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Antioxidant and Antimicrobial Activities of the Volatile Oil of *Ocimum gratissimum* and its Inhibition on Partially Purified and Characterized Extracellular Protease of *Salmonella enteritidis*

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ABSTRACT

The emergence of antibiotic resistant bacteria has made the treatments of pathogenic infections difficult. The discovery that some analytes of plant extracts are active against multi-drug resistant bacteria has opened ways to researching into the antimicrobial activity of these phytocomponents. Studies are designed to determine the antimicrobial and antioxidant potentials of the volatile oil of *Ocimum gratissimum* and its inhibition on partially purified and characterized extracellular protease of *Salmonella enteritidis*. The oil was extracted by hydrodistillation. Its antimicrobial effect was carried out using microdilution method while antioxidant effect was tested against 1,1-diphenyl-2-picrylhydrazyl (DPPH). The caseinolytic activity of the enzyme was studied with oil as inhibitor. The enzyme was partially purified with dialysis and gel filtration. The oil has scavenging activity of $66.98 \pm 1.78\%$ compared to butylated hydroxytoluene (positive control), $53.29 \pm 2.51\%$ (sig. $p < 0.0001$) against DPPH. *Salmonella enteritidis* was inhibited by this oil with IC_{50} of 3.98% (v/v). The enzyme had optimal activities at 45°C and pH 7.5. None of the metallic chloride tested produced any significant increase in the activity of the enzyme. Hg^{2+} and Pb^{2+} were inhibitors of this enzyme. The oil showed a non-competitive inhibition with $K_m = 0.33 \text{ mg mL}^{-1}$, $V_{\text{max}} = 1.25 \times 10^3 \text{ } \mu\text{mol min}^{-1}$ (oil absent) and $V'_{\text{max}} = 2.50 \times 10^2 \text{ } \mu\text{mol min}^{-1}$ (oil present). The highest purification fold = 1.88 and the highest percentage yield = 51.74 as compared to the crude enzyme extract. Therefore, the volatile oil of *Ocimum gratissimum* possessed antimicrobial and antioxidant activities, its inhibition against this protease may be one of the ways of its antimicrobial effect.

Key words: *Ocimum gratissimum*, extracellular protease, antioxidant, antimicrobial, *Salmonella enteritidis*

INTRODUCTION

Medicinal plants are of great importance to the health of individual and it is now being employed as alternative and more safety ways to the failing, compromising and toxic synthetic drugs (Gurib-Fakim, 2006). More importantly, their medicinal indexes have been characterized by both *in vivo* and *in vitro* activities such as teratogenic, abortifacient, laxative, immunomodulation, antioxidant, cardioprotective, hepatoprotective and nephroprotective. These effects have been attributed to the presence of identified and characterized phytocomponents such as terpenes in

essential oils, flavonoids, phenolic compounds, steroids, terpenoids, glycosides, tannins and alkaloids (Zheng and Wang, 2001; Marija *et al.*, 2012; Edeoga *et al.*, 2005). Native medicinal plants have long been used in form of concoctions, decoctions, fumes, steams, bathe, foods, chewing and incisions to treat various ailments (Okwu, 2001). Recently, the discovery of these phytoconstituents has geared the tune of ethnomedicine towards advanced development that will facilitate the isolation, extraction, purification and clinical trials (Kong *et al.*, 2003). Essential oils are generally volatile aromatic coloured liquids extracted from plants. Of interest, is the clinical use of this oil to treat wounds and skin infections, aid digestions, stimulate smooth muscle contraction, keep away insects/fly and as food additives (Lawrence, 1997; Tisserand and Balacs, 1995; Burt, 2004). Essentially, they are becoming a central focus in ethnomedicine where alternative medicines are adopted for the treatment and management of clinical ailment especially those associated with pathogens. In this light, volatile oils of medicinal plants may have been found to possess antimicrobial (Larrondo *et al.*, 1995), antifungal (Boyraz and Ozcan, 2006), anti-inflammatory (Caldefie-Chezet *et al.*, 2004), antiparasitic (Pessoa *et al.*, 2002; Oliveira *et al.*, 2009), cardioprotective (Lahlou *et al.*, 2004) and anti-infarctive (Buchbauer *et al.*, 1993) properties. *Ocimum gratissimum* (Labiatae) are medicinal plants that have long been used in Ayurvedic medicine in Asia and Africa for the treatment of body ailments. The essential oils from this plant are characterized with high contents of eugenol, bisabolene, terpinene, cymene, caryophyllene, thymol and others (Adams, 2005; Dubey *et al.*, 2000; Oliveira *et al.*, 2009). In Nigeria, the leaves of this plant have been used in making soup, insect repellent, extracted water from the leaves as worm expellant and bowel decongestion, as concoction and in herbal formulation (Effraim *et al.*, 2003; Odugbemi, 2006). The organic and aqueous extracts of the species of this medicinal plant and its volatile oil have been shown to possess radioprotective (Uma Devi *et al.*, 2000), cardioprotective (Sharma *et al.*, 2001), antibacterial (Nakamura *et al.*, 1999), antinfarction (Buchbauer *et al.*, 1993), antidiarrheal (Adebolu and Oladimeji, 2005), antifungal (Faria *et al.*, 2006; Silva *et al.*, 2005), antiviral (Chiang *et al.*, 2005), anti-inflammatory and analgesic (Sahouo *et al.*, 2003), hypotensive (Interaminense *et al.*, 2005) and cardiovascular (Lahlou *et al.*, 2004) properties. The leaves of this plant have been incorporated into different types of soups, herbal formula and concoctions with the belief that it heals different kinds of human ailments/discomforts and as such, it is used as laxative (Madeira *et al.*, 2002), anti-haemorrhoid, blood and tissue purifier. It would have been possible probably because of the homeostatic action of this plant extract in the body (Akah, 1990).

Salmonella are peritrichous gram-negative bacilli *Enterobacteriaceae* characterized with anaerobic chemoorganotrophic facultative activities (Balliton and Ralphk, 2004; Ryan and Ray, 2004). *Enteritidis*, one of the serotypes of *Salmonella* (Centres for Disease Control and Prevention, 2004) is responsible for majority of reported cases of zoonotic salmonellosis (Gast and Beard, 1990a, b; Gast and Holt, 2000; Gast and Benson, 1995). *Salmonella enteritidis* is involved in the aetiology of some diseases including fever, paratyphoid fever and foodborne illness (Ryan and Ray, 2004; Binkin *et al.*, 2007), hence it is easily contacted through raw and half-cooked foods, drinks, poultry and egg products (Gantois *et al.*, 2009). The pathogenicity of this bacterium is a function of many immunogenic pathotoxins which are endogenously released during pathogen infection, integration and colonization. Among the mechanism designed for immune evasion is the release of extracellular protease which helps in the digestion of heterogeneous epithelial and endometrial surface proteins, elastin, collagen, receptor proteins and immunogenic proteins.

Microbial proteases are proteolytic enzymes responsible for the breaking down of proteins or proteoconjugate molecules. Infectious bacteria possess extracellular protease by which they

breakdown the host proteins like surface expressed proteins, glycoproteins, defensive proteins, signalling proteins and anchoring proteins. Most pathogenic organisms secrete both intracellular and extracellular proteases with which, they accomplish their physiologic activities. These proteases may also contribute to the virulent nature of the pathogens (Todar, 2012). The ability of each causative organism to elicit its pathogenicity is a function of its virulence factors (Lantz and Ciborowski, 1994; Keen, 2012). This may probably facilitate their favourable host-pathogen interaction leading to invasion, integration and colonization of the host tissues (Priest, 1977; Todar, 2012). *Salmonella enteritidis* secretes quite a lot of extracellular proteases that make it very virulent and shows a high level of antibiotic resistance thus indicating the need for a more novel and different approach towards fighting this microbe. Apart from the biotechnological application of these proteases, researches are directed at targeting these enzymes and related others with phytopharmaceutical agents capable of inhibiting their activity thus prevent the pathogens from causing infections (Anwar and Saleemuddin, 1998, 2000; Gupta *et al.*, 2002; Kelleher and Juliano, 1984; Keen, 2012; Lantz and Ciborowski, 2005).

Presently, *Ocimum gratissimum* is used in most local dishes/foods to achieve a variety of purposes; many literatures have shown that the volatile oil of *Ocimum gratissimum* has antioxidant and antimicrobial activity which can serve as a good inhibitory agent against the activity of pathogenic organisms (Adesegun *et al.*, 2013). Therefore, this study is designed to re-establish the antioxidant properties of the volatile oil of this plant and more importantly to examine the mode of its inhibition on the activity of extracellular protease of *Salmonella enteritidis*.

MATERIALS AND METHODS

Plants materials: *Ocimum gratissimum*, whole plant, was obtained at Ojo Local Government Area of Lagos State, Nigeria as green foliage and was air-dried for 5 days under a shade. The plant was identified and authenticated at the Department of Botany, Faculty of Science, Lagos State University, Ojo Lagos State, Nigeria.

Microorganisms: *Salmonella enteritidis* used in this study was obtained from the Nigeria Institute of Medical Research (NIMR), Yaba, Lagos, Nigeria and maintained on nutrient agar at 4°C.

Extraction of volatile oil from *Ocimum gratissimum*: The volatile oil of *Ocimum gratissimum* was extracted by hydrodistillation method (Lawrence and Reynolds, 2005). The air-dried *Ocimum gratissimum* plant leaf was cut into pieces and packed into the 5 L 34/35 quick fit round bottom flask containing 1.5 L distilled water with fixed Clevenger. The oil was extracted at a steady temperature of 70°C for 3 h and the oil was collected over 2 mL n-hexane. The extracted oil was stored at 4°C until it was tested.

Antioxidant activity of the volatile oil of *Ocimum gratissimum*: The antioxidant activity of the volatile oil of *Ocimum gratissimum* was carried out by the process described by Koleva *et al.* (2002) with little modification. Briefly, 2-20 µL of the volatile oil at 30% (v/v) concentrations in acetone (99.5%) was added to constant volume of 80 µL methanolic solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH) (0.2 mg mL⁻¹) and mixed thoroughly and the absorbance was read at 517 nm after 30 min at room temperature. Butylated hydroxytoluene (BHT) was used as positive controls. The experiment was repeated three times. The IC₅₀ value denotes the

concentration of an antioxidant which is required to scavenge 50% of DPPH free radicals. The percentage radical scavenging activity of the volatile oil and BHT were estimated using the following equation:

$$\frac{A_0 - A_1}{A_0} \times 100$$

where, A_0 is the absorbance of DPPH solution in the absence of antioxidant and A_1 is the absorbance of DPPH solution in the presence of antioxidant (the volatile oil and BHT).

Antimicrobial activity of the volatile oil of *Ocimum gratissimum*: The volatile oil extracted from *Ocimum gratissimum* leaves was tested for antimicrobial activity using microbroth dilution spectrophotometric method (Akujobi and Njoku, 2010) with little transformation. A pure colony of *Salmonella enteritidis* was added to 200 μ L susceptible test broth containing two fold serial dilutions of the essential oil in the microtitre plate (21.5 cm by 17 cm). The plate was covered and incubated at 37°C for 24 h. After 24 h, each inoculum in a microwell was re-inoculated into a fresh nutrient broth and inhibition of the bacteria was spectrophotometrically monitored at 620 nm using a microplate reader after 18 h of anaerobic incubation at 37°C. The percentage growth inhibition of bacteria was determined using the following equation:

$$\frac{A_0 - A_1}{A_0} \times 100$$

where, A_0 is the absorbance of the well in the absence of volatile oil and A_1 is the absorbance of the well in the presence of volatile oil. The IC_{50} value of the volatile oil against the growth of *Salmonella enteritidis* was graphically extrapolated and it denotes the concentration of the oil % (v/v) that reduced the growth of this bacterium by half.

Extraction of crude enzyme from *Salmonella enteritidis* isolate: As described by Makino *et al.* (1981) with little modification, a colony of *Salmonella enteritidis* was inoculated into 5 mL of nutrient broth inside McCartney bottle. It was incubated for 24 h at 37°C. The inoculum was centrifuged (Kendros PicoBiofuge, Heraeus) at 9000 rpm for 10 min at room temperature. The supernatant (enzyme extract) was decanted and stored in a sample bottle at 4°C until it was used.

Protein determination: Total protein of the crude enzyme extract was determined using Lowry *et al.* (1951) method. Briefly, 5.0 mL of alkaline solution containing a mixture of 50 mL of solution A (20 g sodium trioxocarbonate IV and 4 g sodium hydroxide in 1 L) and 1 mL of solution B (5 g copper II tetraoxosulphate VI pentahydrate and 10 g sodium-potassium tartrate in 1 L) was added to 0.1 mL of crude enzyme extract and mixed thoroughly. The solution was allowed to stand for 10 min at room temperature and 0.5 mL of freshly prepared Folin Ciocalteu's phenolic reagent (50% v/v) was added. The solution was mixed thoroughly and the absorbance was read at 750 nm after 30 min at room temperature. Bovine Serum Albumin (BSA) at 0.20 mg mL⁻¹ was used as standard protein.

Enzyme activity of the crude extract: The extracellular protease of *Salmonella enteritidis* was assayed using Jenssen *et al.* (1994) with little transformation. Protease activity was carried out by adding 5.0 mL of casein solution (0.6% w/v in 0.05 M Tris buffer at pH 8.0) to 0.1 mL of the crude enzyme extract and the mixture was incubated for 10 min at 37°C. The reaction mixture was stopped by adding 5.0 mL of a solution containing 0.11 M trichloroacetic acid, 0.22 M NaCl and 0.33 M acetic acid mixed in ratio 1:2:3. The turbid solution was filtered and 5.0 mL of alkaline solution was added to 1.0 mL of the filtrate followed by 0.5 mL of freshly prepared Folin Ciocalteau's phenolic reagent after 10 min of thorough mixing. The absorbance was read at 750 nm after 30 min. L-tyrosine solution (0.20 mg mL⁻¹) was used as standard for the protease activity. A unit of protease activity was defined as the amount of enzyme required to liberate 1.0 µmol of tyrosine in 60 sec at 37°C. The specific activity was expressed in units of enzyme µmol min⁻¹ mg⁻¹ protein.

Characterization of the extracellular protease

Determination of optimum temperature: As described by Makino *et al.* (1981) with little modification, 5.0 mL of 0.6% casein in 0.05 M Tris buffer at pH 8.0 was mixed with 0.1 mL of crude enzyme extract and the enzyme assay was carried out at temperature range of 30-60°C for 10 min. The reaction was stopped and enzyme activity was carried out at each stage of temperature.

Determination of optimum pH: The method adopted was described by Makino *et al.* (1981) with little modification. This was carried out by adding 5.0 mL of 0.6% w/v casein solution in 0.05 M Tris buffer (pH ranges from 6.0-9.0) to 0.1 mL of the crude enzyme extract and the enzyme assay was carried out at 37°C for 10 min. The reaction was stopped and enzyme activity was carried out at each stage of the pH.

Inhibitory assay of the volatile oil of *Ocimum gratissimum*: As adopted from Makino *et al.* (1981), 0.1 mL of the crude enzyme extract and 0.1 mL of 3.5% v/v of the volatile oil in 0.5% v/v Tween 80 (BDH) solution were added concomitantly to different concentration of casein solution (0.2-1.0% w/v) in 0.05 M Tris buffer at pH 8.0 and the reaction mixture was mixed and incubated at 37°C for 10 min. The reaction was stopped by adding 5.0 mL of a solution containing 0.11 M trichloroacetic acid, 0.22 M NaCl and 0.33 M acetic acid mixed in ratio 1:2:3. Protease assay was carried out and the procedure was repeated without an inhibitor.

Effect of metallic ions: As described by Jahan *et al.* (2007) with little modification, the activity of extracellular protease was carried out in the presence of 1.0 mM chloride solutions of Mg²⁺, Fe²⁺, Fe³⁺, Ca²⁺, Co²⁺, Pb²⁺, Mn²⁺, Hg²⁺, K⁺, Zn²⁺ and Cu²⁺. Briefly, to 0.1 mL of the crude enzyme extract, 1.0 mL of each chloride salt solution and 5.0 mL of different concentration of casein solution (0.2-1.0% w/v) in 0.05 M Tris buffer at pH 8.0 were concurrently added and the reaction mixture was mixed and incubated at 37°C for 10 min. The reaction was stopped by adding 5.0 mL of a solution containing 0.11 M trichloroacetic acid, 0.22 M sodium chloride and 0.33 M acetic acid mixed in ratio 1:2:3. Protease assay was then carried out in each of the inhibitory stage.

Purification of the extracellular protease

Dialyses: Salting out process was carried out on the crude enzyme extract by dialyzing 55% (NH₄)₂SO₄ saturated solution of the crude enzyme extract using SIGMA Dialysis Tubing Cellulose Membrane, D9402, for 48 h and thereafter, centrifuged (Kendros PicoBiofuge, Heraeus)

at 5000 g for 5 min. The sediment was reconstituted using buffer solution. Total protein and enzyme assay were carried out on the part of the reconstituted enzyme solution while other part was used for gel filtration.

Gel filtration: Sephadex G-100 was soaked for 72 in deionized water. It was poured gently into the columns (30 mL×2 cm) and the packed gel form a bed length of 20 cm. Small amount of sodium azide was added to prevent pathogen contamination. The flow rate was 1 mL/3 min. Fifty elution samples were collected and in each (3 mL) total protein and enzyme activity were carried out.

Statistical analysis: The antioxidant activity of the volatile oil and BHT was evaluated with a paired t-test correlation analysis. The values were presented as Mean±SEM and the values were considered significant at p<0.05.

RESULTS

The antioxidant activity against DPPH, antimicrobial and inhibitory effects of the volatile oil of *Ocimum gratissimum* plant were carried out on the extracellular protease of *Salmonella enteritidis*. The antioxidant activity of the volatile oil was shown in Table 1. The average radical scavenging activity of the volatile oil was 66.98±1.78% while BHT was 53.29±2.51%. The average percentage radical scavenging activity of volatile oil of *Ocimum gratissimum* against DPPH was significantly higher (p<0.0001) than the BHT. Similarly, the antioxidant activity of the volatile oil of this plant showed a distinct higher activity per antioxidant volume against DPPH when compared with the activity of BHT (Fig. 1).

The percentage growth inhibition of the volatile against *Salmonella enteritidis* was shown in Fig. 2. The IC₅₀ of the volatile oil was 1.58% (v/v). The highest and lowest percentage growth inhibitions were 64.0 and 18.0, respectively.

Table 1: Total antioxidant activity of the volatile oil of *Ocimum gratissimum*

Antioxidant	Radical scavenging activity (%)	IC ₅₀
Volatile oil	66.98±1.78 ^a	0.084
BHT (positive control)	53.29±2.51 ^b	0.028

The values are presented as Mean±SEM, n = 2. ^{a,b}The means are statistically different at p<0.0001 when compared with each other

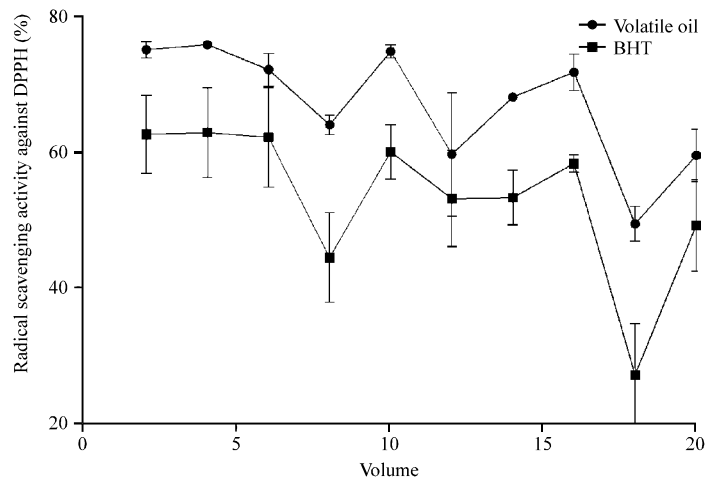


Fig. 1: Antioxidant activity of the volatile oil of *Ocimum gratissimum* as compared to BHT

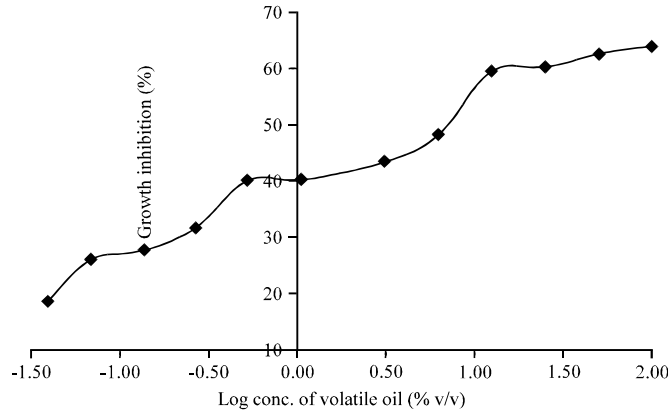


Fig. 2: Growth inhibition by the volatile oil of *Ocimum gratissimum* against *Salmonella enteritidis*

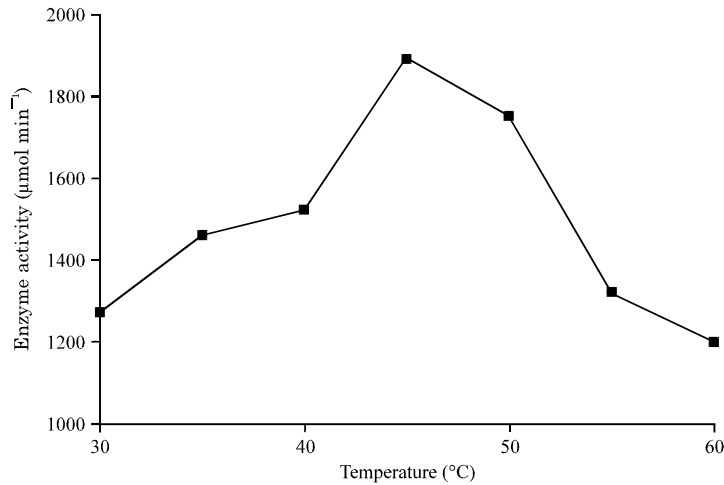


Fig. 3: Effect of temperature on the activity of the extracellular protease of *Salmonella enteritidis*

Figure 3 and 4 show the effects of temperature and pH on the activity of the extracellular protease of *Salmonella enteritidis*. The enzyme exhibited highest activity of 1894 $\mu\text{mol min}^{-1}$ at 45 $^{\circ}\text{C}$ and 2150 $\mu\text{mol min}^{-1}$ at pH 7.5, respectively. The activity of this enzyme was relatively stable between pH of 6.5 and 8.5 (Fig. 4).

Figure 5 shows the activity of the extracellular protease in the presence of metallic chlorides. The figure offers three classes of activity. The Hg^{2+} and Pb^{2+} were potent inhibitors, reducing the activity of this protease by 4 $\frac{1}{2}$ folds. The Ca^{2+} , Co^{2+} , Fe^{2+} and Fe^{3+} were moderate inhibitors while Mg^{2+} , Mn^{2+} , Zn^{2+} , Cu^{2+} and K^{+} showed no significant effect ($p > 0.05$) on the activity of this enzyme. While, these metals may not have effectively modulated the activity of this enzyme, it might be possible that such metals like Mg^{2+} , Mn^{2+} , Zn^{2+} , Cu^{2+} and K^{+} may be needed in some other protein activities needed for their virulence. Therefore, the extracellular protease of *Salmonella enteritidis* may not be a metalloprotein or may be using metals not included in this study.

The Fig. 6 shows a double reciprocal plot for the caseinolytic activity of the extracellular protease of *Salmonella enteritidis* in the presence of the volatile oil of *Ocimum gratissimum* as inhibitor. The plot showed a non-competitive inhibition by the volatile oil. The V_{max} in the presence

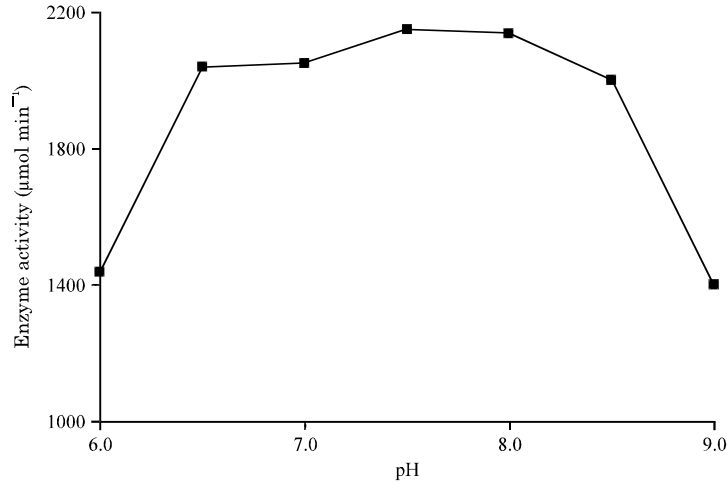


Fig. 4: Effect of pH on the activity of the extracellular protease of *Salmonella enteritidis*

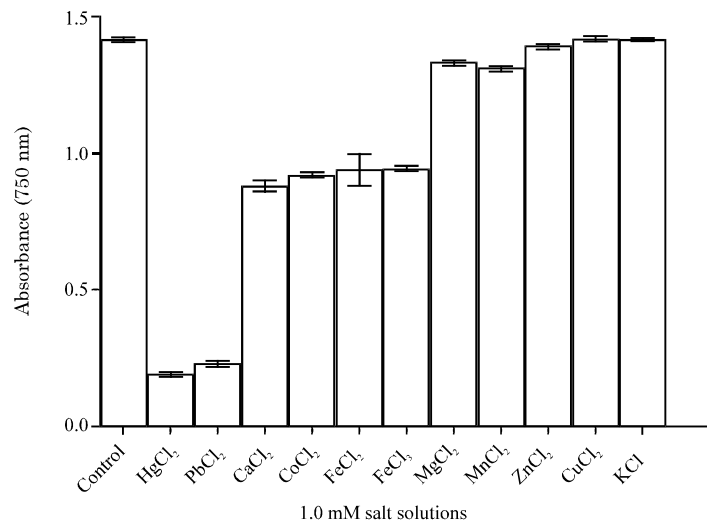


Fig. 5: Effects of metallic chloride salt solution on the caseinolytic activity of the extracellular protease of *Salmonella enteritidis*

and absence of the oil was $250 \mu\text{mol min}^{-1}$ and $250 \mu\text{mol min}^{-1}$, respectively. There was a 5-fold reduction in the activity of this enzyme in the presence of the volatile oil, with K_m of 0.33 mg mL^{-1} .

Figure 7 shows the elution chromatogram of the extracellular protease of *Salmonella enteritidis* using Sephadex G-100. The plot comprised both total protein and enzyme activity axes. By the nature of the pore size on the gel, a peak each indicated the presence of monomeric protein with an enzyme activity in the partially purified enzyme extract was revealed. A more stringent Sephadex may reveal the true nature of the protein and probably additional activity.

The purification of the extracellular protease of *Salmonella enteritidis* was shown in Table 2. The highest purification fold and percentage yield were 1.88 and 51.74, respectively as compared to the crude extract. Similarly, the highest specific activity obtained was $29.78 \mu\text{mol min}^{-1} \text{ mg}^{-1}$ protein as compared to the crude enzyme extract with specific activity of $15.83 \mu\text{mol min}^{-1} \text{ mg}^{-1}$ protein. Hence, there was a two-fold increase in the activity of this enzyme.

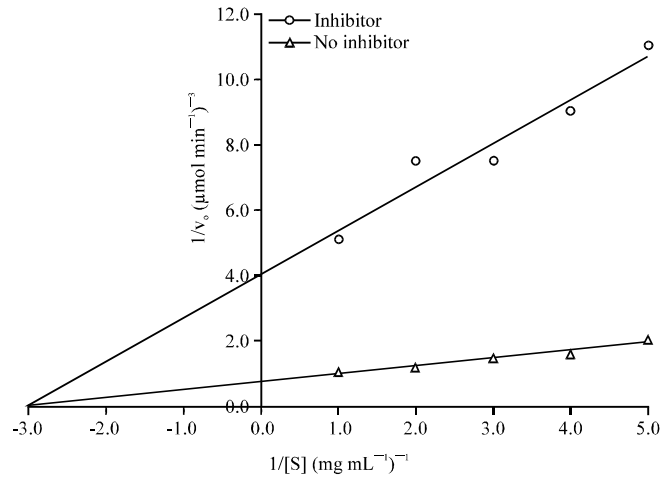


Fig. 6: Lineweaver Burk plot of enzyme activity in the presence of volatile oil of *Ocimum gratissimum* as inhibitor

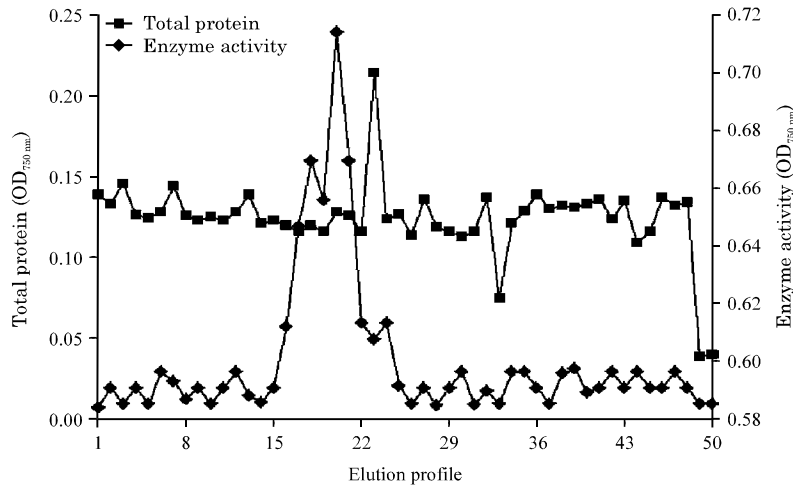


Fig. 7: Elution profile for Sephadex G-100 gel filtration

Table 2: Summary of purification procedure

Purification steps	Total protein (mg)	Total activity (μmol min ⁻¹)	Specific activity (μmol min ⁻¹ mg ⁻¹ protein)	Yield (%)	Purification fold
Crude enzyme extract	120	1900	15.83	100.00	1.00
55% (NH ₄) ₂ SO ₄ precipitation	69	1400	20.29	73.68	1.28
Sephadex G-100	33	983	29.78	51.74	1.88

DISCUSSION

The volatile oil of *Ocimum gratissimum* has been shown to inhibit *Salmonella enteritidis*. This work has also shown a significantly higher antioxidant activity of this oil as compared to BHT. A significant antioxidant activity of this oil has also been obtained in a work carried out by Saliu *et al.* (2011). *Ocimum gratissimum* is a potential medicinal plant that yields volatile oils of different chemical components. Craveiro *et al.* (1981) and Lemos *et al.* (2005) reported some

chemical components and active ingredients found in the oils of this plant and these include eugenol, linalol, methyl cinnamate, camphor and thymol. It has been demonstrated that the eugenol isolated from *Ocimum gratissimum* exhibited antimicrobial activity (Ntezurubanza *et al.*, 1984; Nakamura *et al.*, 1999; Iwalokun *et al.*, 2003; Lemos *et al.*, 2005; Adeola *et al.*, 2012; Saliu *et al.*, 2011). Furthermore, the presence of polyphenols, flavonoids orientin and vicenin in the species of these plants has made it to possess cardioprotective and radioprotective activities (Sharma *et al.*, 2001; Panda and Naik, 2009; Uma Devi *et al.*, 2000). Its antimicrobial activity as shown in this study, might have probably been because of the presence of eugenol and its conjugates. Eugenol from plant materials is best extracted by steam distillation. Adebolu and Salau (2005) used three different aqueous extracts of *Ocimum gratissimum* (cold, hot and steam) on four different pathogenic microorganisms, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhimurium* and *Salmonella enteritidis* and only the steam distillation extract showed inhibitory activity.

The extracellular protease of *Salmonella enteritidis* was a thermosensitive protein whose activity diminished sharply beyond 45°C. Contrarily, this enzyme showed a relative transitional stability to the effect of pH, this may be one of the reasons why this pathogen can withstand pH fluctuation in the GIT apart from the highly acidic content of the stomach. After evading the gastric microacidic environment as a result of multiple gene copies of amino acid decarboxylase and NH₄⁺/H⁺ transmembrane protein (Foster and Spector, 1995), the release of multiple gene copies of proteases to break down epithelial glycoproteins and immunogenic proteins may be another strategy designed by this organism in order to survive in the intestine, urethra and rectal of the infected patients. Most extracellular proteases of enteric pathogenic disease-causing bacteria have been found to exhibit optimal activities at 40-50°C and pH 6.5-8.5 (Lee *et al.*, 2002; Makino *et al.*, 1981; Adeola *et al.*, 2012). The extracellular protease of *Salmonella enteritidis* seemed not to depend on the availability of metallic ions to function. Meanwhile, metals like Mg²⁺, Mn²⁺, Zn²⁺, Cu²⁺, Ca²⁺ and K⁺ are very important for cellular activities including signalling, membrane transport, DNA formation and stabilization, enzyme catalysis and many other cellular functions while heavy metals like Pb²⁺ and Hg²⁺ are generally inhibitors of enzymes/proteins.

The non-competitive mode of inhibition exhibited by the volatile of *Ocimum gratissimum* against the activity of the extracellular protease of *Salmonella enteritidis* has complemented its antibacterial activity. Though, it does not compete with the substrate nevertheless, it has an ability to bind to other sites on the active enzyme and in the protein thereby creating misfit of the enzyme for the true substrate. This invariably decreases the catalytic activity of the enzyme. Meanwhile, the oil may not bear any component resembling the substrate for this protease however; it may interact with the regulatory sites of the enzyme thereby altering the catalytic turnover rate of this enzyme. This, therefore, suggests one of the ways by which most of the plant extracts exhibit their antimicrobial activity.

The mode of purification adopted in this study proved to be relatively effective because twice the enzyme activity and half of the total protein were obtained with the combination of dialysis and gel filtration only. Further studies are needed on this protease to determine its molecular weight and nature of its active site.

CONCLUSION

The essential oil of *Ocimum gratissimum* possessed antioxidant, antimicrobial and inhibitory properties that can be adopted to inhibit the growth of *Salmonella enteritidis*, a pathogenic

microorganism and can, therefore, serve as an alternate antimicrobial drug to the present falling and compromising antibiotics. Further studies and clinical trials may be carried out to evaluate the potential of this oil as an antimicrobial and antioxidant agent against a wide range of microorganisms as well as the isolation and purification of the active agents in it to aid the manufacture of new natural drugs.

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