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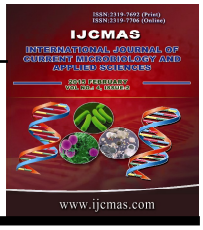

Original Research Article

Evaluation of Antioxidant Activities of Ethanol Leaf Extracts of *Cymbopogon citratus* and *Hyptis spicigera* in Mice Exposed to *Plasmodium berghei*

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Keywords

Plasmodium berghei, Superoxide dismutase, Reduced glutathione, Catalase, Peroxidase, *Cymbopogon citratus* and *Hyptis spicigera*

A B S T R A C T

Antioxidants effect of ethanol leaf extract of *Cymbopogon citratus* and *Hyptis spicigera* in mice exposed to *plasmodium berghei* was carried out using 48 albino mice. The albino mice were infected with *plasmodium berghei*. *Plasmodium berghei* infected blood was collected from the infected mouse and injected into all the mice. The mice were randomly assigned into four groups A, B, C and D. Group A with 6 mice was used as negative control and was given only feed and water while group B was used as positive control and was treated with chloroquine. Group C and D were sub-divided into C1, C2, C3, D1, D2, and D3 with 6 mice each. The sub-groups were treated with 200, 400 and 800mg/kg body weight of *Cymbopogon citratus* and *Hyptis spicigera* extracts respectively for seven days. Mice from all the groups were allowed free access to water and feed. After seven days, the mice were sacrificed and their liver collected and analyzed to check for the effectiveness of the extracts on antioxidant indices using spectrophotometer. The result obtained showed a significant ($P<0.05$) increase in superoxide dismutase, reduced glutathione, catalase and peroxidase activities in mice treated with *Cymbopogon citratus* and *Hyptis spicigera* extracts. The result also revealed that there was significant ($P<0.05$) decrease in lipid peroxidation activity in mice treated with *Cymbopogon citratus* and *Hyptis spicigera* when compared with controls. This indicated the effectiveness of the use of the plants in the management of oxidative stress caused by malaria.

Introduction

An antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons or hydrogen from a substance to an oxidizing agent. Oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions. When the chain reaction occurs in a cell, it can cause damage or death to the cell. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions. They do this by being oxidized themselves, so antioxidants are often reducing agents such as ascorbic acid, vitamin C, vitamin A, and vitamin E as well

as enzymes such as catalase, superoxide dismutase, various peroxidases, lipid peroxidation and glutathione (Bjelakovic *et al.,* 2007). Insufficient levels of antioxidants, or inhibition of the antioxidant enzymes, cause oxidative stress and may damage or kill cell. Antioxidants are widely used in dietary supplements and have been investigated for the prevention of diseases such as cancer, coronary heart disease and even altitude sickness (Sies, 2001).

Antioxidants are classified into two broad divisions, depending on whether they are soluble in water (hydrophilic) or in lipids (hydrophobic). In general, water-soluble antioxidants react with oxidants in the cell cytosol and the blood plasma, while lipid-soluble antioxidants protect cell membranes from lipid peroxidation. These compounds may be synthesized in the body or obtained from the diet (Angelo *et al.,* 2004). The different antioxidants are present at a wide range of concentrations in body fluids and tissues, and may be obtained from diets or synthesized in the body through various intracellular mechanisms. An antioxidant could be preventive or chain breaking. Preventive antioxidants (catalase, glutathione, peroxidase, vitamin C) inhibit the initial production of free radicals including ROS, while chain breaking antioxidants (superoxide dismutase, vitamin E) inhibit the damaging phase of ROS (Valko *et al.,* 2007). Recent biochemical advances have been focused on antioxidants and their potency in minimizing the damaging effects of free radicals, as well as their roles in potentiating drug efficacy. Some biochemical changes regarding the oxidant and antioxidant levels in malaria infected patients have been observed (Kulkarni *et al.,* 2003).

Glutathione is a cysteine-containing peptide found in most forms of aerobic life. It is not required in the diet and is instead synthesized in cells from its constituent amino acids. Glutathione has antioxidant properties since the thiol group in its cysteine moiety is a reducing agent and can be reversibly oxidized and reduced. In cells, glutathione is maintained in the reduced form by the enzyme glutathione reductase and in turn reduces other metabolites and enzyme systems, such as ascorbate in the glutathione-ascorbate cycle, glutathione peroxidases and glutaredoxins, as well as reacting directly with oxidants. Tocopherols and tocotrienols (vitamin E): is the collective name for a set of eight related tocopherols and tocotrienols, which are fat-soluble vitamins with antioxidant properties (Packer *et al.,* 2001).

Vitamin C is an antioxidant, which is important for ones skin, bones, and connective tissue. It promotes healing and helps the body absorb iron (Azzi, 2007). Vitamin C comes from fruits and vegetables. Good sources include citrus, red and green peppers, tomatoes, broccoli, and greens. Vitamin A is an antioxidant, which plays roles in reducing the effect of free radicals, molecule produced when one's body breaks down food, or by environmental exposures like tobacco smoke or radiation. It also plays roles in vision, born growth and cell formation. Catalases are enzymes that catalyse the conversion of hydrogen peroxide to water and oxygen, using either an iron or manganese cofactor (Chelikani *et al.,* 2004).

Superoxide dismutases (SODs) are a class of closely related enzymes that catalyze the breakdown of the superoxide anion into oxygen and hydrogen peroxide. SOD enzymes are present in almost all aerobic cells and in extracellular fluids (Johnson and Giulivi 2005). Superoxide dismutase enzymes contain metal ion cofactors that,

depending on the isozyme, can be copper, zinc, manganese or iron. In humans, the copper/zinc SOD is present in the cytosol (Johnson and Giulivi, 2005).

Peroxidase has been found well suited for the preparation of enzyme conjugated antibodies, due in part to its ability to yield chromogenic products, and in part to its relatively good stability characteristics. Peroxidase labeled immunoglobulins have been used successfully as immunohistological probes for the demonstration of tissue antigens, and in enzyme amplified immunoassay systems for the quantitative determination of soluble and insoluble antigens (Grey *et al.*, 2003) have described novel soluble peroxidase-antiperoxidase techniques for immune histochemistry and immunoassay (Grey *et al.*, 2003). Haem peroxidases (or haem peroxidases) are haem-containing enzymes that use hydrogen peroxide as the electron acceptor to catalyse a number of oxidative reactions.

Lipid peroxidation refers to the oxidative degradation of lipids. It is the process in which free radicals "steal" electrons from the lipids in cell membranes, resulting in cell damage. This process proceeds by a free radical chain reaction mechanism. It most often affects polyunsaturated fatty acids, because they contain multiple double bonds in between which lie methylene bridges (-CH2-) that possess especially reactive hydrogen (Ostrea and Enrique, 1999). As with any radical reaction, the reaction consists of three major steps: Initiation, Propagation termination (Ostrea and Enrique, 2010).

Initiation is the step in which a fatty acid radical is produced. The most notable initiators in living cells are reactive oxygen species (ROS), such as OH• and (HO_2), which combines with a hydrogen atom to make water and a fatty acid radical. The fatty acid radical is not a very stable molecule, so it reacts readily with molecular oxygen, thereby creating a peroxyl-fatty acid radical. This radical is also an unstable species that reacts with another free fatty acid, producing a different fatty acid radical and lipid peroxide, or cyclic peroxide if it had reacted with itself. This cycle continues, as the new fatty acid radical reacts in the same way (Ostrea and Enrique, 2010). When a radical reacts with a non-radical, it always produces another radical, which is why the process is called a "chain reaction mechanism". The radical reaction stops when two radicals react and produce a non-radical species. This happens only when the concentration of radical species is high enough for there to be a high probability of collision of two radicals. Living organisms have different molecules that speed up termination by catching free radicals and, therefore, protecting the cell membrane. One important such antioxidant is vitamin E. Other anti-oxidants made within the body include the enzymes superoxide dismutase, catalase, and peroxidase (Marnett, 1999).

Malaria is a mosquito-borne infectious disease of the blood caused by the parasite, *Plasmodium sp*. It spreads through the bite of infected female *Anopheles* mosquito and is endemic in tropical and sub-tropical regions including parts of America, Asia and Africa. Malaria represents a medical emergence because it may rapidly progress to complication and death without prompt and appropriate treatment. The malarial infection remains a devastating global problem with an estimated 300 – 500 million cases occurring annually (WHO, 2000), killing between 1–3 million people each year. Malaria infection has the ability to activate the immune system which causes the release of reactive oxygen species (ROS)

with the potency of inducing oxidative damage and cell destruction (Kremsner *et al.,* 2000). The body however, has a number of mechanisms to minimize the cellular effects of ROS. These defense mechanisms include the production of antioxidants. Antioxidants may be obtained from diets or synthesized in the body through various intracellular mechanisms. An antioxidant could be preventive or chain breaking. Preventive antioxidants (catalase, glutathione, peroxidase, and vitamin C) inhibit the initial production of free radicals including ROS, while chain breaking antioxidants (superoxide dismutase, uric acid, vitamin E) inhibit the damaging phase of ROS (Valko *et al.,* 2007).

Plasmodium berghei is a unicellular protozoan parasite that infects mammals other than human. It is one of the four Plasmodium species that has been described in African murine, the others being: *Plasmodium chabaudi, P. vinckei, P. yoelii* (Vincke and Lips, 1948). They are not of direct concern to man practically; the interest of man in these parasites is that they are practical model organisms in the laboratory for the experimental study of human malaria (Vincke and Lips, 1948). Rat malaria parasites are used in many research institutes for studies aiming at development of new drugs or vaccine against malaria (Amino *et al.,* 2005). Like all malaria parasites of mammals, *Plasmodium berghei* is transmitted by anopheles mosquitoes and it infects the liver after being released into the bloodstream by a bite from an infected female mosquito. After a few days of development and multiplication, these parasites leave the liver and invade erythrocytes. The multiplication of the parasite in the blood causes damage to essential organs of the host such as lungs, liver, spleen, and it may also affect the brain and can be the cause of cerebral complications in laboratory mice. These symptoms are to a certain degree comparable to symptoms of cerebral malaria in patients infected with the human malaria parasite, *Plasmodium falciparum* (Hall *et al.,* 2005). *Plasmodium berghei* is found in the forests of Central Africa, where its natural cyclic hosts are the thicket rat (*Grammomys surdaster*) and the mosquito (*Anopheles dureni*) the vector. These Rodent malaria parasites are used in many research institutes for studies aiming at the development of new drugs or a vaccine against malaria.

Plants are considered as one of the most valuable sources of food, medicine and drug for prevention of illness and maintenance of human health. In Northern Nigeria, many indigenous plants are widely consumed as food or home remedies especially for the treatment or management of common diseases. The importance of plants in medicine remains even of greater relevance with the current global shift to obtain drugs from plants sources, as a result of which attention has been given to the medicinal value of herbal remedies for safety efficiency and economy (Glombitiza *et al.,* 1993). A plant as a source of herbal medicine is the oldest form of medicine known to mankind. It was the mainstay of many earlier civilization and still the most widely practiced form of medicine in the world today. *Cymbopogon citractus* and *Hyptis spicigera* are also one of those plants that serve the purpose of prevention of illness, maintenance of human health and management of Disease (medicinal plants).

Cymbopogon citratus is a perennial grass that grows spontaneously around the world, mainly in the tropical and savannah regions. The genus *Cymbopogon* includes around 30 grass species, most of all native from the Old World (Figueirinha *et al.,* 2010). The

name *Cymbopogon* is derived from the Greek words kymbe (boat) and pogon (beard), referring to the flower spike arrangement. *Citratus* derives from the ancient Latin, meaning lemon-scented leaves. *Cymbopogon citratus* is native from the southwest Asia and, now, it grows spontaneously around the world, mainly in the tropical and savannah regions. Perennial herb, it grows forming dense clumps of up to 3 m tall, with short rhizomes. Its leaves are erect, glabrous plane, more than 1m long, 5-15mm wide, whiter upper face and closed edge in the base, with rough margins and membranaceous or arid ligules 4–5mm long. Erect inflorescences, usually in pairs of terminal spiciform racemes 30–60cm long. Sessile small spikes, canaliculated ventral side, 4.5-5.0mm long, 0.8 -1.0mm wide, ciliated margins. Equal or sub equal glumes. The inferior glume is lance-shaped, bicarinated, with bilobulated apex and with acutely curved margins from the middle upwards. The superior glume is lanceolated, 4.3-4.5mm long, usually 1- nervate. Sterile lanceolated lemma, 3.5mm long, 2- nerved, ciliated (Baldacchino *et al.,* 2013). In the folk medicine of Brazil it is believed to have anxiolytic, hypnotic and anticonvulsant properties (Blanco *et al.,* 2009). But at least one study has found no effect on humans. Laboratory studies have shown that *Cymbopogon citractus* has cytoprotective, antioxidant, and anti-inflammatory properties in vitro, as well as anti-fungal properties (Bastos *et al.,* 2010).

Cymbopogon citractus essential oil (Citronellol) possesses antihypertensive properties and has been shown lower blood pressure in rats by a direct effect on the vascular smooth muscle leading to vasodilation (Bastos *et al.,* 2010). In a small randomized controlled trial, an infusion made from *Cymbopogon citratus* was used as an inexpensive remedy for the treatment of oral thrush in HIV/AIDS patients (Wright *et al.,* 2009).

Hyptis spicigera is an erect aromatic herb, up to 1 m in height with a terminal inflorescence in which the seeds are packed in quadruplets or more in the flowers. The seeds are very small and light and are very tiny brown/black seeds that clustered in groups of fours, fives or even more which are encased in each flower that make up the inflorescence. It is commonly called Black Beni seed or black sesame. The whole plant is used in traditional stores/barns to protect cowpea and maize against damage by Callosobruchus species (Lambert *et al.,* 1985) by resource poor West-African farmers. It is also locally used as mosquito repellent by burning of the whole plant in enclosures (Dalziel, 1937). The seeds and the leaves are eaten as vegetables because of its spicy aroma. Several researchers have reported on the volatile oils and the seeds of *Hyptis spicigera* to possess insecticidal, pharmacological and biological activity (Othira *et al.,* 2008).

The peroxide value of 1.95 meq. H_2O_2 showed the oil to be stable to oxidative degradation cause by over exposure to oxygen, heating and improper storage. Peroxide value depends on a number of factors such as oxidation by oxygen, extraction methods and storage (Dhellot *et al.,* 2006). *Hyptis spicigera* seed oil with high linoleic levels is also potentially useful as food additive and can be converted to gamma-linolenic acid that could be used as a dietary supplement to increase the production of anti-inflammatory l-series prostaglandins.

The vitamin E content of *Hyptis spicigera* oil is relatively high (186.15 mg mL-1). Vitamin E is antioxidants which have been correlated with lowering of cholesterol levels. Though the oil is rich in vitamin E, there is an insufficient research finding on

the medicinal properties of *Hyptis spicigera* oil (Wretenjo and Karlberg, 2002). *Hyptis spicigera* seed oil is rich in linoleic fatty acid and vitamin E and therefore is a potential source of vitamin E and linoleic acid which could find use in reducing the risk of coronary heart disease (CHD). It is used locally as mosquito repellent by burning of the whole plant in closures and the whole plant is used in traditional stores to protect cowpea and maize against damage by *Callosobruchus* species (Lambert *et al.*, 1985). The study therefore evaluates the comparative effect on antioxidant activities of *Cymbopogon citractus* and *Heptis spicigera* leaves extracts from Abakaliki, Nigeria in albino rats.

Material and Methods

Collection and preparation of *Cymbopogon citractus* and *Heptis spicigera* leaves extracts

Fresh leaves of *Cymbopogon citractus* and *Heptis spicigera* where collected from Ogwudu-ano village in Umuogudu-oshia Ngbo, Ohaukwu Local Government Area of Ebonyi state,Nigeria and was classified by a taxonomist Prof., S. Onyekwasi at Department of Applied Biology, Ebonyi State University, Abakaliki, Nigeria. A part was also deposited in the hebarium for reference purposes.

The leaves of *Cymbopogon citractus* and *Heptis spicigera* leaves extracts were washed thoroughly under running tap water, shade dried and pulverized, using a grinding machine. 200g of *Cymbopogon Citractus* and *Hyptis spicigera* were soaked in 1200mls of ethanol and were allowed for 24hours respectively. The mixtures were filtered using clean sieve cloth and the filtrates evaporated and dark past extract were formed. The extract were stored in dried containers and stored in refrigerator for further usage.

Experimental animals

A total of 48 mice weighing were used for the study. They were purchased from the Pharmacy Department University of Nigeria, Nsukka, Nigeria and acclimatized (for 7 days) and maintained at normal room temperature in the Experimental Animal House of the Faculty of Biological Sciences, Ebonyi State University, Abakaliki, Nigeria. They were housed in stainless mice cages and allowed access to water and food *ad-libitum.* At the start of the experiment, all the animals were weighed and subsequently at week intervals. The mice were randomly assigned into four groups A, B, C and D. Group A with 6 mice was used as negative control and was given only feed and water while group B was used as positive control and was treated with chloroquine. Group C and D were sub-divided into C1, C2, C3, D1, D2, and D3 with 6 mice each. The sub-groups were administered 200, 400 and 800mg/kg body weight of *Cymbopogon citratus* and *Hyptis spicigera* extracts respectively for seven days. Mice from all the groups were allowed free access to water and feed.

Experimental Design

In this experiment, a total of 48 mice were used. The mice were randomly assigned into four groups A, B, C and D. Group A with 6 mice was used as negative control and was given only feed and water while group B was used as positive control and was treated with chloroquine. Group C and D were sub-divided into C1, C2, C3, D1, D2, and D3 with 6 mice each. The sub-groups were administered 200, 400 and 800mg/kg body weight of *Cymbopogon citratus* and *Hyptis spicigera* extracts respectively for seven

days. Group A**:** Received no extract. Group B: mice received chloroquine once for 7 days. Group C1: Mice received ethanol extract of *Cymbopogon citratus* leaf of 200mg/kg body weight daily for 7days. Group C2: Mice received ethanol extract of *Cymbopogon citratus* leaf of 400mg/kg body weight daily for 7days. Group C3: Mice received ethanol extract of *Cymbopogon citratus* leaf of 800mg/kg body weight daily for 7 days. Group D1: Mice received ethanol extract of *Hyptis spicigera* leaf of 200mg/kg body weight daily for 7days. Group D2: Mice received ethanol extract of *Hyptis spicigera* leaf of 400mg/kg body weight daily for 7days. Group C3: Mice received ethanol extract of *Hyptis spicigera* leaf of 800mg/kg body weight daily for 7days. After the treatment period (7 days), the mice of all the groups were sacrificed. The mice were dissected and 3 ml of whole blood drawn through cardiac puncture. The livers were collected using specimen bottles in Normal saline and were stored in refrigerator for further use. The collected livers were homogenized using mortar and pestle and stored until required. The blood was dispensed into centrifuge tubes and centrifuged at 5000 rpm for 10 minutes. After centrifugation, the serum was then separated from the blood cells and used for assay of serum reduce glutathione level, catalase, superoxide dismutase and Lipid Peroxidation activities were determined using the method described by Oyedemi *et al.* (2010) while peroxidase activity was determined using the method described by Reddy *et al.,* (1985). A Statistical analysis: Data obtained were subjected to a one way analysis of variance ANOVA using the General Liner Model procedure of SAS (version 6.04) (SAS Institute, 1994). Comparison of significant treatment means was by least significance differences (LSD) as outlined by Obi (2002).

Results and Discussion

The treatment of mice with ethanol leaf extracts of *Hyptis Spicigera* and *Cymbopogoon citratus* on the body weights of mice showed that there was no significant ($p>0.05$) increase in the body weight of mice within 7 days of treatment as shown in table 1 and 2. The treatment mice with ethanol Leaf extracts of *Cymbopogon citratus* and *Hyptis spicigera* 200,400, and 800 mg/kg body weight to mice significantly ($P<0.05$) increased the level of reduced glutathione (GSH), peroxidase activity, activity of superoxide dismutase (SOD) and catalase (CAT) activity in mice in doses dependent manner while the administration of the same extracts significantly ($P<0.05$) decreased the activity of lipid peroxidation marker(MDA) in a doses dependent manner as shown in tables 3–7. The results showed that there was no significant ($P>0.05$) difference in the oxidative stress indices among the mice treated with the extracts at different doses (Tables 3–7). The treatment of mice with ethanol leaf extracts of *Hyptis Spicigera* and *Cymbopogoon citratus* on the body weights of mice showed that there was no significant ($p>0.05$) increase in the body weight of mice within 7 days of treatment as shown in table 1 and 2.

The treatment mice with ethanol Leaf extracts of *Cymbopogon citratus* and *Hyptis spicigera* 200,400, and 800 mg/kg body weight to mice significantly ($P<0.05$) increased the level of reduced glutathione (GSH), peroxidase activity, activity of superoxide dismutase (SOD) and catalase (CAT) activity in mice in doses dependent manner while the administration of the same extracts significantly ($P<0.05$) decreased the activity of lipid peroxidation marker(MDA) in a doses dependent manner as shown in tables 3–7. The results showed that there was no significant ($P>0.05$) difference in the oxidative stress indices among the mice

treated with the extracts at different doses (Tables 3–7).

Table.1 Effects of ethanol leaf extracts on *Hyptis Spicigera* on the body weights of mice

Plant/Dosage (mg/kg)	initial wt (g)	Final wt (g)	change in wt (g)	%change in wt
0	33.0±1.23[b]	27.67±2.32[a]	-5.33	-16.15
200	19.50±2.38[a]	19.25±03.59[a]	-0.25	-1.28
400	23.50±4.38[b]	22.75±3.86[a]	-1.75	-9.21
800	19.00±4.83[b]	17.25±4.24[a]	-1.75	-9.21
CQ	20.25±3.40[a]	20.50±1.73[a]	0.25	1.24

SD, n = 6 mice per group. Values in same column with superscripts are significantly different from each other at P<0.05.

Table.2 Effects of ethanol leaf extracts of *Cymbopogoon citratus* on the body weights of mice

Plant/Dosage (mg/kg)	initial wt (g)	Final wt (g)	change in wt (g)	%change in wt
0	33.00±1.73[b]	27.67±2.52[a]	-5.33	-16.15
200	33.00±1.73[a]	32.00±2.00[a]	-1.00	-3.03
400	24.00±1.00[a]	25.00±3.51[a]	1.00	4.17
800	19.33±2.08[a]	17.25±4.24[a]	0.00	0.00
CQ	20.25±3.40[a]	20.50±1.73[a]	0.25	1.24

SD, n = 6 mice per group. Values in same column with superscripts are significantly different from each other at P<0.05.

Table.3 SOD activities of mice treated with ethanol leaf extracts of *Cymbopogon citratus* and *Hyptis spicigera*

Treatment	***H. specigera***	***C. citratus***
0 mg/kg	0.16±0.3[a]	0.16±0.3[a]
200mg/kg	0.46±0.26[ab]	0.21±0.5[a]
400mg/kg	0.56±0.27[b]	0.29±0.10[a]
800mg/kg	0.56±0.32[b]	0.43±0.36[a]
CQ 5mg/kg	0.21±0.05[ab]	0.21±0.8[a]

Values are expressed as Mean ± S.D, n = 6 mice per group. Values in the same row with different superscripts are significantly different from each other at P< 0.05

Table.4 Catalase activities of mice treated with ethanol leaf extracts of *Cymbopogon citratus* and *Hyptis spicigera.*

Treatment	***H. specigera***	***C. citratus***
0 mg/kg	0.09±0.01[a]	0.09±0.01[a]
200mg/kg	0.17±0.05[ab]	0.26±0.04[b]
400mg/kg	0.30±0.15[b]	0.29±0.02[b]
800mg/kg	0.30±0.07[b]	0.29±0.04[b]
CQ 5mg/kg	0.15±0.08[ab]	0.15±0.8[a]

Values are expressed as Mean± S.D, n = 6 animals per group. Values in some row with different superscripts are significantly different from each other at P<0.05

Table.5 Peroxidase activities of mice treated with ethanol leaf extracts of *Cymbopogon citratus* and *Hyptis spicigera*

Treatment	***H. specigera***	***C. citratus***
0 mg/kg	0.19±0.08[a]	0.19±0.08[a]
200mg/kg	0.26±0.10[ab]	0.68±0.10[b]
400mg/kg	0.31±0.19[ab]	0.95±0.12[a]
800mg/kg	0.43±0.16[ab]	0.29±0.19[a]
CQ 5mg/kg	0.47±0.22[b]	0.47±0.22[a]

Values are expressed as Mean± S.D, n = 6 animals per group. Values in same row with different superscripts are significantly different from each other at P<0.05

Table.6 Reduced glutathione level of mice treated with ethanol leaf extracts of *Cymbopogon citratus* and *Hyptis spicigera.*

Treatment	***H. specigera***	***C. citratus***
0 mg/kg	0.24±0.31[a]	0.24±0.31[a]
200mg/kg	0.66±0.02[b]	0.50±0.19[b]
400mg/kg	0.67±0.05[b]	0.71±0.03bc
800mg/kg	0.72±0.03[b]	0.83±0.14c
CQ 5mg/kg	0.68±0.03[b]	0.68±0.03[b]

Values are expressed as Mean± S.D, n = 6 animals per group. Values in same row with different superscripts are significantly different from each other at P<0.05

Table.7 Lipid peroxidation activities of mice treated with ethanol leaf extracts of *Cymbopogon citratus* and *Hyptis spicigera*

Treatment	***H. specigera***	***C. citratus***
0 mg/kg	0.68±0.18[b]	0.68±0.18c
200mg/kg	0.11±0.06[b]	0.49±0.56a[b]
400mg/kg	0.11±0.01[b]	0.48±0.15a[b]
800mg/kg	0.07±0.07[b] 0.23±0.08[a]	
CQ 5mg/kg	0.22±0.05[a]	0.22±0.05bc

Values are expressed as Mean± SD, n = 6 animals per group. Values in same row with different superscripts are significantly different from each other at P<0.05.

This increase in glutathione, catalase, peroxidase and superoxide dismutase may be as a result of inhibition in the proliferation of the plasmodium berghei and reacting oxygen species by the extracts unlike negative control (induced but not treated) which there is increase in lipid peroxidation, and the standard drug chloroquine (group E) positive control which gave the highest effect after treatment, This slight difference may be as a result of crude nature of the extract. Aja (2014) earlier reported that *Moringa oleifera* seed and leaf extracts significantly (P <0.05) increased serum superoxide dismutase

(SOD), glutathione (GSH) and catalase (CAT) levels in rat.

According to Settaf *et al.,* 2000 the treatment of rats with hepatic injury after subjecting them to ischemia and reperfusion brought about normalization of plasma GSSG/GSH ratio, a good index of an antioxidant. This increase in antioxidant activity of *Hyptis spicigera* and *Cymbopogon citratus* after administration of plant extracts shows efficient protective function of the plants. Ladan *et al.,* (2010) reported that *Hyptis Spicigera* oil is stable to oxidative degradation caused by exposure to oxygen, heat and improper storage because of peroxide value of1.9 meq. H_2O_2. He also observed that this plant contain palmitic acid (16.0 %) and linoleic acid (71.85%) with a particular interest of fighting against cardiovascular diseases and Vitamin E which have being correlated with lowering of cholesterol level. Though the oil is rich in vitamin E, there is an insufficient research finding on medicinal property of *Hyptis Spicigera* (Dhellot *et al.,* 2006).

It was also observed that after the treatment of the animals with *Hyptis spicigera* and *Cymbopogon citratus* leaf extracts, there was an increase in the physical activity of the mice which were infected with *plasmodium berghei* after treatment, which showed that the leaf extract of *Hyptis spicigera* and *Cymbopogon citratus* have anti-malaria effects. Meera *et al.*, (2009) observed that the leaf extract of *O. basilicum linn* and *Trigonella foenum linn* against H_2O_2 and CCl_4 showed excellent activities of antioxidants properties by reducing oxidative stress and free radicals and also decrease in lipid peroxidation in goat liver. Also according to the work of (Iribhogbe *et al*, 2013) during evaluation of anti-plasmodial effect of antioxidant micronutrient on plasmodium berghei infected mice, they recorded a significant ($P<0.05$) anti-plasmodial activity which suggested that this may be due to potentiating of erythrocyte membrane stabilization, therefore the anti-plasmodial activity of *Hyptis Spicigera* and *Cybopogon Citratus* may be as a result of its possession of antioxidant micronutrients with the ability to potentiate erythrocyte membrane stabilization.

This study therefore revealed that ethanol leaf extracts of *Cymbopogon Ctractus* and *Hyptis Spcigera*, have antioxidant properties and may be useful in the treatment malaria and management of oxidative stress.

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