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# Assessment of antioxidant and toxicological effect of *Allium sativum* extracts in rat liver *in vitro*

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**ABSTRACT**

*The antioxidant and toxicological effect of Allium sativum extract was investigated in rat liver in vitro. The effect of the antioxidant potential of the extract on 1,1-diphenyl-2-picryhydrazyl (DPPH) and hydroxyl radicals (OH*) was assessed while the toxicological effect of the extract on antioxidant enzymes such as reduced glutathione (GSH) and superoxide dismutase (SOD) as well as catalase (CAT) was also evaluated. The results show that aqueous and ethanolic extracts of Allium sativum cloves demonstrated significant ($p<0.05$) scavenging effect on DPPH and OH* radicals. It also indicates synergistic effect of the plant extract on antioxidant defense enzymes with concomitant increase in their activities compared to control. Therefore, Allium sativum could offer protection against free radical-induced oxidative stress and related diseases.*

**Keywords**: *Allium sativum*, antioxidant enzymes, free-radicals, oxidative stress.

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## INTRODUCTION

Oxidative stress occurs where there is imbalance between the productions of reactive oxygen species and the biological system's ability to readily detoxify the reactive intermediate and repair the resulting damage [1]. Antioxidants modulate the pathophysiology of chronic inflammation to a reasonable extent [2]. Antioxidants are needed to scavenge and in order to prevent the formation of reactive oxygen species. Exposure to free radicals from variety of sources has led organisms to develop defense mechanism [3] against free radical induced-oxidative stress and such enzymatic defense mechanisms include glutathione, glutathione peroxidase, catalase, superoxide dismutase. The balance between the activities and intracellular levels of these antioxidants is essential for the survival of the organisms [4].

Myriads of in vitro and in vivo studies reported that organo-sulphur and polyphenolic compounds protect against oxidative stress [5]. Some of these medicinal plants have been investigated for their antioxidative potentials in the treatment of oxidative stress related diseases [6]. Many of the metabolites from these plants especially flavonoids exhibited potent antioxidant activity in vitro and in vivo [7]. The reactive oxygen species and free radical mediated reaction are involved in degenerative or pathological conditions such as aging, cancer, rheumatoid, arthritis, conorary heart disease and diabetes. Therefore, this research is planned to assess the toxicological and antioxidant potential of *Allium sativum* extract in rat liver in vitro.

## MATERIALS AND METHODS

**Collection of sample**
The sample (garlic cloves) was freshly harvested from the garlic plants on farmland in Iseyin, Oyo State, Nigeria. The sample was identified and authenticated with herbarium number UHAE 2013/109 by a plant scientist in the Department of Plant Science, Ekiti State University, Nigeria.

**Chemicals and reagents**
Chemicals and reagents used such as trichloro acetic acid (TCA), 1,1-diphenyl-2-picryl-hydrazyl (DPPH), 1,10-phenanthroline, gallic acid, Folin Ciocalteau's reagent were procured from Sigma Aldrich, Inc. 2-nitro-5-thiobenzoic acid, sodium azide, Ellman's reagent and all other analytical grade chemicals were purchased from E. Merck (Germany).

**Preparation of extract**
The crude garlic cloves were macerated, ground into paste and then dissolved in distilled water and thoroughly filtered to obtain the aqueous extract. The ethanolic extract was obtained after the garlic cloves were dried using freeze dryer, milled into powdery form and ran through Soxhlet-Apparatus with 1L 70% ethanol as reagent.

**Determination of DPPH radical scavenging potential of *Allium sativum* extract**
**Procedure:** The free radical scavenging potential of hydroalcoholic extract of *Allium sativum* was measured by 1,1-diphenyl-2-picryl-hydrazyl using method of (8). 0.1mM solution of DPPH prepared was added to 3ml of the extract suspension in water at different concentrations.

After 30min of incubation, absorbance was read at 517nm while ascorbic acid was used as reference material and the tests were carried out in triplicate and the results averaged where percentage reduction in absorbance was calculated.

**Determination of hydroxyl radical (OH*) scavenging potential of *Allium sativum* extract Procedure:** Freshly prepared 100µl of extract was added to a reaction mixture containing 12µl, 20mM deoxyribose, 400µl, 0.1M phosphate buffer at pH 7.4, 40µl, 20mM hydrogen peroxide and 40µl, 500µM FeSO4, and the volume made up to 800µl with distilled water. The reaction mixture was then incubated at $37^0$C for 30min and the reaction was stopped by the addition of 0.5ml of 2.8% TCA followed by the addition of 0.4ml of 6% TBA solution (9). The test tubes were subsequently incubated in boiling water for 20min and the absorbance was read at 532nm.

**Determination of effect of *Allium sativum* extract on reduced glutathione (GSH) concentration in rat liver**
**Procedure:** Garlic extract (0.2ml) was added to 1.8ml of distilled water and 3ml of sulphosalicylic acid. The mixture was centrifuged at 3000 xg for 4min and 0.5ml of the supernatant was added to 4.5ml of Ellman's reagent. A blank was prepared with 0.5ml of dilute precipitating agent and 4ml of phosphate buffer with 0.5ml of Ellman's reagent. The absorbance was taken within 30mins of colour development at 412nm against blank and the concentration of GSH was extrapolated from the GSH standard curve using method by (10).

**Determination of effect of *Allium sativum* extract on superoxide dismutase activity in rat liver**
**Procedure:** Garlic extract (0.2ml) was added to 2.5ml of 0.05M carbonate buffer of pH 10.2 to equilibrate in the spectrophotometer. The reaction was initiated by the addition of 0.3ml of freshly prepared 0.3mM adrenaline to the mixture which was quickly mixed by inversion. The reference cuvette contained 2.5ml buffer, 0.3ml of adrenaline and 0.2ml distilled water. The increase in absorbance at 480nm was monitored every 30secs for 150secs (11).

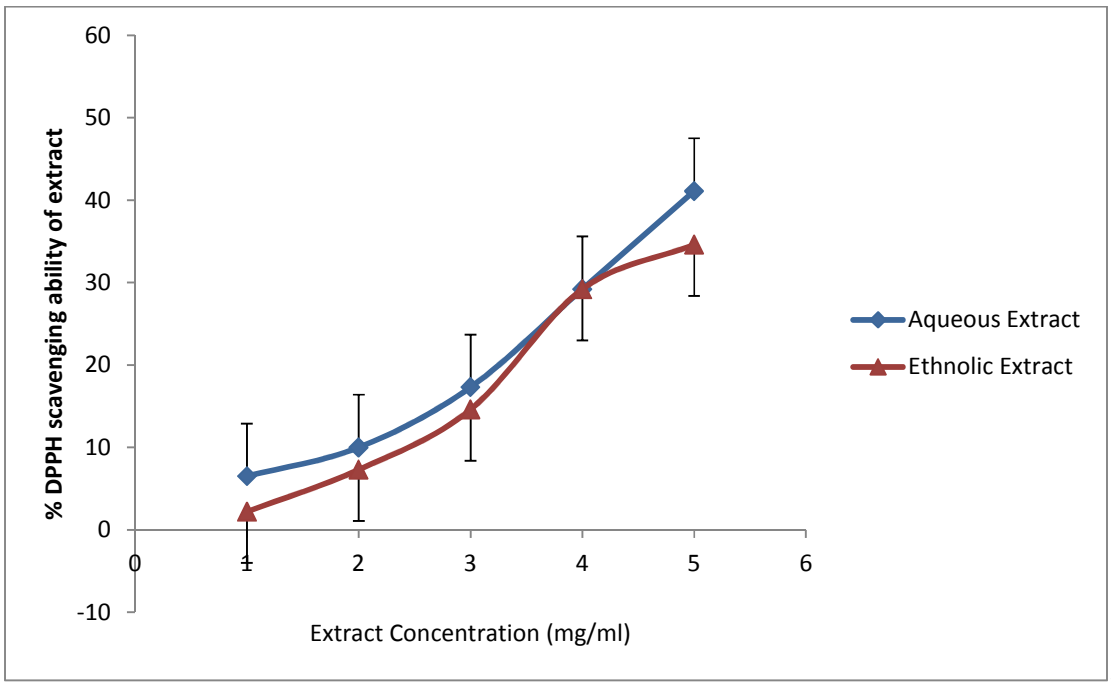
**Determination of effect of *Allium sativum* extract on catalase activity in rat liver**
Procedure: Garlic extract 70µl was mixed with 920µl sodium phosphate buffer containing 0.1mM EDTA at pH 7.0. The reaction was started by adding 10µl of hydrogen peroxide. The decrease in hydrogen peroxide concentration was taken by reading the absorbance at 240nm at 10secs intervals for 180secs (12) method was used to determine the catalase activity:

Catalase activity (Units/mg) = change in absorbance at 240nm/initial 45sec.

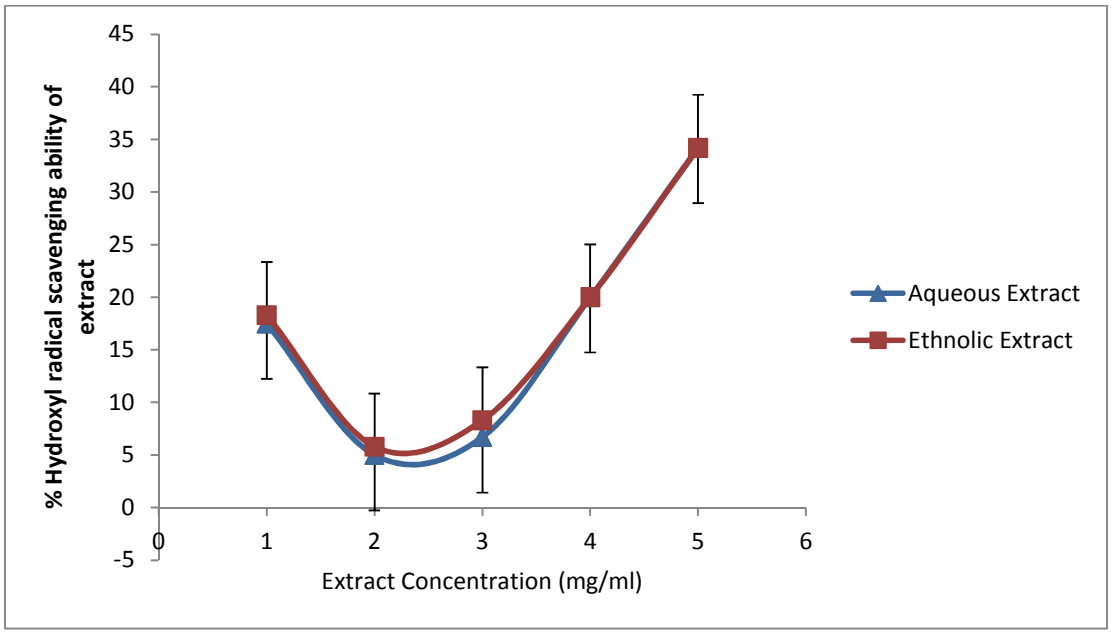
**Statistical Analysis**

Data were expressed as Mean ± SEM of mean. Comparison between values obtained in control and treated groups of animals were performed with One-way Analysis of Variance (ANOVA) and ($p<0.05$) was considered significant.

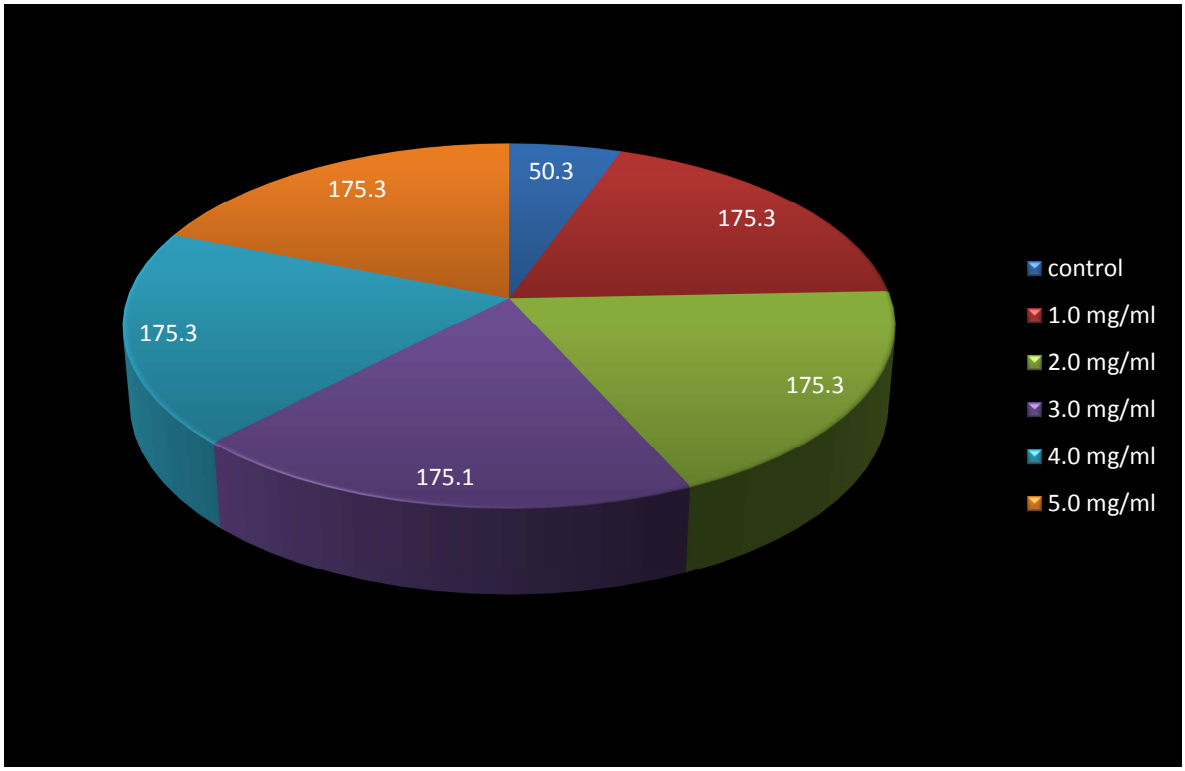
## RESULTS



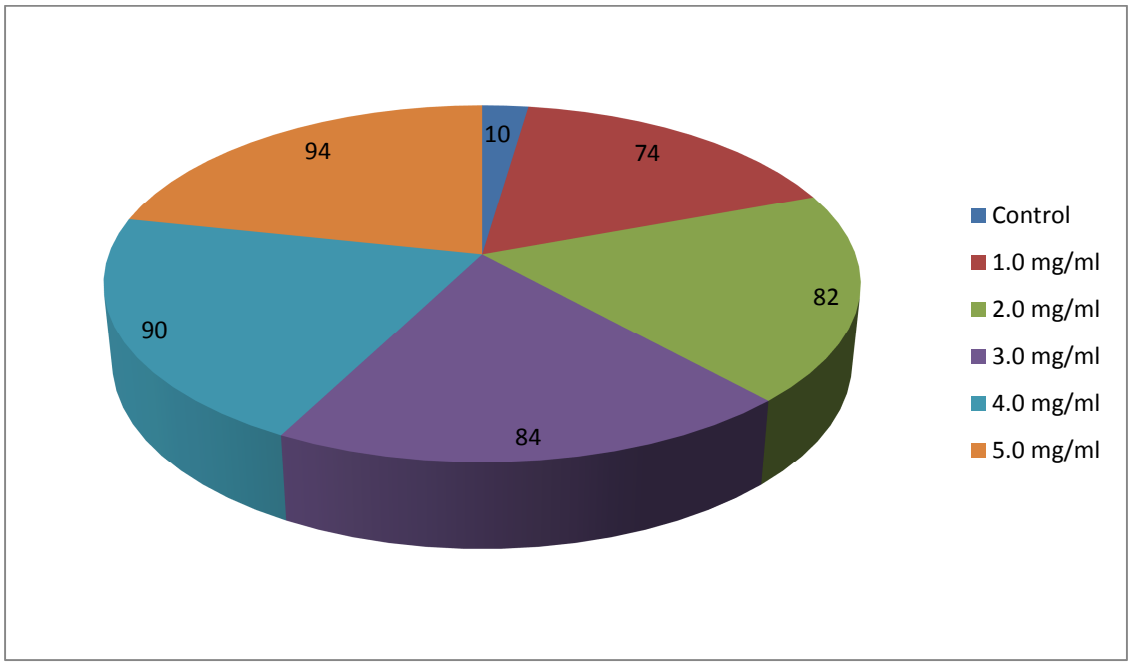

**Scavenging effect of extracts of *Allium sativum* cloves on DPPH radical**



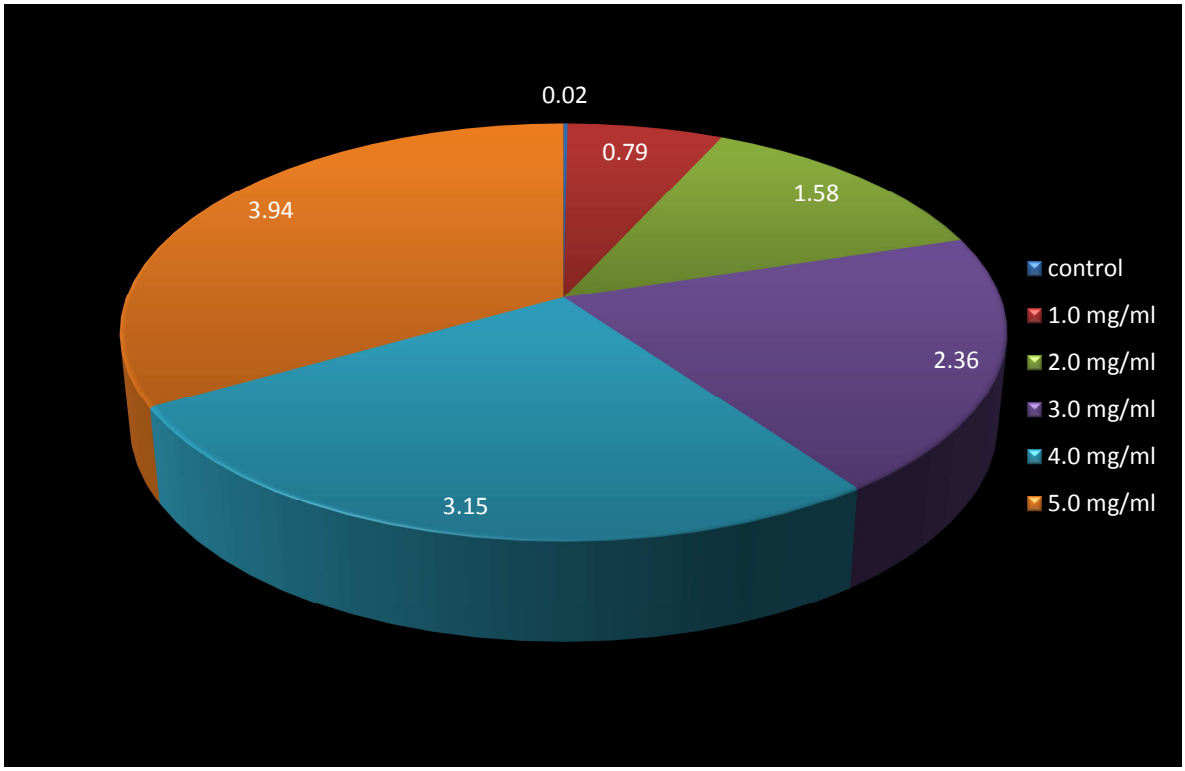

**Scavenging effects of extracts of *Allium sativum* cloves on hydroxyl radical**




**Effect of *Allium sativum* aqueous extract on concentration level of reduced glutathione**




**Effect of *Allium sativum* aqueous extract on superoxide dismutase activity**




**Effect of *Allium sativum* aqueous extract on catalase activity**

## DISCUSSION

The aqueous extract of *Allium sativum* cloves exhibited higher percentage of DPPH scavenging ability than the ethanolic extract.The strong inhibition displayed on these radicals could be linked to the extract inherent phenolic compounds which are capable of donating electrons or transferring hydrogen atoms to neutralize free radicals, thus, could be a promising therapeutic agent to treat oxidative stress-induced pathological conditions. A similar trend of inhibition by the extracts was also observed against hydroxyl radicals where the extracts demonstrated same inhibitory strength. This is contrary to findings of (Ojo *et al*., 2014) who reported that ethanolic extract of *Ocimum gratissimum* exhibited higher inhibitory potentials against nitric oxide and hydroxyl radicals (13). Hydroxy radical have been implicated in the oxidative damage of DNA, proteins and lipids (14). The formation of hydroxyl radicals in biological systems have been attributed to interactions of metal ions such as ferrous with hydrogen peroxide (15). Their inhibitory potentials increased with increased concentration of the extracts. Oxidative damage can result when the critical balance between free radical generation and antioxidant defense is unfavourable. The oxidative damage could be retarded by endogenous defense systems such as reduced glutathione (GSH), glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT). These enzymes work in concert to detoxify the free radicals (16). Hydrogen peroxide has been reported to cause oxidative injury to the cell where it is being produced by myriad of metabolic pathways (17). The result from this research indicates synergistic effect of the extract on these antioxidant enzymes with concomitant increase in their activities corresponding to increase in extract concentration. This effect could be attributed to the presence of selenium and zinc in the extract which generally activate the defensive antioxidant enzymes, thus, prevent the rise in concentration of superoxide anions and hydrogen peroxide that normally cause cellular assault.

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