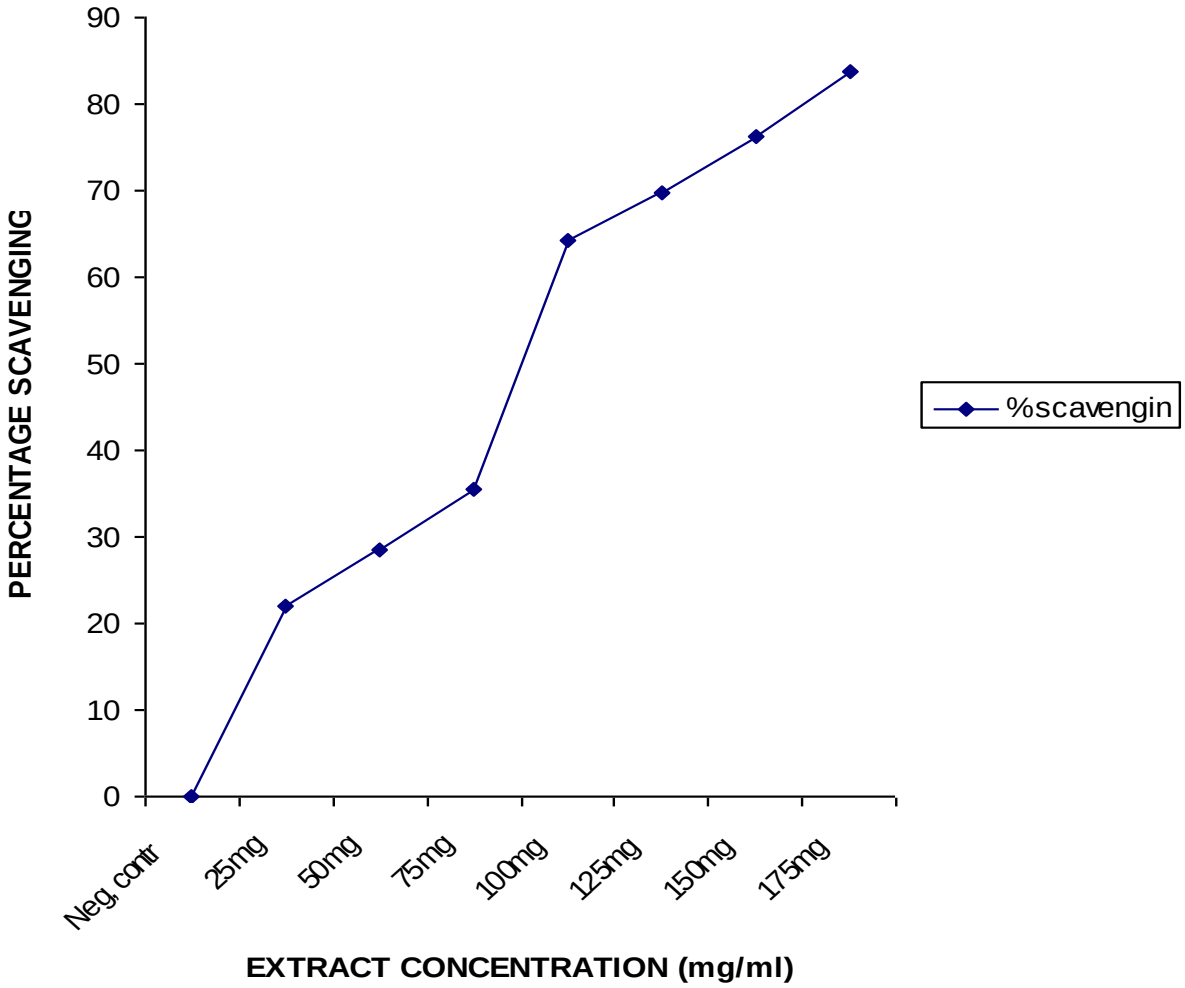

Figure 3.2: DPPH RADICAL SCAVENGING ACTIVITY OF ETHANOLIC ROOT EXTRACT OF *Agave sasilana*

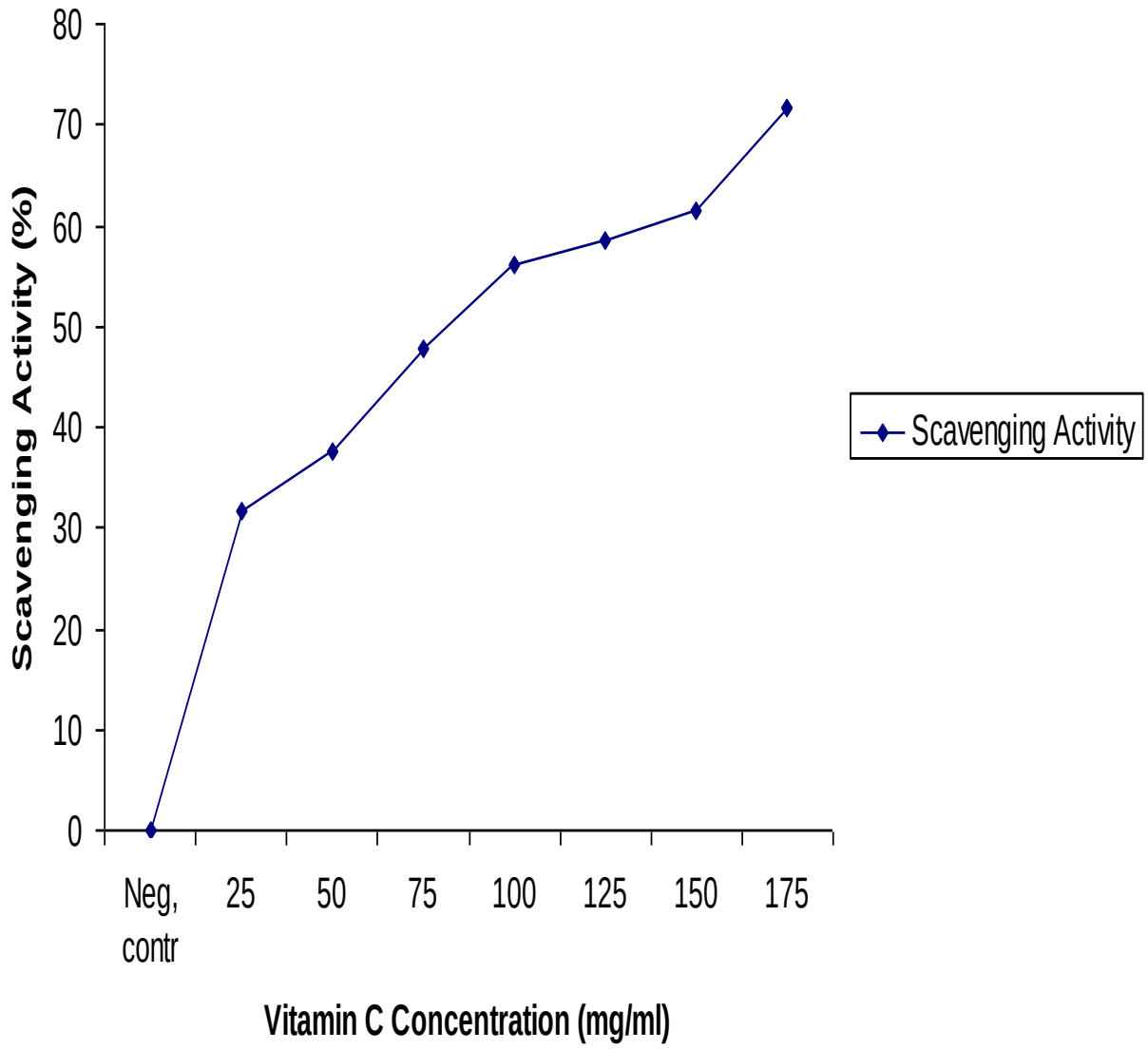

Figure 3.3: DPPH RADICAL SCAVENGING ACTIVITY OF THE STANDARD (VITAMIN C)

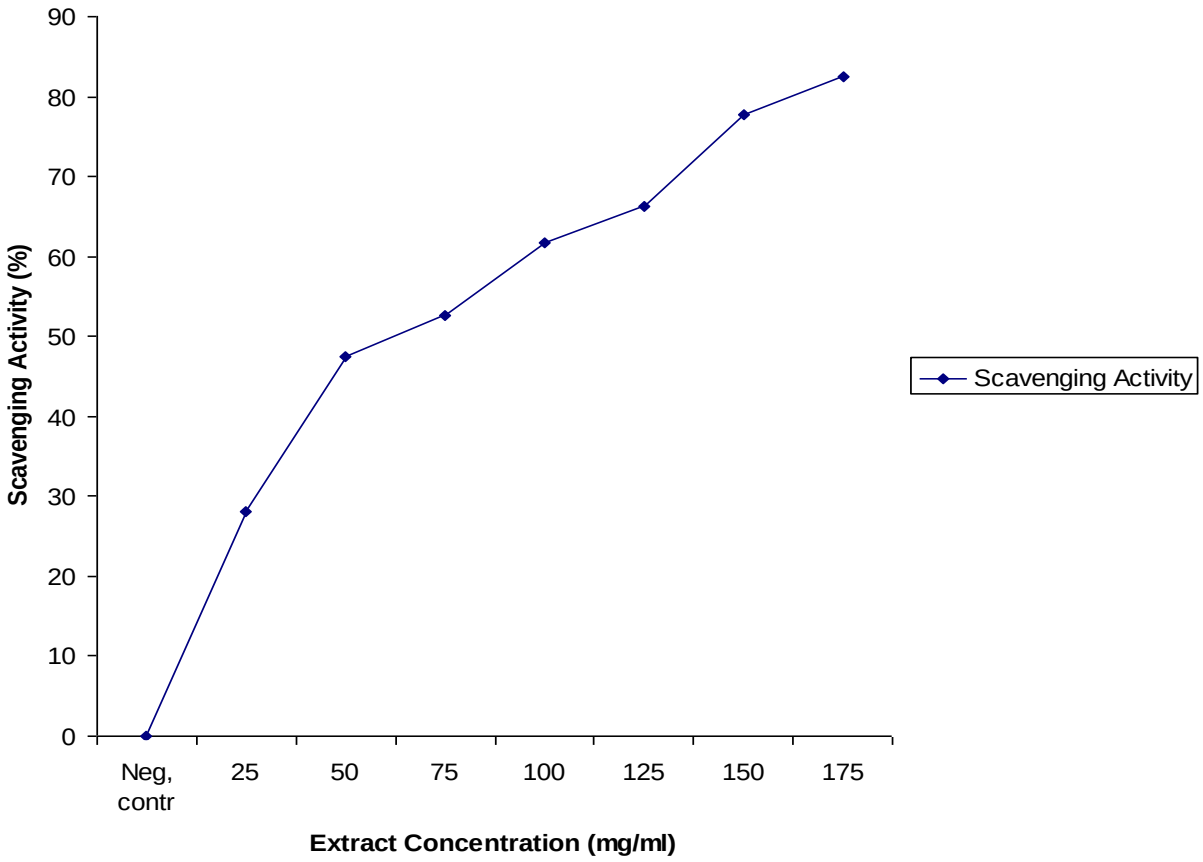

FIGURE 3.4: ANTI-LIPID PEROXIDATIVE PROPERTIES OF ETHANOLIC ROOT EXTRACT OF *Agave* sasilana

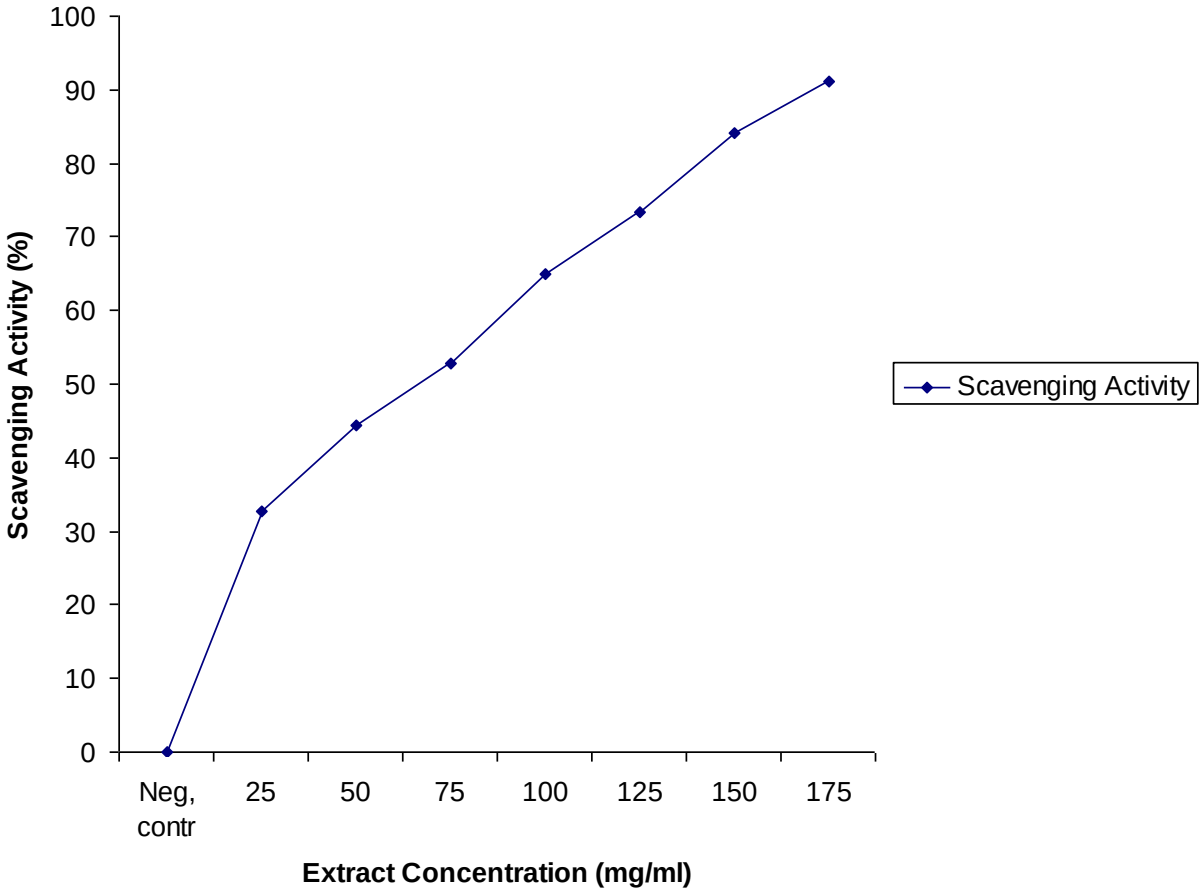

FIGURE 3.5: NITRIC OXIDE RADICAL INHIBITION ACTIVITIY OF THE ETHANOLIC ROOT EXTRACT OF *Agave sasilana*

Bioactivities of the Five Fractions

The biological activities of the five fractions with respect to antioxidant and lipid profile were shown in the figures below.

Fraction A which contains mainly flavonoids produced statistically significant ($p<0.5$) increase in the antioxidant activity and statistically significant ($p<0.05$) reduction in plasma cholesterol when compared to the control group. Fraction B contains mainly alkaloids which also produced significant ($p<0.05$) increase in antioxidant activity and significant ($p<0.05$) reduction in plasma

lipid profile when compared to the control group. Fraction C contains primarily saponins and produced high significant reduction in plasma cholesterol and significant ($p<0.05$) increase in antioxidant activity compared to the control group. Fraction D contains mainly tannins and it produced significant ($p<0.05$) increase in antioxidant activity and significant ($p<0.05$) reduction in plasma lipid profile. Fraction E contains mainly Anthraquinones which produced statistically significant ($p<0.05$) increase in antioxidant activities.

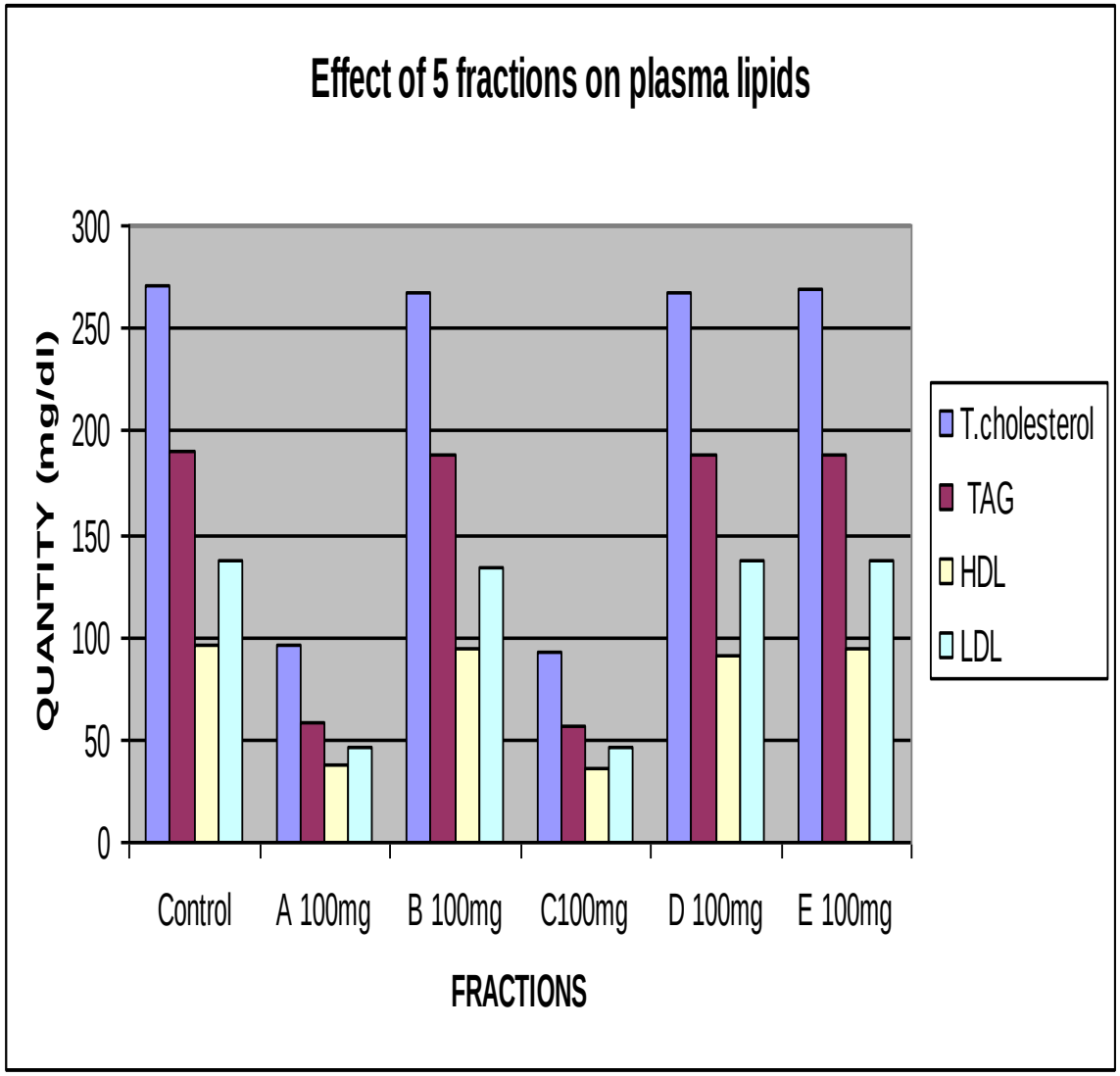

Figure 3.6 Effect of the fractions on the plasma lipid profile

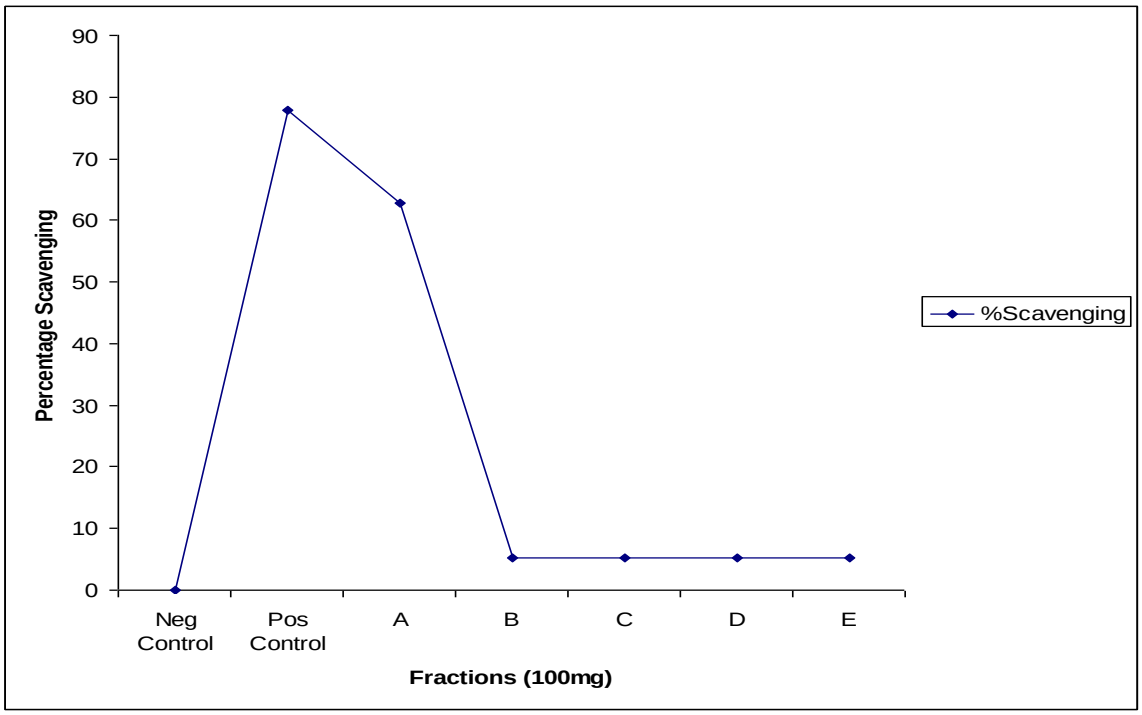

FIGURE 3.7: RESULT OF ANTI-LIPID PEROXIDATION PROPERTY OF FIVE FRACTIONS

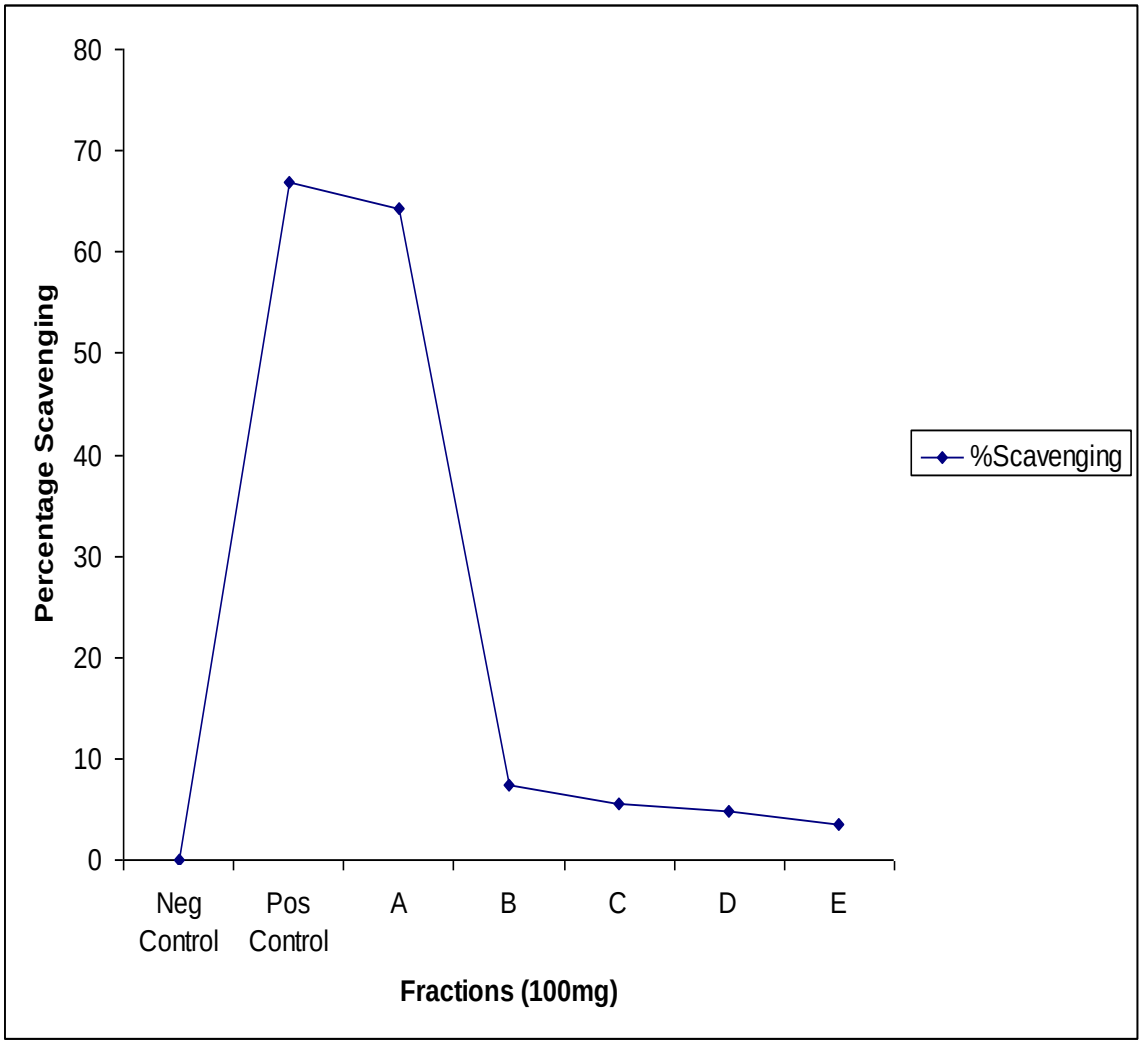

FIGURE 3.8: RESULT OF DPPH RADICAL SCAVAGING ACTIVITY OF THE FIVE FRACTIONS

4. Discussion

A lot of research shows that free radicals and oxidative stress are one of the instrumental features in the causation and progression of numerous ailments including cancer and diabetes.[21] Naturally, free radicals are removed by antioxidant enzymes such as superoxide dismutase (SOD) and catalase which defend the body from oxidative stress.[22] The decrease in the action of these redox markers can result to a rise in superoxide anion, hydrogen peroxide, and hydroxyl radicals leading to widespread lipid peroxidation in the animals.[23] It has been revealed that ethanolic root extract and fractions of *Agave sasilana* contains high flavonoids that are phenolic compounds that have hydroxyl groups in their structure increasing their antioxidative effect.[24] These compounds could be important in enhancing the redox status in animals treated with the root extract. The study also showed that ethanolic root extract of *Agave sasilana* is rich in vitamin C as presented in figure 3.3. Vitamin C is a water-soluble antioxidant. As water soluble antioxidant it is in a unique position to

scavenge aqueous peroxyl before these destructive substances have a chance to damage lipids. It works synergistically with vitamin E and glutathione peroxidase to stop free radical chain reaction. It also helps the body to absorb iron and breakdown histamine, the inflammatory components of many allergic reactions.[25]

In the present study, the activity of the plasma lipid profile (high density cholesterol (HDL), triacylglycerol (TAG), total cholesterol, low density cholesterol (LDL) were significantly ($p< 0.05$) reduced in the treated groups (25 mg/kg to 175 mg/kg b.wt of ethanolic root extract of *Agave sasilana*) which shows that the extract and its fractions were able to lower the lipid profile in the albino rats, which can be attributed to the phytochemical constituents of the ethanolic root extract and fractions of *Agave sasilana*, which implies that Agave sasilana can be used to prevent cardiovascular complications arising from hyperlipidemia.[26]

5. Conclusion

The present study investigated the antioxidant and lipid profile of ethanolic root extract and fractions of *Agave sasilana* in albino rats. The study establishes the antioxidant activity and hypolipidemic effect of ethanolic root extract and fractions of *Agave sasilana* in albino rats, by decreasing the concentration of lipid peroxidation products and increasing the activity of the antioxidants markers which is valuable in the treatment of diseases implicated by free radicals.

Ethical Approval

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropiate ethics committe.

Acknowldgement

We sincerely appreciate all that made this work successful.

Competing Interests

Authors have declared that no competing interests exist.

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