

Antioxidant and Antibacterial Activities of *Ocimum gratissimum*

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ABSTRACT

Aim: The study was planned to investigate the efficiency of ethanol extract of *Ocimum gratissimum* on pathogens obtained from clinical samples using standard microbiological procedures and also determined the antioxidant capacity of the plant extract.

Study design: *Ocimum gratissimum* was harvested and air dried for 6-7days. The plant material was milled into fine powdered. Ethanol served as extraction solvent, and after extraction, the obtained plant extract was weighed and preserved under anti-microbial condition. DPPH radical scavenging activity, anti-lipid peroxidation activity and nitric oxide scavenging activity served as marker for determination of antioxidant capacity. For anti-bacterial activity, clinical strains of microorganism used for the anti-microbial study includes *Proteus mirabilis*, *Streptococcus pneumonia*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Salmonella species*. The disc agar method was used and zone of inhibition recorded also minimum inhibitory concentration was determined.

Place and duration of study: the study was conducted at Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria, College of Natural Sciences, Department of Biochemistry. The study lasted for 3weeks, which began November 2013 and ended December 2013.

Result: The IC₅₀ for nitric oxide yielded 56.91 ± 0.33 at $800 \mu\text{g/ml}$, for anti-lipid peroxidation it yielded 54.76 ± 1.35 at $400 \mu\text{g/ml}$ but for DPPH, the results were less when compared with the standard agent Ascorbic Acid. For its antibacterial activity, the results at different graded concentrations (500, 250, 125, 62.25, 31mg/ml) of the ethanol extract containing 500mg/ml showed he highest mean \pm SD zones of inhibition against the clinical pathogens while the discs containing 31mg/ml revealed least inhibitory zones for *Escherichia coli*, *Staphylococcus aureus*, *Salmonella spp.*, *Proteus mirabilis* and 62.25mg/ml for *Pseudomonas aeruginosa* and *Streptococcus*

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pneumonia.

Conclusion: The results obtained from this study implies that the ethanol extracts of *Ocimum gratissimum* could be useful in the treatment of infections and for scavenging free radicals and its effectiveness increases with increasing concentration.

Keywords: *Ocimum gratissimum*, Bacteria strain, Nitric oxide, DPPH, Lipid peroxidation.

INTRODUCTION

Our interest lies with the properties of this plant to inhibit and attack reactive oxygen species. The role of therapeutic plants in disease prevention has been credited to the anti-oxidant properties of their parts usually linked to an extensive variety of amphipathic molecules, mostly termed polyphenolics¹. Reactive free radicals such as superoxide anion (O_2^-), hydroxyl radical ($OH\cdot$) and peroxy radical ($ROO\cdot$) are mostly reactive and are known to be a biological specie in reducing molecular oxygen. Damage mediated by free radicals results in the disturbance of membrane fluidity, lipid peroxidation, protein denaturation, oxidative DNA and alteration of platelet functions which have generally been linked with many chronic health problems such as cancers, inflammation, aging and atherosclerosis².

Antioxidants are both natural and synthetic compounds with the ability to scavenge free radicals and inhibit oxidation processes³. Many synthetic compounds have ability to scavenge free radicals (such as Butylated Hydroxy Anisole (BHA), and Butylated Hydroxy Toluene (BHT) are very effective and are used for industrial processing but they possess some side effects and toxic properties to human health.

Anti-oxidants play an important function in protecting the body against free radicals. They achieve this by stopping the formation of new free radicals species,

converting older ones to free radicals, less toxic molecules that can be easily mopped up and preventing radical chain reaction⁴. The principle function of anti-oxidants is in suspending the oxidation of other molecules, by inhibiting the initiation or propagation of oxidizing chain reactions by free radicals and thereby reducing oxidative damage to human body⁵.

Anti-microbial substances are these substances that are capable of stopping or inhibiting the growth of microorganism⁶. Presence of microbes causes food spoilage and results in decline of the quality and quantity of processed food products. Plant biological active fractions obtained from herbs have been used for growth inhibition of pathogenic microbes because of their anti-microbial activity⁷.

Phytochemical studies of this plant have shown that it has alkaloid, tannin, phytates, flavonoids and oligosaccharides in abundance. The plant cyanogenic content is well low and tolerable⁸. The volatile aromatic oil obtained from the leaves comprise mainly of thymol (32–65%) and eugenol. It also contained other compounds like xanthenes, terpenes and lactones⁹.

In traditional medicine, *Ocimum gratissimum* is widely used throughout the Western part of Africa as a febrifuge, antimalarial and anticonvulsant. Oil obtained from leaf extraction has been found

to have antiseptics, antibacterial and antifungal activities⁹.

The methanol extracts of *Ocimum gratissimum* has revealed a DPPH scavenging action of 84.6% at 250 $\mu\text{g/ml}$ and reducing activity of 0.77 at 100 $\mu\text{g/ml}$ and ascorbic acid, 0.79 at 60 $\mu\text{g/ml}$ serves as standards for DPPH scavenging activity and reducing potential. These findings indicate that *Ocimum gratissimum* has good antioxidant activity¹⁰.

Presence of phenol indicates that *Ocimum gratissimum* could act as anti-inflammatory, anti-clothing, anti-oxidant immune enhancers and hormone modulators. Phenols have ability to block enzymes that cause inflammation, and can also modify prostaglandin pathways leading to protection of platelets from clumping¹¹.

MATERIALS AND METHODS

Collection of plant material

Ocimum gratissimum used in the research was obtained from their natural habitats in Arochukwu, Abia State, Nigeria. The plant materials were validated by Dr. G. Osuagwu of the Department of Plant Science and Biotechnology, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, and herbarium numbers MOUAU/COLNAS/PSB/2013/11/19 assigned to the plant material with samples kept for upcoming references. The leaves of these plants were dried at room temperature for six to seven days. The dried plants were milled and kept in polythene bag in absence of sunlight and oxygen until required.

Bacterial strains

Clinical strains of microorganism used for the anti-microbial study were obtained from the microbiology laboratory of the Federal Medical Centre (FMC), Umuahia, Abia State. They include: *Proteus*

mirabilis, *Streptococcus pneumonia*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Salmonella species*.

Preparation and concentration of ethanol extracts

Fifty gram (50g) of the milled *Ocimum gratissimum* was weighed into four (4) different beakers; 200ml of 100% Ethanol was added to each beaker and left for 72 hours(3days). After 72 hours, the extracts were filtered using Whatman No. 1 filter paper with the addition of 100ml of ethanol to each beaker containing the sample in order to increase the concentration of the filtrate that contains the active compound. The filtrate was allowed to heat in water bath at 50°C to concentrate it better. After complete evaporation of ethanol, the extract was weighed and stored under anti-microbial conditions.

Free radical scavenging activity

Nitric oxide scavenging activity

2.0ml of 10mm sodium nitroprusside and 5.0ml of phosphate buffer were mixed with 0.5ml of different concentrations of the plant extracts and incubated at 25°C for 150minutes. After the incubation, 2ml of the incubated solution was added to 2ml of Greiss reagent (1% sulphanilamide, 0.1% α -naphthyl-ethylenediamine dihydrochloride and 3% phosphoric acid) and incubated at room temperature for a period of 30 minutes. The absorbance of the pink chromophore formed by the diazotization of nitrite with α -naphthyl ethylene diamine dihydrochloride was measured at 540nm. Ascorbic acid was used as standard, sodium nitroprusside as control and results were expressed as percentage inhibition of nitric oxide. All determinations were performed in triplicates.

$$\% \text{ inhibition} = \frac{A \text{ control} - A \text{ sample}}{A \text{ control}} \times 100$$

Anti – lipid peroxidation activity

This was determined by the method described by Dinakarn *et al.*,¹². Ethanol extracts of *Ocimum gratissimum* were used in various concentrations (25, 50, 100, 200, 400 and 800 μ g/ml). Liver obtained from a fresh sacrificed animal from the Veterinary Medicine Laboratory, Michael Okpara University of Agriculture, Umudike was used for the determination of anti-lipid peroxidation activity. The liver was made homogenate by a mechanical grinder. 3ml of liver homogenate was added with 100 μ l of 15mM ferric chloride and was shaken for 30 minutes. From collected mixture, 100 μ l was added with 1ml of different concentrations of plant extracts individually in different test tubes. The same procedure was followed for standard and blank. Ascorbic acid was used as standard and TBARS as control. All the test tubes were incubated for 4 hours at 37 $^{\circ}$ C. After incubation, 20% trichloroacetic acid (TCA) was added to all the test tubes containing the mixture in 1:1 ratio and was centrifuged for 30 minutes. The supernatant liquid was collected and 0.6% thiobarbituric acid (TBA) was added in 1:1 ratio and heated for 1 hour in a water bath. The mixture was cooled and absorbance measured at 530nm. The percentage of anti-liquid peroxidation activity was calculated using the formula.

$$\% \text{ inhibition} = \frac{(A \text{ control} - A \text{ sample})}{A \text{ control}} \times 100$$

Determination of DPPH radical scavenging activity

Preparation of DPPH solution,
 1mMol/L of DPPH = 0.394g of DPPH
 0.5mMol/L of DPPH = 0.197g of DPPH

$$\begin{aligned} 1000\text{ml} &= 0.197\text{g} = 197\text{g} \\ 150\text{ml} &= x \end{aligned}$$

$$x = \frac{150 \times 197}{1000} = \frac{2955}{100} = 29.55\text{mg}$$

This was carried out according to the 2, 2, diphenyl – 2 – picrylhydrazyl (DPPH) assay system (Mensor *et al.*, 2001). Extracts at different concentrations was mixed with 2ml of absolute ethanol (100%). 1ml of DPPH was added to the solution and shaken immediately. The mixture was allowed to stand at room temperature in the dark for 30 minutes. The absorbance of the resulting mixture was measured at 518nm and converted to percentage antioxidant activity (AA%) using the formula:

$$\% \text{ inhibition} = \frac{(A \text{ control} - A \text{ sample})}{A \text{ control}} \times 100$$

DPPH free radical was used as control.

Anti-bacterial activity

The disc agar diffusion method was used. The test organism (1:100) dilution of an 18h broth cultures were inoculated onto nutrient agar plates with sterile cotton swabs soaked in the inocula. Disc of different extract concentrations were placed firmly on the surface of the inoculated agar plates and incubated at 37 $^{\circ}$ C for 18 hours under aerobic conditions. Zones of inhibition were measured and recorded in millimeters.

Determination of minimum inhibitory concentration¹³

The minimum inhibitory concentration (MIC) is the concentration giving the least inhibitory activity and below which there is no further inhibition. It is therefore the concentration giving the lowest possible zones of inhibition. Two fold serial dilutions were prepared to obtain a 31 – 500mg/ml concentration range. Sterile discs

were immersed into the different dilutions. Absorption was allowed for 30mins. A pair of sterile forceps was used to pick the paper discs and placed on the inoculated plates. The portions were labelled, and the plates incubated at 37⁰c for 24hrs. Zones of growth inhibition were measured and the MIC determined.

RESULT

See table 1-5 and figure 1-3.

DISCUSSION

Oxidative stress which is caused by insufficient capacity of biological system to neutralize excessive free radical product has been associated with all kinds of human diseases and aging¹⁴. Plant derived natural antioxidants are better than synthetic ones because they show defensive roles against oxidative stress involved in the generation of disease such as cancer and other coronary disorders without inducing any side effect.

Ocimum gratissimum contains phytochemicals (Phenol in abundance) and this plant chemical has defensive and medicinal properties that protects from disease¹⁵. As a vegetable, it helps in the removal of carcinogens there by inducing phase 2 biotransformation.

Ethanol extracts of *Ocimum gratissimum* were used at varying concentration (25, 50, 100, 200, 400, 800µg/ml) for anti-lipid peroxidation activity. The experiment was performed to analyze the anti-hyperlipidemic effect of the extract on rat liver, homogenate from 25–800 µg/ml by measuring the levels of malondialdehyde (MDA) which is produced upon acidic decomposition of lipid peroxides. The analysis of data indicates that the anti-lipid peroxidation activity was maximum at 800µg/ml.

Nitric oxide scavenging ability exhibited maximum inhibition at 800µg/ml.

the maximum conc. of inhibition was found to be 47.62% for ascorbic acid and 56.91 for the extract showing that the extract possess anti-oxidative agents.

In this study, *Ocimum gratissimum* ethanol extract revealed in-vitro antioxidant properties when tested with DPPH radical scavenging assay. Antioxidants react with DPPH radical converting it to 1, 1-diphenyl-2-picryl hydrazine. This is achieved due to its hydrogen donating ability at a fast rate. The level of discoloration reveals the scavenging potential of the antioxidant. The IC₅₀ was lower when compared with Ascorbic acid even at the highest Concentration Used. Therefore, the anti-oxidant activity increases is proportional to a corresponding increase in the concentration of the extracts of *Ocimum gratissimum*.

This study therefore shows that the ethanol extract possesses significant antioxidant activities, and that the scavenging effect increases with increasing concentration. The highest scavenging effect revealed by the extract was due to the high concentration of the active compound present in the plant material.

Ethanol extracts at different concentrations (500, 250, 125, 62.25 & 31mg/ml) exhibited antibacterial activity against the test organisms. This was also observed in a dose dependent manner, zone of inhibition was strong related with concentration. *S. aureus* had the highest zone of inhibition following the result of this study.

From the study it was observed that the inhibitory effects of the extracts were dose dependent on both the bacteria. The inhibitory role of the plant oil is more active compared to the leaves ethanol extract¹⁶ and the result of this study conform whit his work.

The findings from this study explain the use of the plant in folklore medicine and also in nutritional purposes. The modes of action of the extract was dose-dependent on the bacterial, the higher the extract

concentration, the higher the inhibition and this agrees with the work of Oboh¹⁷.

The antibacterial activities of ethanol extracts of *Ocimum gratissimum* were compared to that of ciprofloxacin, a broad spectrum antibiotic used as standard. Leaf ethanol extracts used in this work revealed inhibition of both gram positive and gram negative bacteria (table 4.4). This indicates that it possess a broad spectrum anti-bacterial activity. *S. aureus* and *E. coli* are organisms indicated in nosocomial infection¹⁸ hence, the antagonistic property of this leaf to such organisms is vital in the clinical controlling of nosocomial infections.

This natural herb is therefore effective in controlling microbial growth by selectively inhibiting bacterial growth, protein synthesis, cell wall and membrane synthesis, nucleic acid synthesis and the essential metabolic pathways that exist in the bacterium.

The basis for the anti-bacterial activity can be credited to the presence of phyto-compounds like saponin, tannin, anthraquinone which had been reported to have antimicrobial and medicinal activity¹⁹.

CONCLUSION

The antibacterial and antioxidant activities of this extract provide justification for the chemotherapeutic utilization of these herbs, because from the present investigation, it may be concluded that ethanol extracts of *Ocimum gratissimum* possessed potent anti-bacterial & anti-oxidant agent.

Further works are on-going to characterize the specific bioactive compound responsible for these findings.

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Competing interests

The author affirms that there is no contending interest and all authors listed partook actively in the study and approved the final version of the manuscript for publication.

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Table 1. Result of anti-lipid peroxidation

Conc. ($\mu\text{g}/\text{mg}$)	Mean of inhibition \pm SEM	IC_{50}
Extract 25	31.17 \pm 0.79*	400 $\mu\text{g}/\text{ml}$
50	7.14 \pm 1.37*	
100	16.67 \pm 1.38*	
200	33.33 \pm 1.37*	
400	54.76 \pm 1.35*	
800	58.73 \pm 2.10*	
Standard Ascorbic Acid (100 $\mu\text{g}/\text{ml}$)	47.62 \pm 1.37	

TBARS was used to identify lipid peroxidation.

Table 2. Result of nitric oxide scavenging activity

Conc. ($\mu\text{g}/\text{mg}$)	Mean of inhibition \pm SEM	IC ₅₀
25	25.31 \pm 0.33*	800 $\mu\text{g}/\text{ml}$
50	29.38 \pm 0.12*	
100	31.48 \pm 1.90*	
200	30.00 \pm 1.70*	
400	34.69 \pm 0.25*	
800	56.91 \pm 0.33*	
Ascorbic Acid (100 $\mu\text{g}/\text{ml}$)	47.62 \pm 1.37	

The IC₅₀ value represents the concentration of the extracts that caused 50% inhibition of radical formation.

Table 3. Result of 2, 2-diphenyl-1-picryl hydrazyl scavenging activity

Conc. ($\mu\text{g}/\text{mg}$)	<i>Ocimum gratissimum</i>	Ascorbic acid	IC ₅₀
25	9.61 \pm 4.48	92.15 \pm 0.36	>400 $\mu\text{g}/\text{ml}$
50	12.43 \pm 2.12	94.08 \pm 0.80	
100	12.43 \pm 2.50	94.78 \pm 0.68	
200	16.43 \pm 1.46	96.38 \pm 4.31	
400	25.43 \pm 1.83	99.33 \pm 0.40	

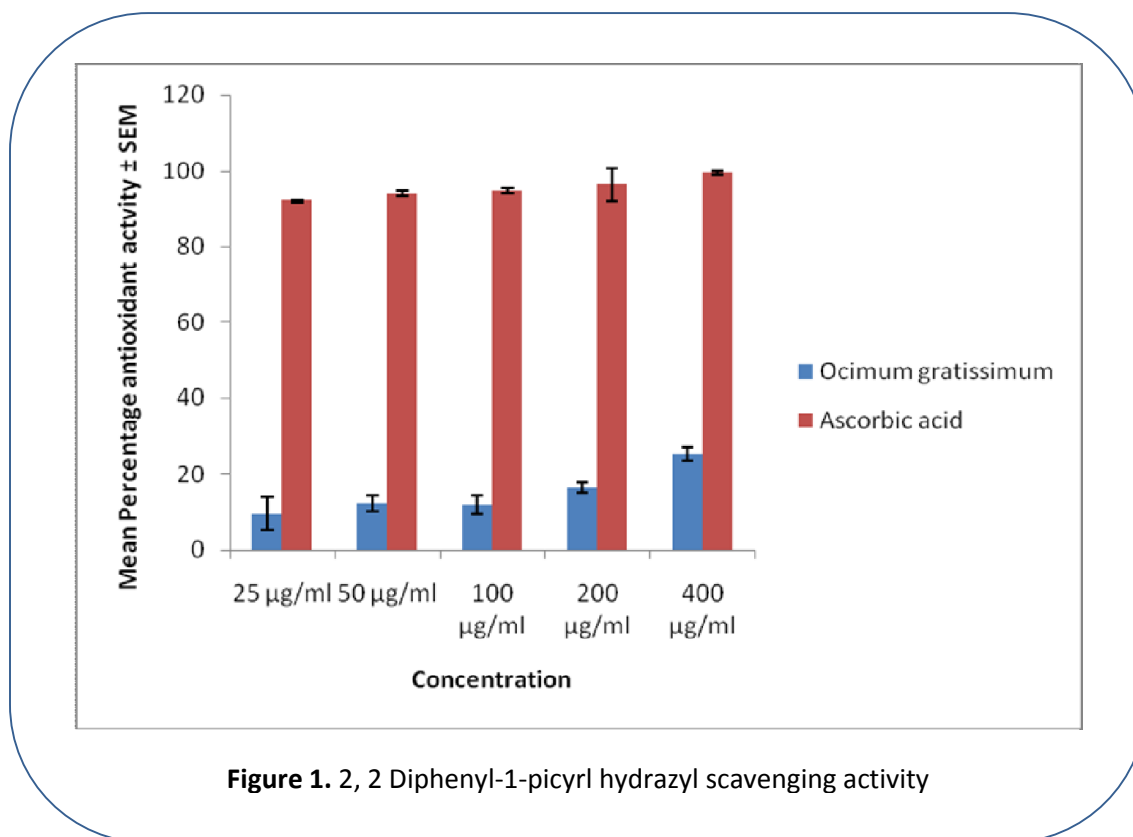
The extract possesses an antioxidant activity that is less potent when compared with the standard. Therefore, an increase in concentration is required for an increase in the radical scavenging effect.

Table 4. Result of the antibacterial activity of *Ocimum gratissimum* at different concentrations

Pathogen	500mg/ml	250mg/ml	125mg/ml	62.25mg/ml	31mg/ml	MIC mg/ml
<i>E. coli</i>	9.61 \pm 1.41		5.5 \pm 0.71	1.5 \pm 0.71	0	62.25
<i>S. aureus</i>	21.5 \pm 0.71	10.5 \pm 0.71	6 \pm 1.41	2.5 \pm 0.71	0	62.25
<i>S. pneumoniae</i>	13.5 \pm 0.71	12.5 \pm 0.71	0	0	0	250
<i>P. aeruginosa</i>	10.5 \pm 0.71	5.5 \pm 0.71	0	0	0	250
<i>S. Spp</i>	20.5 \pm 0.71	4.5 \pm 0.71	7 \pm 1.41	3.5 \pm 0.71	0	62.25
<i>P. mirabilis</i>	43.5 \pm 1.41		5 \pm 1.41	1.5 \pm 0.71	0	62.25

Table 5. Antibacterial activity of the plant extract (*Ocimum gratissimum*) & Ciprofloxacin

Pathogen	Zone of inhibition of <i>Ocimum gratissimum</i> (1000mg)	Zone of inhibition of ciprofloxacin (500mg)
<i>E. coli</i>	26.5 ± 0.71	46 ± 2.8
<i>S. aureus</i>	35 ± 1.41	45 ± 1.41
<i>S. pneumoniae</i>	23. ± 1.41	39. ± 1.41
<i>P. Aeruginosa</i>	19 ± 1.41	28 ± 1.41
<i>S. Spp</i>	31 ± 1.41	43 ± 1.41
<i>P. mirabilis</i>	26 ± 1.41	44 ± 0.71

**Figure 1.** 2, 2 Diphenyl-1-picryl hydrazyl scavenging activity

The antibacterial activities of ethanolic extracts of *Ocimum gratissimum* leaves were compared to that of Ciprofloxacin, a broad spectrum antibiotic used as standard. Results are below.

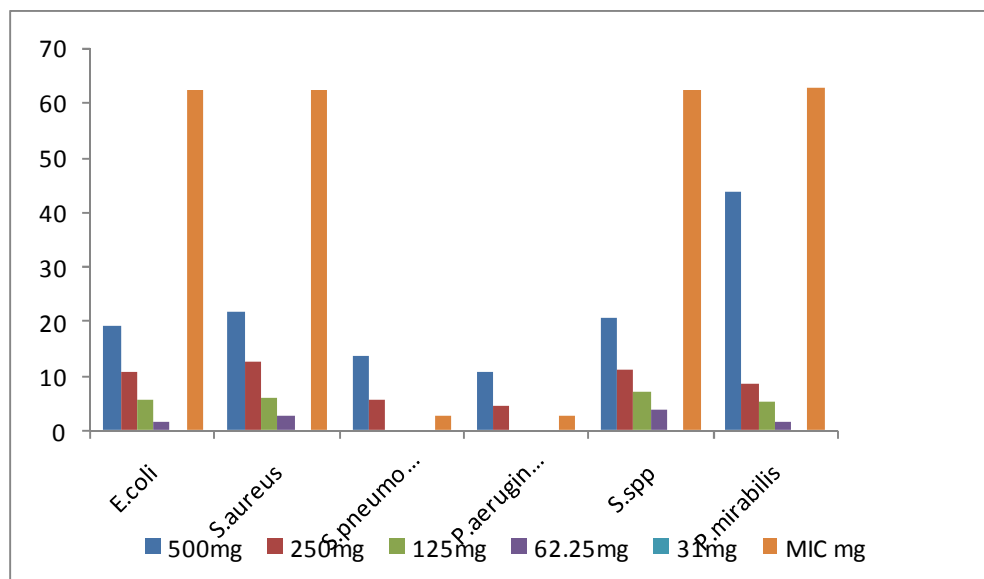


Figure 2. Antibacterial activity of *Ocimum gratissimum* at different concentrations

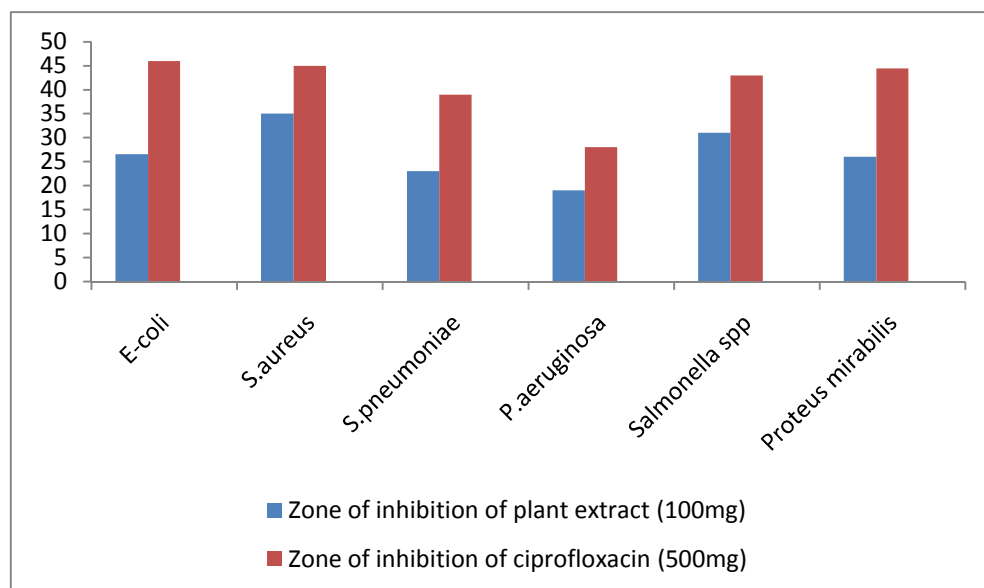


Figure 3. Antibacterial activity of the plant extract (*Ocimum gratissimum*) and ciprofloxacin

Antibacterial activities of the ethanol extracts of *Ocimum gratissimum* showed a wide range of activity on the pathogens from clinical sources. The discs containing 500mg of the ethanol extracts showed the highest Mean \pm SD zones of inhibition while the discs containing 31mg showed the least inhibitory zones.