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EVALUATION OF ANTIOXIDANT AND ANTI-INFLAMMATORY POTENTIAL OF METHANOL EXTRACT OF *OCIMUM GRATISSIMUM* LEAF

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ABSTRACT

The rich history of traditional medicine, rooted in the use of medicinal plants across diverse cultures for thousands of years. *Ocimum gratissimum*, commonly known as African basil is renowned for its versatile medicinal properties. This study evaluated the *in-vitro* antioxidant and anti-inflammatory activities of the methanol extract *Ocimum gratissimum* leaf. The study employed various standard assays for evaluating antioxidant and anti-inflammatory, which includes hydrogen peroxide, nitric oxide scavenging, lipid peroxidation, reducing power, heat-induced hemolysis, protein denaturation, proteinase inhibitory, and lipoxygenase inhibition activities. The results indicate significant antioxidant and anti-inflammatory potential methanol extract *Ocimum gratissimum* leaf in a concentration dependent manner. The anti-inflammatory potential revealed hemolysis inhibition (4.20 – 18.33%), inhibition of protein denaturation (25.50 – 64.10%), proteinase inhibition (14.80 – 30.01%) and lipoxygenase inhibition (8.19 – 30.95%). of as a valuable resource in traditional medicine. *Ocimum gratissimum's* methanol leaf extract demonstrated notable antioxidant activities, as evidenced by its ability to scavenge hydrogen peroxide, inhibit nitric oxide, and mitigate lipid peroxidation. Additionally, the extract exhibited anti-inflammatory properties through inhibition of heat-induced hemolysis, protein denaturation, proteinase, and lipoxygenase activities. Therefore, usage of *Ocimum gratissimum* leaf as potential sources of safe and effective therapeutic agents for ameliorating oxidative and inflammation related disease should be encouraged.

KEYWORDS: *Ocimum gratissimum,* anti-oxidant, anti-inflammation, oxidative stress.

INTRODUCTION

Traditional medicine has a rich history spanning thousands of years, with medicinal plants serving as its ancient foundation, employed across diverse cultures globally (Khan, 2014). The accumulated wisdom about the positive impacts of these plants has been shared within communities over centuries. In contemporary pharmacotherapy, natural products are integral, with numerous modern drugs deriving inspiration from traditional herbal medicine (Patwardhan *et al*., 2008). The World Health Organization WHO (2008), reports that approximately 80% of individuals in developing nations rely on traditional herbal mixtures for treating various ailments. In many African villages, reliance on traditional herbal remedies persists due to cultural acceptance, accessibility, and affordability. Such remedies, deeply rooted in indigenous knowledge, are often deemed effective for maintaining health and treating diverse diseases (Bandaranayake, 2006; Bodeker *et al*., 2005).

The exploration of active compounds within plants, facilitated by methods like GC-MS, has led to the discovery of medicinal drugs effective against conditions such as cancer and Alzheimer's disease (Sheeja *et al*., 2007). Phytochemical analysis, particularly through techniques like GC-MS, is crucial for understanding the bioactive components in medicinal plants (Koduru *et al*., 2006). Higher plants continue to be rich sources of bioactive compounds crucial for human health, known for their non-phytotoxic nature, systemic effectiveness, and biodegradability (Kushik *et al*., 2002). The global interest in studying traditional plants and their medicinal value has surged in recent times. These plants are increasingly investigated due to their strong pharmacological activities, economic viability, and low toxicity (Prashant *et al*., 2010). The growing fascination with plant-derived drugs is fueled by the belief that herbal medicine is safer and more reliable compared to expensive orthodox medicine, which may have adverse side effects (Jigna and Sumitra, 2006).

Researchers have traditionally acknowledged the diverse therapeutic effects of *Ocimum gratissimum*. The exploration of active compounds in plants has resulted in the discovery of novel medicinal drugs, offering effective safeguarding and treatment capabilities against a range of diseases. *Ocimum gratissimum*, commonly referred to as African basil, stands as a versatile medicinal herb, widely embraced for its diverse therapeutic attributes. Within West African households, it is a trusted remedy for addressing gastrointestinal issues like diarrhea, colitis, and stomach ulcers (Oppong Bekoe *et al*., 2021). In Benin, this botanical gem serves

dual purposes, finding applications in both medicinal and culinary realms, with its esteemed antibiotic qualities (Kpètèhoto *et al*., 2020). The leaves and seeds of this plant boast a rich tapestry of ethnomedicinal properties, celebrated for their antimicrobial, antidiabetic, and antioxidant prowess (Ugbogu *et al*., 2021b). Its reputation extends further to encompass chemopreventive and anticarcinogenic virtues, elevating its status as a prized asset in traditional medicine (Priyanka *et al*., 2018). The present study aimed at bioactive compound profiling, *in-vitro* antioxidant and anti-inflammatory activity of *O.gratissimum* methanol leaf extract.

MATERIALS AND METHOD

Chemicals / Reagents

All chemicals and reagents used were good and of analytical grade.

Plant Collection

Fresh plant of *Ocimum gratissimum* was collected from Ekeapkara Osisioma, Abia State Nigeria. The plants were identified and authenticated by a Plant Taxonomist at the Michael Okpara University of Agriculture Umudike and deposited in the herbarium of the same institution.

Extract preparation and fractionation

Five hundred grams (500g) of powdered leaves were macerated in 2.5L of methanol at room temperature for 72h. It was continuously mixed and then filtered using filter paper (Whatman size No.1). The filtrate was dried in a water bath at 45ºC and the concentrate was kept in air tight bottle at 4°C until used (Unegbu *et al*., 2017).

In vitro antioxidants

Determination of hydrogen peroxide scavenging capacity

The hydrogen peroxide (H_2O_2) scavenging activity of the extract was determined by the method of (Srinivasan *et al*., 2007) with slight modification. A solution of hydrogen peroxide (20mM) was prepared in phosphate buffered saline (pH 7.4). Different concentrations of plant extract and standard ascorbic acid solution (200, 400, 600 and 800 µg/ml) in methanol were prepared. 1ml of each was separately added to H_2O_2 solution (2ml) and mixed. Each tube was allowed to stand for 10min after which absorbance at 230 nm of UV-visible spectrophotometer (Jasco V-630) was read against a blank solution containing phosphate buffer in place of H_2O_2. The percentage inhibition activity was calculated as follows.

% inhibition = [(A0–A1)/A0]×100.

Where A0 is the absorbance of the control, and A1 is the absorbance of extract/standard. All the tests were performed in triplicates.

Determination of reducing power

The method described by Kumaran and Karunakaran (2007) was used. Different concentrations of plant extract and standard ascorbic acid solution (200, 400, 600 and 800 µg/ml in methanol) were prepared. 1mL of each was mixed separately with 0.2M phosphate buffer (2.5 mL, pH 6.6) and 1% potassium ferricyanide [K3Fe(CN)6] (2.5 mL). The mixture was incubated at 50°C for 20min. A portion (2.5 mL) of 10% trichloroacetic acid was added to the mixture, which was then centrifuged at 3,400 g for 10 min at room temperature. The upper layer of solution (2.5 mL) was mixed with 2.5 mL distilled water and 0.5 mL of 0.1% $FeCl_3$ solution. The absorbance, which reflects increase in reducing power, was measured at 700 nm using UV-visible spectrophotometer (Jasco V-630). All the tests were performed in triplicates.

Determination of nitric oxide (NO) scavenging activity

The NO scavenging activity of the extract was determined by the method of Daljit and Priyanka (2010). An aliquot (6mL), of 5 mM sodium nitroprusside solution was mixed with 6ml of different concentrations (200, 400, 600 and 800 µg/ml) of extract and standard vit C and incubated at 25^{o}C for 150minutes. The reaction mixture (0.0ml) was removed at 30 min intervals, and mixed with an equal 0.5 mL of Griess reagent (1 % sulphanilamide, 2 % phosphoric acid, and 0.1 % napthylethylene diamine dihydrochloride). The absorbance of the mixture was then read at 546 nm using UV-visible spectrophotometer (Jasco V-630). All the tests were performed in triplicates.

Determination lipid peroxidation assay

Inhibitory Capacity of Extract on Lipid Peroxidation by Malondialdehyde (MDA) assay was determined colorimetrically using thiobarbituric acid (TBA), as described by Okolie *et al*. (2009) with slight modifications. 1g of egg albumin was homogenized with 100mL of iced physiological saline for 15min. The homogenate was centrifuged at 8000g for 5 min. 1mL of the supernatant was then added to 2.0mL of TCA-TBA –HCl reagent (15 % TCA, 0.375 % TBA, 0.25 M HCl) and 1ml of the sample, the solution was mixed thoroughly and placed in a boiling water bath for 15 min. On cooling, the protein precipitate was removed by

centrifugation at 10,000 g for 5min and the absorbance of the clear, pink supernatant fraction was read at 535 nm against reagent blank.

Evaluation of *in Vitro* Anti-Inflammatory Activity

Heat-Induced Hemolysis

This test was carried out as described by Okoli et al. (2008), with some modifications as described in Gunathilake et al. (2018). Briefly, 0.05 mL of blood cell suspension and 0.05 mL of hydromethanolic extracts of leaves were mixed with 2.95 mL phosphate buffer (pH 7.4). The mixture was incubated at 54 ˚C for 20 min in a shaking water bath. After the incubation, the mixture was centrifuged (2500 rpm for 3 min), and the absorbance of the supernatant was measured at 540 nm using a UV/VIS spectrometer. Phosphate buffer solution was used as a control for the experiment. The level of hemolysis was calculated using the following equation based on the Okoli et al. (2008). % inhibition of hemolysis = 100 × (1 − A2/A1).

where A1 = absorption of the control, and A2 = absorption of test sample mixture.

Effect on Protein Denaturation

Protein denaturation assay was done according to the method described by Gunathilake et al. (2018). The reaction mixture (5 mL) consisted of 0.2 mL of 1% bovine albumin, 4.78 mL of phosphate buffered saline (PBS, pH 6.4), and 0.02 mL of extract, and the mixture was mixed, and was incubated in a water bath (37 ˚C) for 15 min, and then the reaction mixture was heated at 70 ˚C for 5 min. After cooling, the turbidity was measured at 660 nm using a UV/VIS spectrometer. Phosphate buffer solution was used as the control. The percentage inhibition of protein denaturation was calculated by using the following formula.

% inhibition of denaturation = 100 × (1 − A2/A1),

where A1 = absorption of the control sample, and A2 = absorption of the test sample.

Proteinase Inhibitory Activity

Proteinase inhibitory activity of the leaf extracts was performed according to the method of Sakat et al. which is modified by Gunathilake et al. (2018). Briefly, the reaction solution (2 mL) consisted of 0.06 mg trypsin, 1 mL of 20 mM Tris-HCl buffer (pH 7.4), and 1 mL test sample (0.02 mL extract 0.980 mL methanol). The solution was incubated (37 ˚C for 5 min), and then 1 mL of 0.8% (*w*/*v*) casein was added, and the mixture was further incubated for an additional 20 min. At the end of incubation, 2 mL of 70% perchloric acid was added to terminate the reaction. The mixture was centrifuged, and the absorbance of the supernatant was measured at 210 nm against buffer as the blank. Phosphate buffer solution was used as

the control. The percentage inhibition of protein denaturation was calculated by using the following formula.

% inhibition of denaturation = 100 × (1 − A2/A1),

where A1 = absorption of the control sample, and A2 = absorption of the test sample.

Lipoxygenase Inhibition Assay

Lipoxygenase inhibition activity of the extracts of leafy vegetables was assayed according to the method of Wu with some modifications as described in Gunathilake et al. (2018). Briefly, a mixture of a solution of sodium borate buffer (1 mL, 0.1 M, pH 8.8) and lipoxygenase (10 µL, final concentration 8000 U/mL) was incubated with 10 mL leaf extract in a 1 mL cuvette at room temperature (30 ± 2 °C) for 5 min. The reaction was initiated by the addition of 10 µL linoleic acid substrate (10 mmol). The absorbance of the reaction solution was measured at 234 nm using a UV/VIS spectrometer (Optima, SP-3000, Tokyo, Japan). Phosphate buffer solution was used as the control, and the percentage inhibition of lipoxygenase was calculated using the following equation.

% inhibition = 100 × (absorbance of the control − absorbance of the sample)/absorbance of the control.

Statistical Analysis

Statistical analysis of the data was carried out with SPSS version 22.0 using One Way Analysis of Variance (ANOVA) with Duncan post hoc test. The statistically analyzed data were reported as Mean ± SEM. A significant difference was accepted at 95% confidence level of probability ($P < 0.05$).

RESULTS AND DISCUSSION

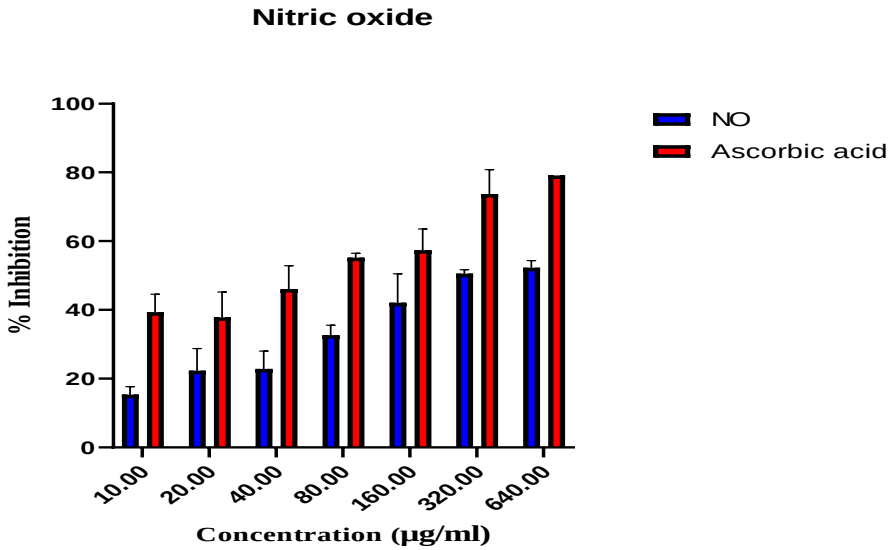

Figure 1: Nitric oxide scavenging activity of the leaf extract of *O.grastissimum.*

The result showed some significant increase (P<0.05) in % nitric oxide inhibition activity of the methanol leaf extract in increasing concentration in comparison to the standard control.

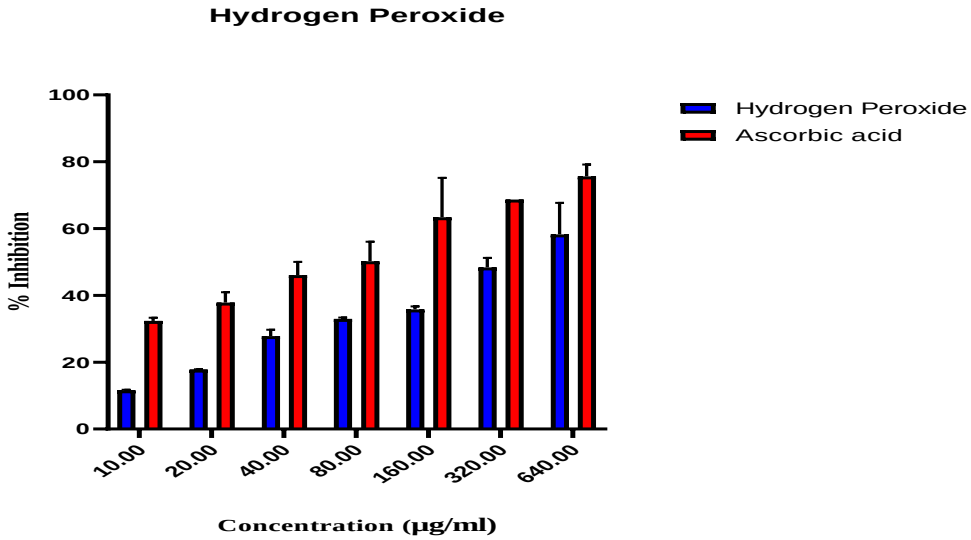

Figure 2: Hydrogen peroxide scavenging activity of the leaf extract of *O.grastissimum.*

The result showed significant increase (P<0.05) in % hydrogen peroxide inhibition activity of the methanol leaf extract in concentration dependent manner.

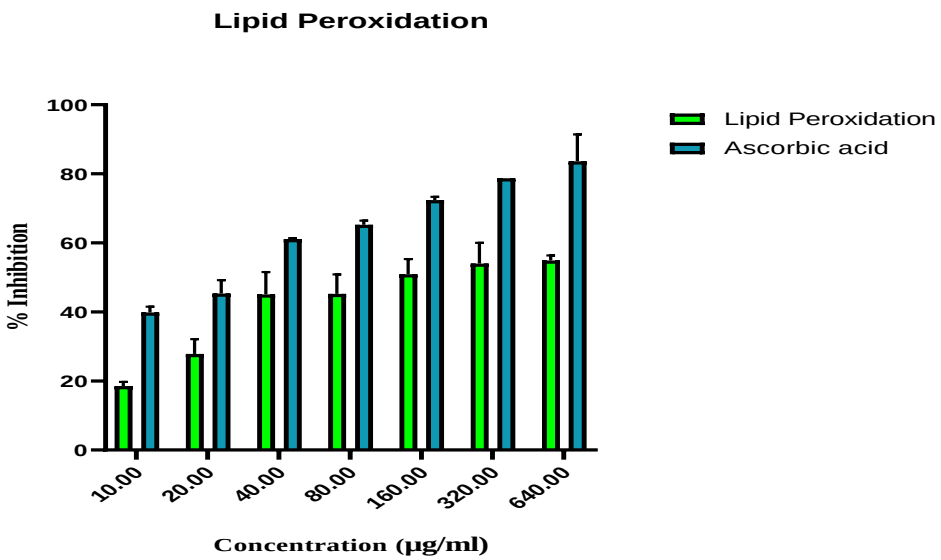

Figure 3: Lipid peroxidizing scavenging activity of the leaf extract of *O.grastissimum*.

The result showed some significant increase (P<0.05) in % lipid peroxidation inhibition activity of the methanol leaf extract in increasing concentration in comparison to the standard control. At 40µg/ml, the scavenging ability of the extract and the standard was significantly lower (P<0.05) when compared to other concentrations.

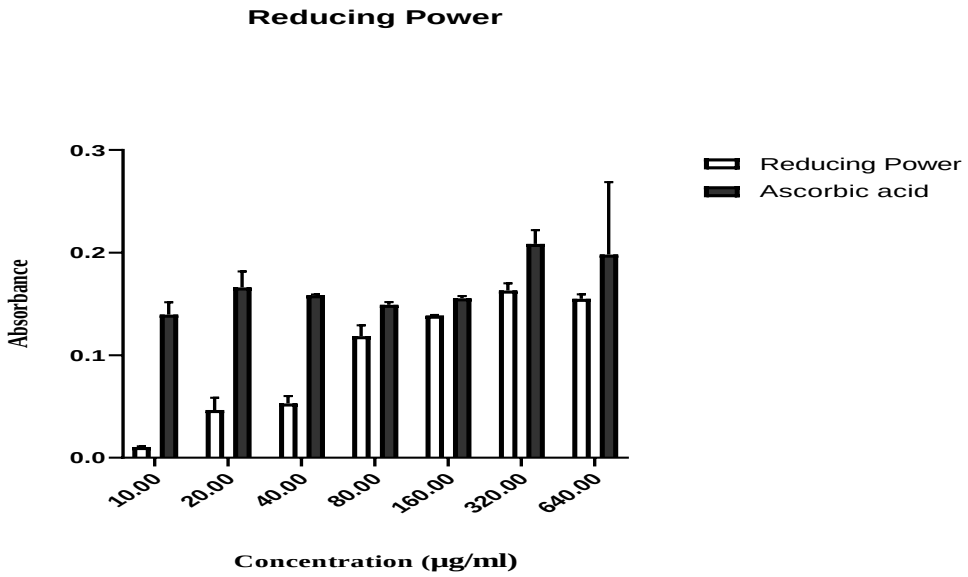

Figure 4: Reducing power potential of the leaf extract of *O. grastissimum*.

The result showed some significant increase (P<0.05) in reducing power scavenging activity of the methanol leaf extract in increasing concentration in at 10, 20 & 40 µg/ml in comparison to the standard control. At 80, 160, 320, 640µg/ml, the reducing power ability of the extract and the standard was non-significant (P>0.05) as shown in the graph above.

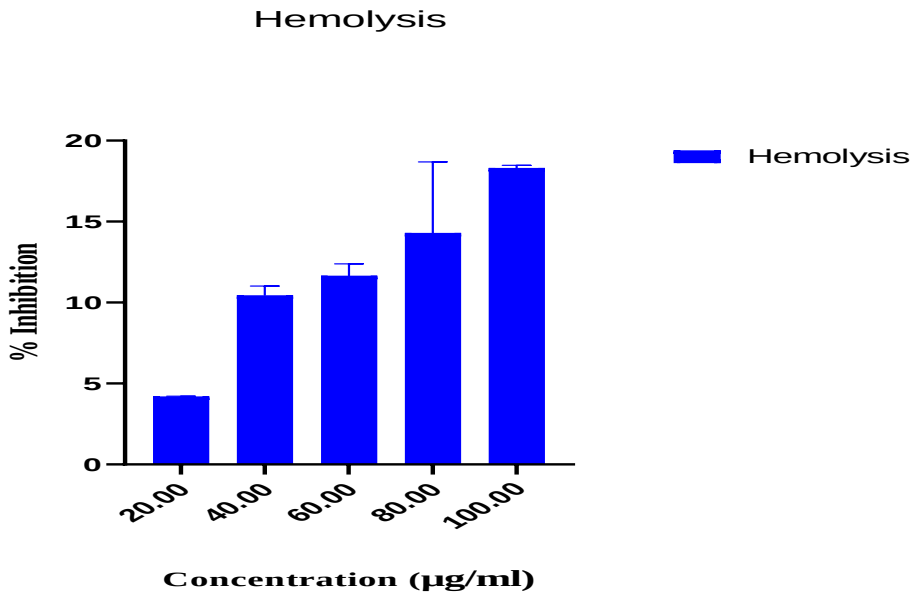

Figure 5: Percentage inhibition of heat-induced hemolysis of the leaf extract of *O. grastissimum*.

The result showed some significant increase (P<0.05) in % hemolysis inhibition activity of the methanol leaf extract in increasing concentration. Result compared each concentration to previous concentrations in their inhibitory activity. Inhibitory activity at 40µg/ml when compared to 20µg/ml was non-significant (P>0.05).

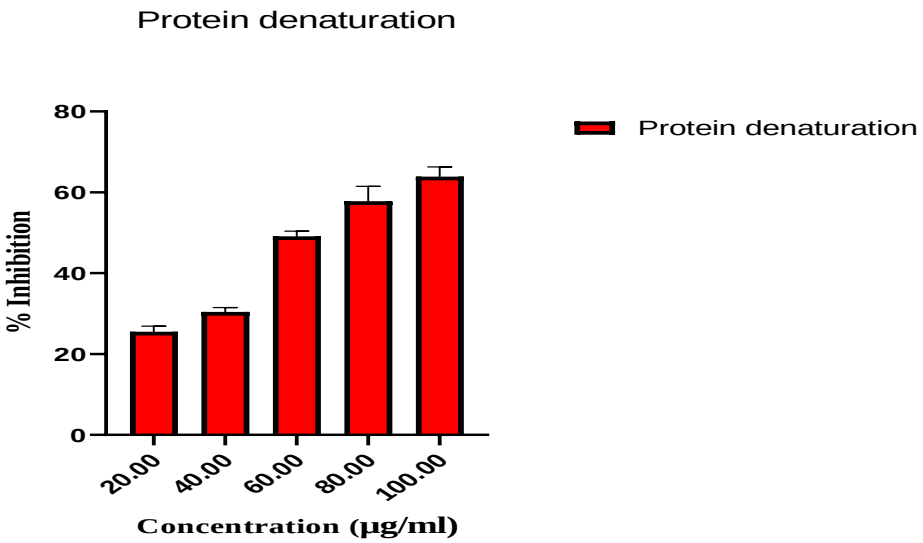

Figure 6: Percentage inhibition of Protein Denaturation of the leaf extract of *O. grastissimum*.

The result showed some significant increase (P<0.05) in % protein denaturation inhibition activity of the methanol leaf extract in increasing concentration. Result compared each

concentration to previous concentrations in their inhibitory activity. Inhibitory activity at 40µg/ml when compared to 20µg/ml was non-significant (P>0.05). However, the % inhibition at 100µg/ml when compared to 80µg/ml was significantly lower (P<0.05).

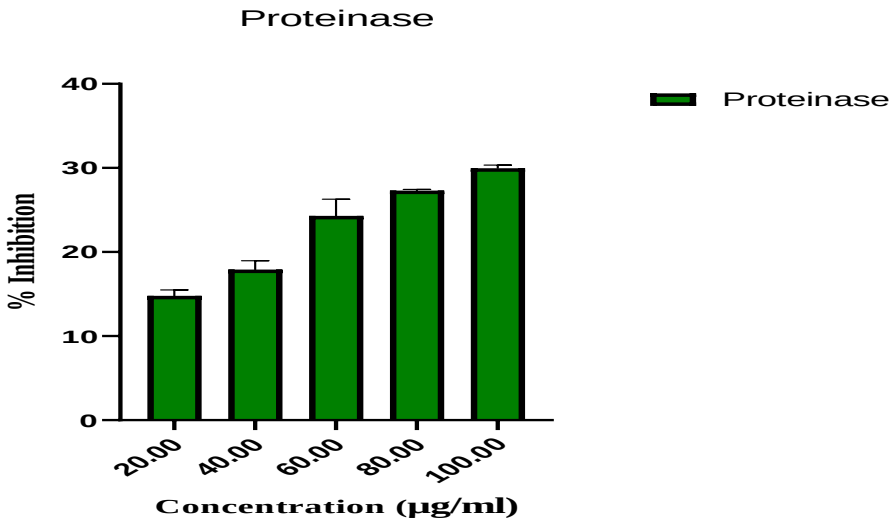

Figure 7: Proteinase Inhibitory activity of the leaf extract of *O. grastissimum*.

The result showed some significant increase (P<0.05) in % proteinase inhibition activity of the methanol leaf extract in increasing concentration. Results compared each concentration to previous concentrations in their inhibitory activity which showed a non-significant (P>0.05) effect except the concentration of 40 & 60µg/ml showed a significant (P<0.05) effect.

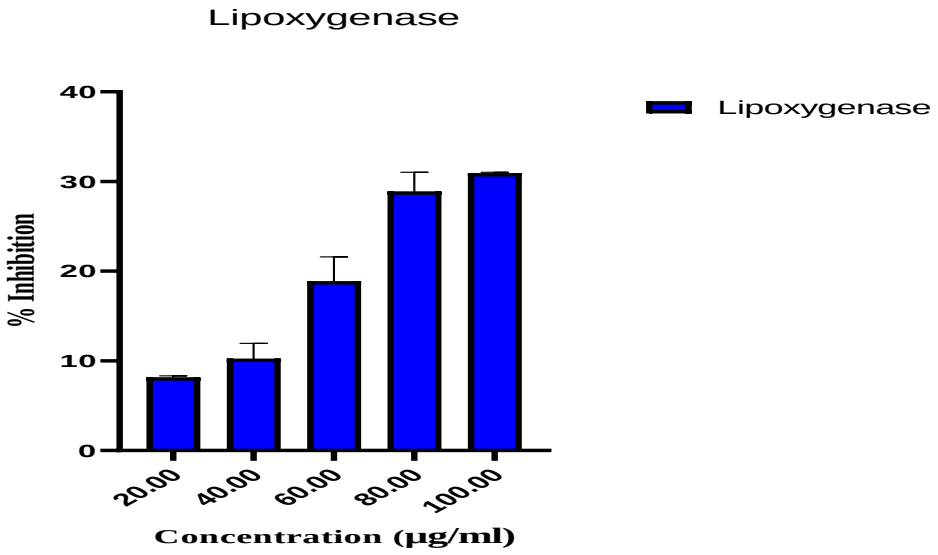

Figure 8: Lipoxygenase Inhibitory activity of the leaf extract of *O.grastissimum*.

The result showed some significant increase (P<0.05) in % proteinase inhibition activity of the methanol leaf extract in increasing concentration. Results compared each concentration to previous concentrations in their inhibitory activity where concentrations of 20µg/ml & 40µg/ml showed a non-significant (P>0.05) also followed by 80µg/ml & 100µg/ml. effect except the concentration of 40, 60 & 80µg/ml showed a significant (P<0.05) effect.

DISCUSSION

In recent years, there has been a growing interest in the search for phytochemicals with anti-inflammatory and antioxidant properties due to their potential therapeutic applications in various chronic and infectious diseases. Both epidemiological and experimental studies have implicated oxidative cellular damage as a primary contributor to conditions such as cardiovascular diseases, cancer, and aging (Halliwell, 2004).

Leukocytes play a crucial role in inflammation, releasing lysosomal enzymes during their defensive actions, causing tissue damage and inflammation. Membrane damage can make cells more susceptible to secondary damage via free radical-induced lipid peroxidation. Regulation of cell volume and water content is influenced by membrane proteins, and damage to these membranes can impact this function (Okoli *et al*., 2008; Umapathy *et al*., 2010). The similarity between red blood cell membranes and lysosomal membranes suggests that inhibiting red blood cell hemolysis may offer insights into the inflammatory process (Umapathy *et al*., 2010). Membrane stabilization can protect against injury, potentially limiting inflammation. The study presented showed that the extract inhibited the lysis of red blood cells induced by heat and hypotonicity.

Additionally, protein denaturation, a process associated with inflammation, was addressed. Nonsteroidal anti-inflammatory drugs (NSAIDs) are known to protect against protein denaturation, and the ability of the studied leaf extracts to prevent protein denaturation suggests potential anti-inflammatory properties.

Proteinases, particularly those associated with leukocytes, play a significant role in tissue damage during inflammation. The study found that the plant extract exhibited antiproteinase (trypsin) activity in a dose-dependent manner, suggesting a potential mechanism for anti-inflammatory effects. Lipoxygenases are key enzymes in the production of leukotrienes, which play a role in inflammatory diseases (Guranthilake *et al*., 2018). Blocking arachidonic acid metabolism by inhibiting lipoxygenase activity and scavenging free radicals generated

during this process are potential anti-inflammatory mechanisms. The extracts inhibited lipoxygenase activity, potentially contributing to their anti-inflammatory activity.

Nitric oxide, while important in inflammatory processes, can be toxic at elevated levels (Neergheen *et al*., 2006). Its reaction with superoxide radicals forms the highly reactive peroxynitrite anion (ONOO−), exacerbating the toxicity. The methanol extract was found to inhibit nitric oxide formation, providing a potential protective mechanism against nitric oxide toxicity. Additionally, increased hydrogen peroxide scavenging activity of the methanol extract was seen, indicating potential antioxidant effects. Lipid peroxidation, induced with AAPH, was used to assess the anti-peroxidative activity of the methanol extract. The extract exhibited great anti-peroxidative ability in concentrations concentration dependent manner.

CONCLUSION

Ocimum gratissimum's methanol leaf extract demonstrated notable antioxidant activities, as evidenced by its ability to scavenge hydrogen peroxide, inhibit nitric oxide, and mitigate lipid peroxidation. Additionally, the extract exhibited anti-inflammatory properties through inhibition of heat-induced hemolysis, protein denaturation, proteinase, and lipoxygenase activities. Therefore, usage of *Ocimum gratissimum* leaf as potential sources of safe and effective therapeutic agents for ameliorating oxidative and inflammation related disease should be encouraged.

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