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Oil of *C. sinensis* inhibits pathogenic bacteria

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Abstract

Context: In spite of great advances observed in modern medicine, plants still make an important contribution to health care. Hence, there is need for unrelenting effort in the exploration of the health benefit of medicinal plants.

Purpose: This work was designed to determine the role of the volatile oil of *Camellia sinensis* on the extracellular protease, which is one of the major virulent factors in the pathogenesis of *Shigella dysenteriae* and its antibacterial effects on eight other enteric bacterial as compared with the antibiotics

Finding: The total antimicrobial effect of the volatile oil (355.0 mm) was significantly higher *($p<0.05$)* than the antibiotics tested (203.0 mm). The minimum inhibitory and bactericidal concentrations of the oils revealed *Escherichia coli* as the most sensitive. However, relatively higher concentrations of the oils is required to achieve similar sensitivity against *Salmonella* and *Shigella species*. The activity of the partially purified extracellular protease, which is one of the virulence factors of *Shigella dysenteriae* was inhibited by the oil from different parts of the plant especially the leaf. The activity of this enzyme increased steadily between pH 7.0 – 8.0 and 40 – 50 ^{o}C.

Summary: The volatile oils possessed antimicrobial activity and showed both competitive and noncompetitive kinetic inhibition of the extracellular protease of *Shigella dysenteriae.*

Implication: The inhibitory action of the oil on protease from *Shigella dysenteriae* suggests the possible mode of action. Volatile oil from *Camellia sinensis,* especially the leaf, may be an important source of antibiotic against these organisms particularly *Shigella dysenteriae.*

Keywords: *Camellia sinensis*, volatile oil, pathogenic bacteria, antibacterial, extracellular protease, *Shigella dysenteriae*

Introduction

In spite of great advances observed in modern medicine, plants still make an important contribution to health care. *Camellia sinensis* (*Theaceae*), a native tea plant to residents of mainland South and Southeast Asia, is today cultivated across the world in tropical and subtropical regions [1].Although, many types of tea can be prepared from this plant but the commonest are the Green (non-fermented) and the Black (fermented) tea leaves. In Nigeria, the leaf of this plant is normally boiled in combination with other plants and is commonly called *"agbo"*. This liquid *(agbo)* from the mixture is usually taken as traditional medicine against gastroenteritis infections (like dysentery, diarrhea, enteritis), malaria fever, asthma, jaundice to mention but few. This practice is common among the *Yorubas*, the major tribe in Western part of the country. Previous reports on the organic extract of the tea leaf have revealed the presence of some chemical components like 2-propanoyl-2H-benzo(*f*) chromene, terpineol, thujene, phellandrene, terpinene, *cis*-methyl isoeugenol, polyphenols and many others[2,3]. In Nigeria, the organic solvents extracted from the leaf of this plant have been reported to have antimicrobial (*Escherichia coli, Listeria monocytogenes, Pseudomonas aeruginosa, Staphylococcus aureus, Salmonella species* and *Shigella species*) and antifungal (*Candida albicans*) properties[4,5]. In addition, immune modulation properties have been reported on the organic solvent extracts from the leaf of this plant. Consequently, the plant is used for the treatment of diseases such as angina pectoris, peripheral vascular and coronary heart diseases[6].

*Shigellae species*are gram-negative, non-spore, non-motile facultative anaerobicbacteria[7] known to cause bacillary dysentery/shigellosis[8]. *Shigella dysenteriae* is commonly spread by contaminated water and food with the release of shiga toxins[9]. Dysentery occurs as a result of persistence opening of cellular membrane carrier and transporter proteins/receptors caused by irreversible shiga toxin-receptors interaction. Shiga toxins act to inhibit protein synthesis within target cells by a mechanism similar to that of ricin toxin[10]. Existing antibiotics for the treatment of this bacterial infection are becoming compromised due to increased resistance. Also, the cost of treatment, habitual unhygienic practices and persistent unsanitary conditions necessitate the need for alternative treatment of shigellosis. Currently, no vaccine is available that can provide adequate protection against many different serotypes of *Shigella*. The pathogenesis of *Shigella dysenteriae* largely depends on its ability to secret toxins and

extracellular proteins/enzymes, which facilitate host specification, adhesion, integration and colonization[11]. According to Meta and Nicole, *Shigella* extracellular protease, as a virulence factor, plays a crucial role in numerous pathological processes through membrane vesicle formation and the release of extracellular exotoxins and hydrolytic enzymes[12].Although, both aqueous and organic solvent extracts from *Camellia sinensis* have been reported to inhibit the growth of *Shigella dysenteriae* and other enteric bacterial in Nigeria[4]the mode of such inhibition is not yet known.
This work was designed to determine the role of the volatile oil of *Camellia sinensis* on the extracellular protease, which is one of the major virulent factors in the pathogenesis of *Shigella dysenteriae* as well as its antimicrobial activities against other eight enteric clinical pathogens.

Materials and Methods

Plant Materials

Camellia sinensis (Tea plant) was obtained locally at Ojo Local Government Area of Lagos State, as green foliage and air-dried for 5 days. The sample of the tea plant was identified and authenticated at the Botany Department, Faculty of Science, Lagos State University, Ojo Lagos, Nigeria.

Microorganisms

The microorganisms used in this work were both Gram positive (Staphylococcus aureus) and Gram negative bacteria, which include Enteroheamorrhagic Escherichia coli (EHEC) (0157:H7) (43895), Staphylococcus aureus (25923), Escherichia coli (25922), Klebsiella pneumoniae (4352), Pseudomonas aeruginosa (27853), Salmonella paratyphimurium (9150), Salmonella typhimurium (700931), Shigella dysenteriae (13313) and Shigella flexneri (29903). These microorganisms from The Nigerian Institute of Medical Research (NIMR), Yaba Lagos, Nigeria were collected on a nutrient agar plates. EHEC, a more pathogenic virulent strain of Escherichia coli was cultured from a woman suffering from bacillary dysentery. The nutrient agar plates were stored at 4^{o}C until they were tested.

Antibiotics

Susceptible antimicrobial sensitivity discs were purchased from a Pharmaceutical store in Ojo Local Government Area of Lagos State, Nigeria. The antibiotic discs were coated with the following drugs: *Ofloxacin* (Travid) – 5µg, *Erythromycin*– 10µg, *Clindamycin*– 5µg, *Ciprofloxacin*– 5µg, *Gentamicin*– 10µg, *Cephalexin*– 30µg, *Cotrimoxazole*– 50µg, *Ampicillin*– 30µg, **Ceftriaxone*– 30µg, *Augumentin*– 30µg. Ceftriaxone, a third generation cephalosporin β-lactam base drug, was arbitrarily assigned as standard antibiotic because it is widely prescribed by Clinicians as a drug of last resort in chronic and acute pathogenic infections, though its safety in neonates remained controversial[13].

Extraction of Volatile Oil

The volatile oil of *Camellia sinensis* tea plant was extracted as described by Wannissorn [14]. Briefly, the five-day-air-dried *Camellia sinensis* tea plant was separated into leaves, stems and roots. Each of the three parts was chopped into pieces and packed into 5L Quick fit (34/35 size) round bottom flask containing 1.5L of distilled water with fixed Clevenger. The oil was extracted steadily at temperature of 70^{o}C for 3hours. As a volatile liquid, the extracted oil was collected in a 2mL*n*-hexane. The oil was dried over anhydrous copper II tetraoxosulphate VI (BDH) to concentrate it and was stored in a screw cap amber colour bottle at 4^{o}C until it was tested.

Antimicrobial Susceptibility Tests

The sensitivity tests of the antibiotics and volatile oils were carried out using agar-gel diffusion technique as described by Wannissorn[14]with slight modification. Briefly, discrete colonies of the microorganisms were obtained on a nutrient agar (BDH)overnight. A colony of each organism was inoculated on a fresh nutrient agar. Then the antibiotic sensitivity disc was gently placed on the culture in each plate. The plates were incubated for 24 hours at 37 ^{o}C. It is worth stating that, a selective medium (*Salmonella-Shigella agar/broth*, BDH) was used to maximize the growth of *Salmonella* and *Shigella species* as specified by the manufacturer. Similarly, sterilized filter paper discs (5.0mm) were soaked with volatile oil for 3 seconds and placed on the culture of each bacterium. The inoculated plates were incubated for 24 hours at 37^{o}C. The magnitude of sensitivity, measured in millimetre, was considered proportional to the diameter of a clear zone (inhibition) around the paper disc.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The MIC and MBC of the volatile oil of *Camellia sinensis* were carried out using microbroth dilution method as described by Wannissorn[14]with little modification. Briefly, a colony of each organism was added to 200µL of susceptible test Muller Hinton broth(with 0.5 %v/v Tween 80 as diluent) containing two-fold serial dilution of the volatile oil in a microtitre plate (21.5 x 17 cm^{2}). The plates were covered, taped and incubated at 37^{o}C for 24hours. The broth from each well was re-inoculated on a freshly prepared Muller Hinton agar (BDH) where MIC and MBC were determined. The MIC was interpreted as the lowest concentration of the oil sample that prevented visible growth of the microorganism in the required medium at standard conditions. The MBC was considered as the lowest concentration of the oil that yielded no colonial growth of the subcultured microorganisms in the required medium under favourable conditions.

Extraction of Crude Enzyme

The extracellular protease of *Shigella dysenteriae* was extracted as described by Sharmi[15]. A colony of *Shigella dysenteriae* was

inoculated into a 5ml *Salmonella-Shigella* broth using McCartney bottle. It was incubated for 24 hours at 37ºCafter which it was centrifuged at 9000rpm for 10 minute at room temperature. The supernatant (enzyme extract) was decanted and stored in a sample bottle at 4ºC until it was tested.

Determination of Total Protein of the Crude Extract

The total protein of the crude extract was determined using Lowry method [16]. The protein was quantified by adding 5.0mL of alkaline solution containing a mixture of 50mL of solution A (20g sodium trioxocarbonate IV and 4g sodium hydroxide in 1 L distilled water) and 1 mL of solution B (5g cupper II tetraoxosulphate VI pentahydrate and 10g sodium-potassium tartrate in 1L distilled water) to 0.1mL of crude extract and mixed. The mixture was allowed to stand for 10 minutes at room temperature and 0.5mL of freshly prepared Folin Ciocalteau's phenolic reagent (50%v/v) was added. The solution was mixed thoroughly and the absorbance was read at 750nm against reagent blank (Spectronic-21, Bausch and Lomb) after 30minutes. Bovine serum albumin was used as standard protein (0.20mg/ml)

Determination of Total Protease Activity of the Crude Extract

The assay for extracellular protease activity of *Shigella dysenteriae* was carried out using Chopra & Mathur [17] method with slight modification. This was done by adding 5.0 mL of casein solution (0.6%w/v in 0.05M Tris buffer at pH 8.0) to 0.1mL of the crude enzyme extract and the mixture was incubated for 10minutes at 37ºC. The reaction was stopped by adding 5.0mL of a solution containing 0.11M trichloroacetic acid, 0.22M NaCl and 0.33M acetic acid mixed in ratio 1:2:3. The turbid solution was filtered and 5.0mL of alkaline solution was added to 1.0mL of the filtrate and mixed thoroughly for 10 minutes.0.5mL of freshly prepared Folin Ciocalteau's phenolic reagent was then added and the absorbance was read at 750nm(Spectronic-21, Bausch and Lomb) after 30minutes. L-tyrosine solution (0.20mg/ml) was used as standard for the protease activity.1.0 unit of protease activity was defined as the amount of enzyme required to liberate 1.0 µmol of tyrosine in 60 seconds at 37ºC. The specific activity was expressed in µmol/min/mg protein.

Dialysis

Salting out process was carried out on the crude enzyme extract. Thirty-five percent $(NH_4)_2SO_4$ saturated solution of the enzyme extract was dialyzed (using SIGMA Dialysis Tubing Cellulose Membrane, D9402) for 48 hours and thereafter centrifuged at 5000 rpm to get the sediment. Then, 50 % $(NH_4)_2SO_4$ saturated solution of the sediment was dialyzed for another 48 hours. Successively, dialysis of 55 % and 65 % saturated solutions of the immediate previous sediment were done for the same periods as the first. In each case both total protein and enzyme assays were carried out.

Gel Filtration

This was carried out by soaking 3.0 g of Sephadex G-100 (BDH) in distilled water for 72 hours. The gel was poured into the chromatographic column (28.0cm by 1.5cm column) and formed a bed length of 23 cm with a flow rate of 1.5mL/min. After, pre-equilibration with 0.05 M Tris buffer, it was then used to separate 65 % $(NH_4)_2SO_4$ dialysate using the same buffer. A total number of 50 elutions were collected. Each elution contained 3.0 mL of eluent and in each of the eluent, both total protein and enzyme assays were carried out. Subsequently, 3.0 g of Sephadex G-75 (BDH) was used with the same procedure to separate the pooled eluents of Sephadex G-100 and in each case, both total protein and enzyme assays were carried out.

Determination of Optimum pH

Optimum pH for the activity of the extracellular protease of *Shigella dysenteriae* was carried out using a method described by Padmapriya[18]with little modification. Protease activity was assayed using 0.6% casein solution in 0.05M Tris buffer solution (pH 6.0 – 9.0) at 37ºC.

Determination of Optimum Temperature

The optimum temperature for the activity of the extracellular protease of *Shigella dysenteriae* was carried out using a method described by Padmapriya[18] with slight modification. Protease activity was assayed under varying temperature conditions (30 – 70ºC) using 0.6% casein solution in 0.05M Tris buffer at pH 8.0.

Inhibitory Assay

The method used was described by Makino [19] with a slight modification. The assay was carried out by adding 0.1mL of the partially purified enzyme and 0.1mL of 3.5%v/v of the volatile oil (in 0.5 %v/v Tween 80 solutions) (inhibitor) concurrently to different concentration of casein solution (0.2 – 1.0%w/v) in 0.05M Tris buffer, pH 8.0 and the reaction mixture was shaken and incubated at 37ºC for 10minutes. 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. The turbid solution was filtered and 5.0 mL of alkaline solution was added to 1.0 mL of the filtrate and mixed thoroughly for 10 minutes. 0.5 mL of freshly prepared Folin Ciocalteau's phenolic reagent was then added and the absorbance was read at 750 nm (Spectronic-21, Bausch and Lomb) after 30 minutes and the protease activity was assayed. This assay was done separately for the volatile oils of the leaf, stem and root. The procedure was again repeated without an inhibitor.

Statistical Analysis

Antimicrobial activities of the volatile oils and the antibiotics were compared using student'st-test and Dunnett's Multiple Comparison

Test with the use of GraphPad Prism version 5.0 and the means were considered statistically different at *p<0.05*

Results

Antimicrobial Activity of Antibiotics

Figure 1 shows the antimicrobial activities of the antibiotics tested. Most of the pathogenic microorganisms tested showed a relatively low sensitivity to the antibiotics. *Salmonella paratyphimurium* showed the highest sensitivity to the antibiotics (FX, ER, CI, GN, CX, CO, AP and OF). Relative sensitivity was also observed in *Pseudomonas aeruginosa* (FX, CI, GN and OF) and *Shigella flexneri* (FX, ER,CI and GN). *Shigella dysenteriae* showed the highest resistance to all antibiotics except FX, while others were either sensitive to two or three antibiotics out of ten used. CX, CO and AP were only sensitive to *Salmonella typhimurium* while AU has no inhibitory effect on all the pathogenic microorganisms tested.

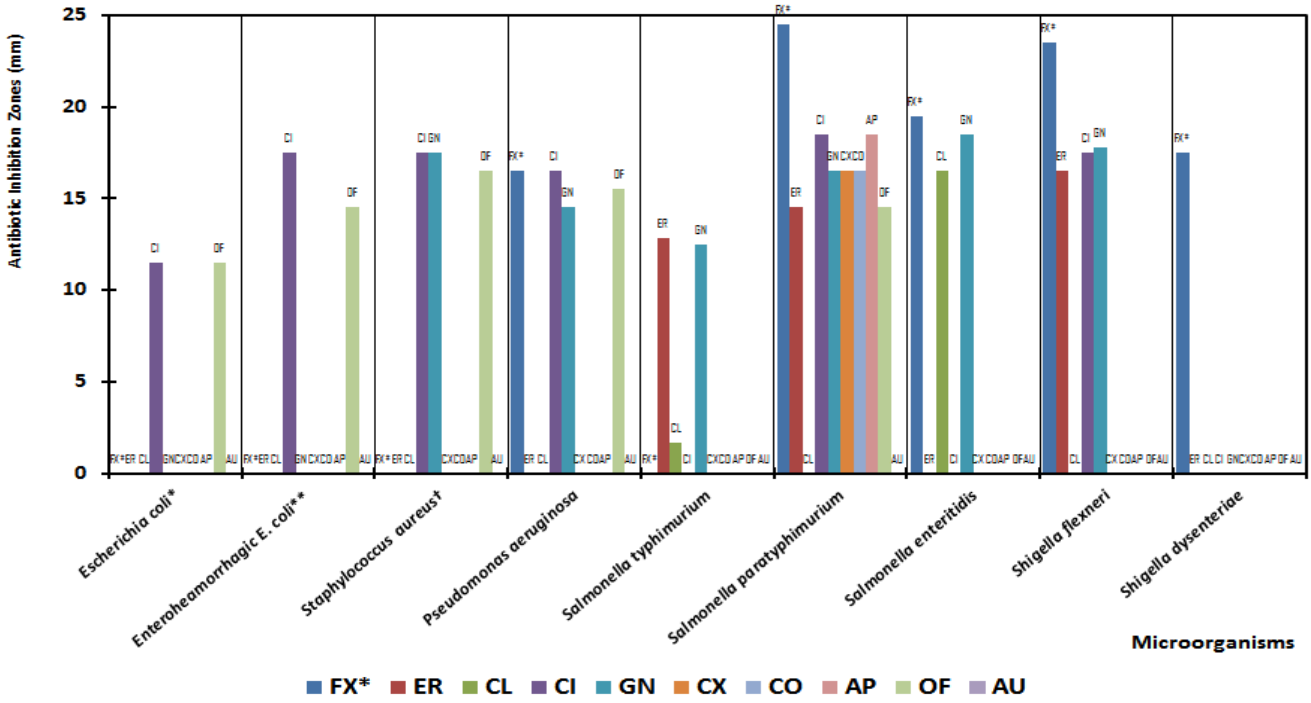

Figure 1: Antimicrobial activities of the antibiotic drugs.

*Generally less pathogenic, **Highly pathogenic and more virulence. †Gram positive bacteria

FX* (Ceftriaxone) exhibited highest antimicrobial activity on *Salmonella paratyphimurium* and *Shigella flexneri* as compared to others *FX (Ceftriaxone), ER (Erythromycin), CL (Clindamycin), CI (Ciprofloxacin), GN (Gentamycin), CX (Cephalexin), CO (Cotrimoxazole), AP (Ampicillin), OF (Ofloxacin), AU (Augumentin)*

Antimicrobial Activity of the Volatile Oils of *Camellia sinensis*

In Figure 2, antimicrobial activities of the volatile oils from the leaf, stem and roots of *Camellia sinensis* plant is presented. Volatile oils from at least two of the three parts of *Camellia sinensis* plant tested, inhibited the growth of the nine strains of bacteria studied. No statistical difference *(p>0.05)* was observed between the average growth inhibition of the enteric bacteria by Ceftriaxone (control drug), ciprofloxacin, gentamicin, ofloxacin, erythromycin, ciprofloxacin and oils from the stem as well as the root of *Camellia sinensis*. The total antimicrobial activity (355.0 mm) of the oil from the leaf *Camellia sinensis* against all the enteric pathogenic organisms used showed a significant growth inhibition *(p<0.05)* compared to the standard antibiotic drug (203.0 mm). Overall, the average total antimicrobial activity of the volatile oil was statistically higher *(p*<0.05*)* than that of the antibiotics. The highest antimicrobial activity recorded was observed in the leaf oil against the growth of *Escherichia coli* and *Shigella dysenteriae*

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the Volatile Oils:Figure 3 shows the inhibitory concentration of the volatile oils of *Camellia sinensis* against nine enteric bacteria. The MIC represents the minimum concentration of the oil that would completely inhibit or yield scanty growth of the bacteria, and if the said concentration (MBC) is doubled, it will become bactericidal. The volatile oil from the leaf had MIC (a) and MBC (b) values of 6.25 %v/v and 12.50 %v/v respectively against *Escherichia coli*. However, moderately high concentration of the volatile oils from the leaf and stem were particularly more effective against *Salmonella* and *Shigella species* than other microorganisms.

(a) Minimum inhibitory concentration and (b) Minimum bactericidal concentration.

*The lowest MIC and MBC values of 6.26 %v/v and 12.5 %v/v respectively were observed against Escherichia coli. It implies that this bacteria was more sensitive to the oil even at low concentration. Similarly, Salmonella and Shigella species were sensitive but at higher concentration of the oils

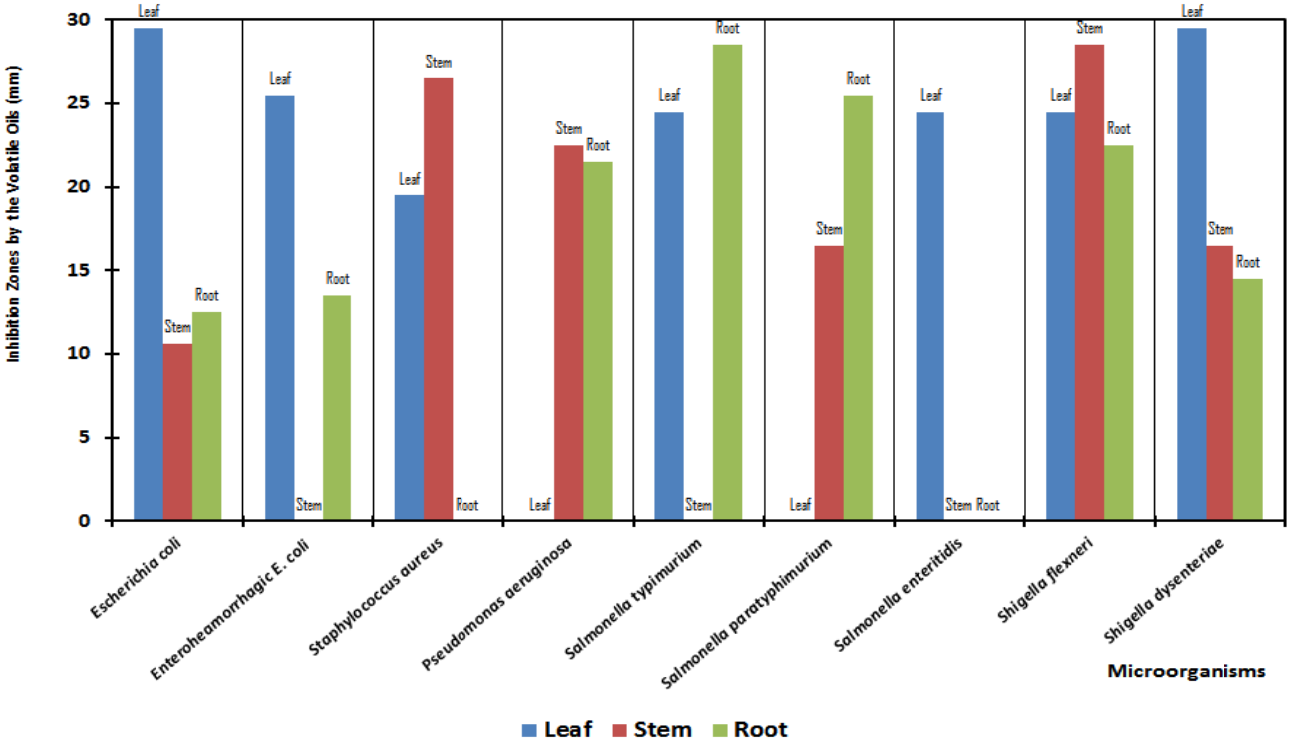

Figure 2: Antimicrobial activities of the volatile oils of the leaf, stem and root of *Camellia sinensis.*

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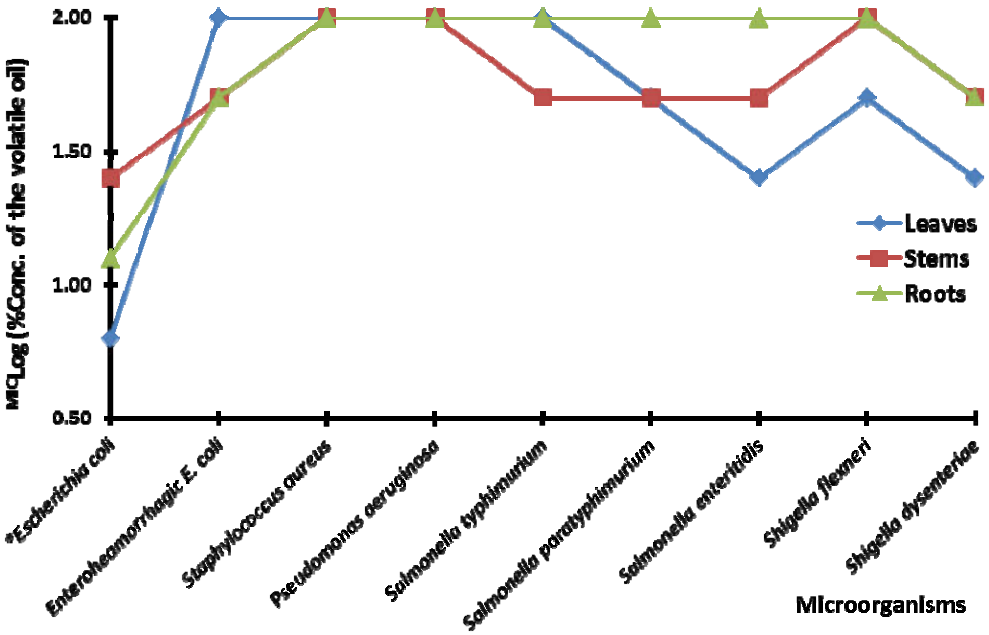


3(b)

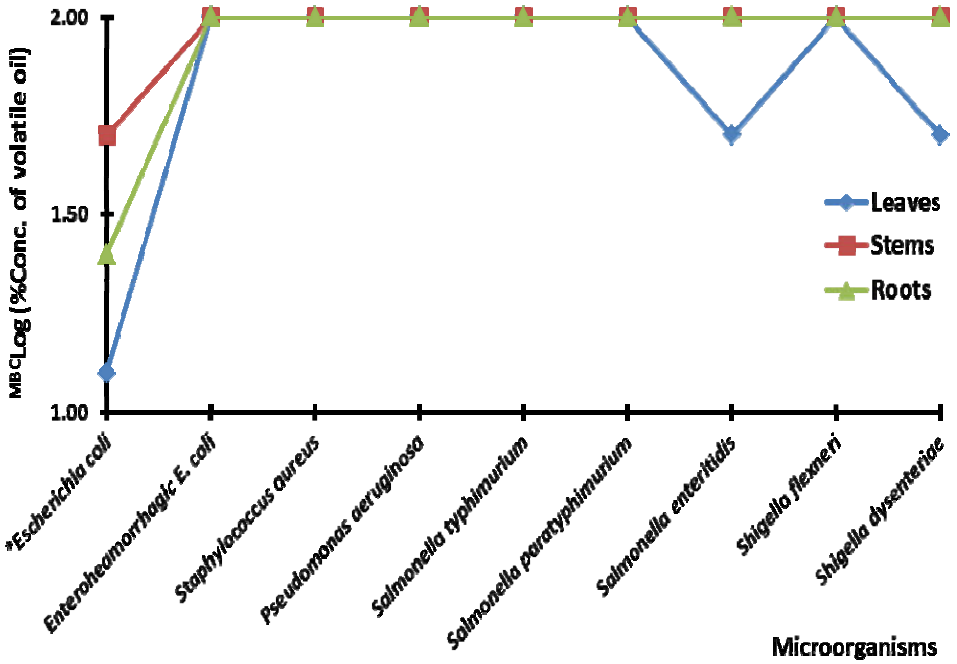


Figure 3: Inhibitory concentration of the volatile oil of *Camellia sinensis* against nine enteric pathogenic bacteria.

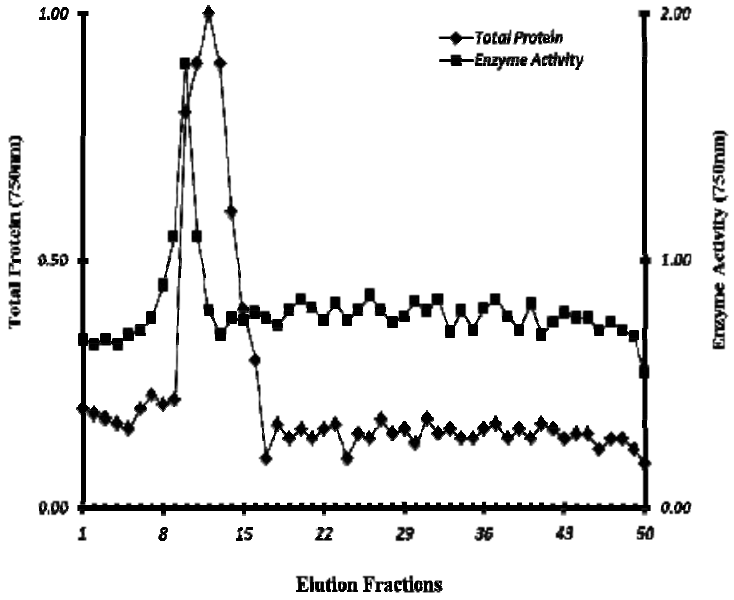
Partial Purification of the Extracellular protease of Shigella dysenteriae

In Table 1, the percentage recovery and purification fold from the crude extracellular protease of *Shigella dysenteriae* are presented. The crude extract was dialyzed successively in four different ammonium sulfate solutions as 35%, 50%, 55% and 65%. The highest percentage yield of 58.5 and purification fold of 22.9 were obtained with 65% $(NH_2)SO_4$ precipitation as compared to the crude extract. However, Sephadex G-75 elution chromatography yielded 90.5 and purification fold of 33.2.Figure 4 (a) and (b) show the elution profiles for size exclusion gel chromatography of the extracellular protease of *Shigella dysenteriae*. Each peak was produced for both total protein and enzyme activity in G-100 elution profile but two peaks were observed for total protein in G-75.
(a) Elution profile using Sephadex G-100 and (b) Elution profile using Sephadex G-75

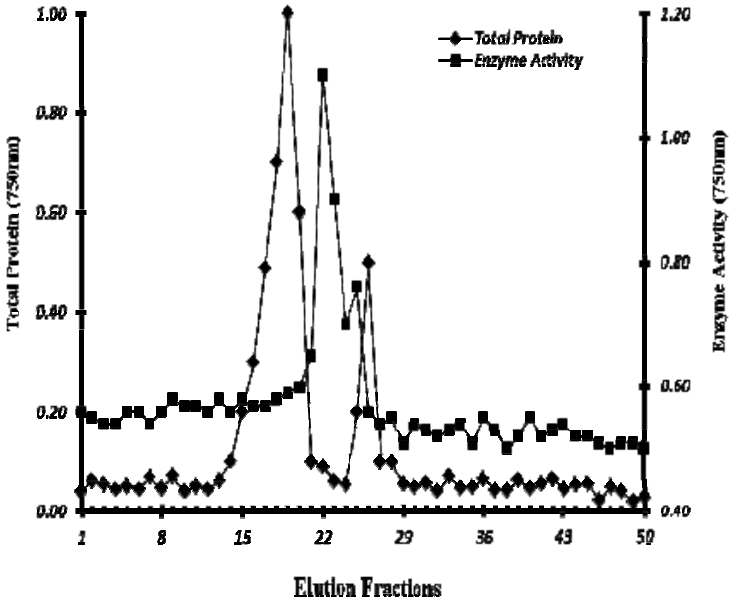
Table 1: Purification analysis of the crude extract of extracellular protease of *Shigella dysenteriae*

Purification Steps	Total Protein (mg)	Total Activity (µmol/min)	Specific Activity (µmol/min/mg protein)	Percentage Yield	Purification Fold
Crude Cellular Extract	0.586	1.998	3.4	100	1.0
35% $(NH_2)SO_4$ precipitation	0.067	1.898	28.3	95.0	8.3
50% $(NH_2)SO_4$ precipitation	0.049	1.711	34.9	85.6	10.3
55% $(NH_2)SO_4$ precipitation	0.026	1.578	60.7	79.0	17.9
65% $(NH_2)SO_4$ precipitation	0.015	1.169	77.9	58.5	22.9
Sephadex G-100	0.014	0.986	70.4	43.3	20.7
Sephadex G-75	0.016	1.808	113.0	90.5	33.2

Highest purification fold of 33.2 corresponding to 90.5% yield from the crude extracellular protease of *shigella dysenteriae* was obtained



(a)



(b)

Figure 4: Size exclusion gel chromatography of the extracellular protease of *Shigella dysenteriae*.
(a) Elution profile using Sephadex G-100 and (b) Elution profile using Sephadex G-75

Characteristic Of Extracellular Protease Of Shigella dysenteriae

Effect of pH: The extracellular protease of *shigella dysenteriae* showed optimum caseinolytic activity of 0.062 μmol/min at pH 8.0 while the activity of the enzyme increased steadily from 7.0 to 8.0. This implies that this organism may survive even relatively alkaline environment (Figure 5).

Effect of Temperature

The extracellular protease of *Shigella dysenteriae* exhibited maximum activity of 0.082 μmol/min at 43.4 ^{o}C. Nevertheless,

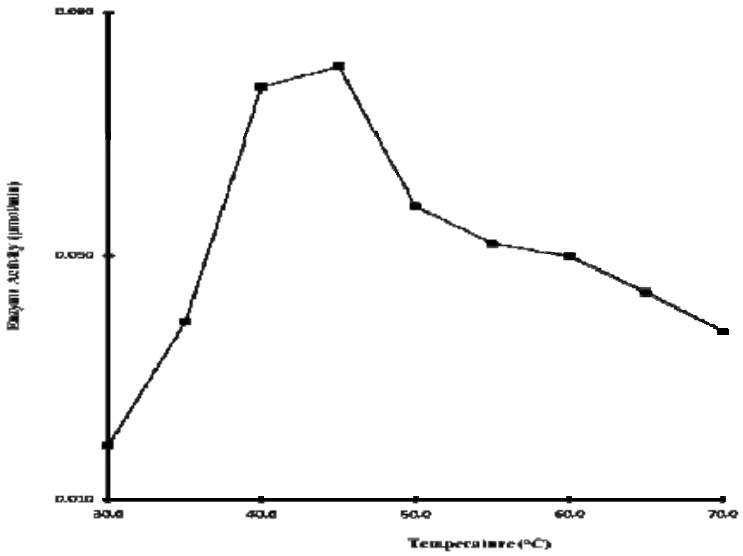


Figure 5: The effect of pH on the activity of extracellular protease of *Shigella dysenteriae*.

temperature above 50 ^{o}C inhibited the activity of this enzyme (Figure 6).

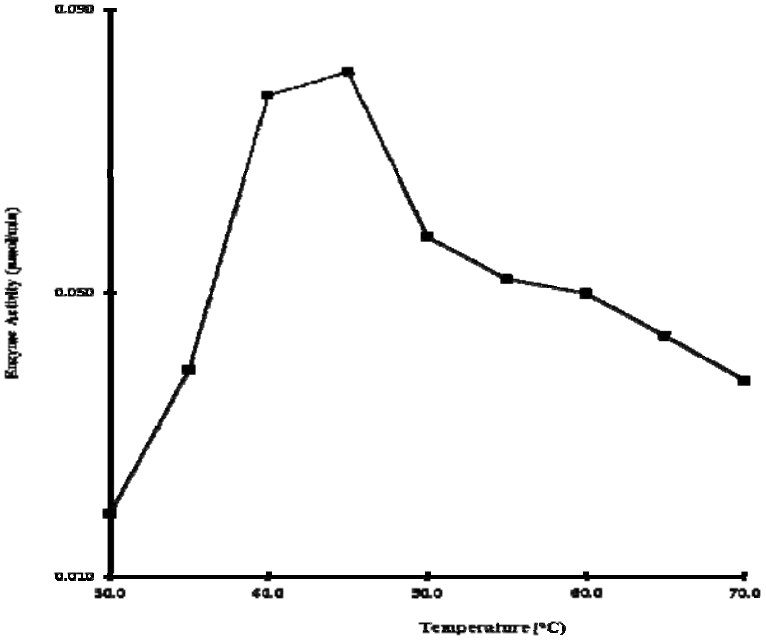
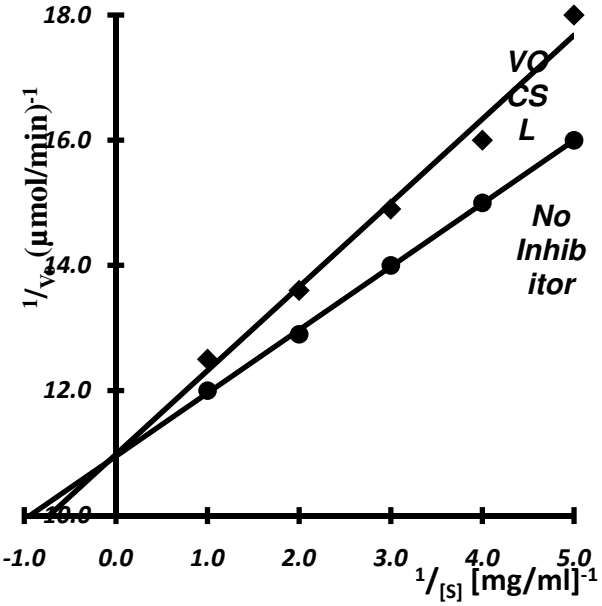
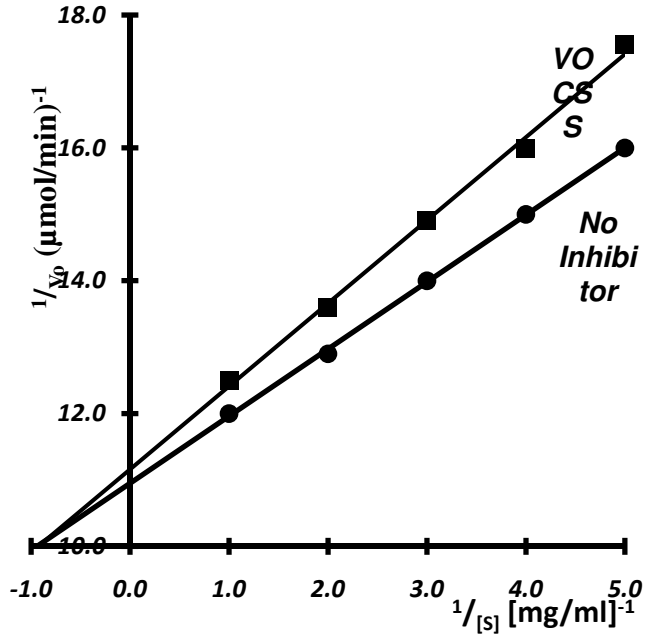


Figure 6: The effect of temperature on the activity of extracellular protease of *Shigella dysenteriae*.

Effect of Volatile Oils as Inhibitor: Inhibitory kinetics of the extracellular protease of *Shigella dysenteriae* was shown in Figure7.



(a)



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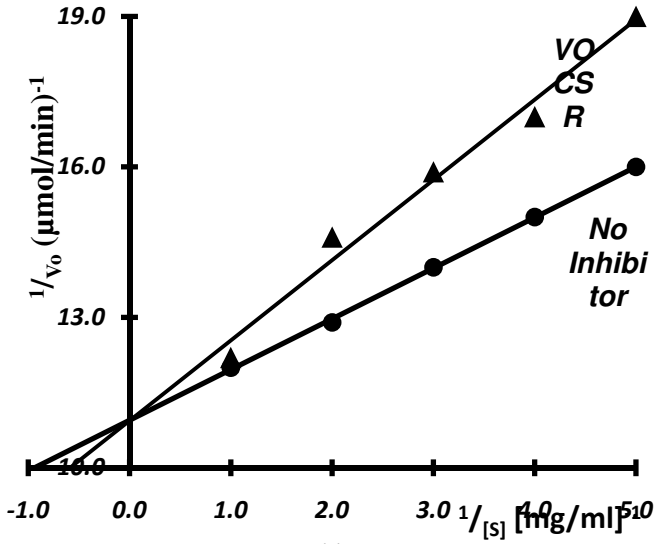

(c)

Figure 7: Lineweaver Burke kinetic plot forthe inhibition of the volatile oils against the activity of the extracellular protease of *Shigella dysenteriae*.

Lineweaver Burke kinetic plot of enzyme inhibition showed VOCSL (volatile oil of *Camellia sinensis* Leaf) and VOCSR (volatile oil of *Camellia sinensis* Root) to have competitively inhibited the extracellular protease of *Shigella dysenteriae* while VOCSS (volatile oil of *Camellia sinensis* Stem) showed a noncompetitive type of inhibition. In competitive inhibition, both axes intersected at the vertical axis resulting in both having the same V_{max} but different K_m while the noncompetitive inhibition showed different V_{max} but the same K_m. Generally, the V_{max} and K_m in the absence of inhibitor were 9.1x 10^{-2} µmol/min and respectively. Overall, in competitive inhibition, the oils from both the leaf and root increased the K_m of this enzyme from 1.11 mg/ml to 1.42 mg/ml and 2.0 mg/ml respectively. However, in noncompetitive inhibition, the oil from the stem reduced the V_{max} from 9.1x 10^{-2} µmol/min to 8.9 x 10^{-2} µmol/min.

Discussion

The use of medicinal plants for curative and management of clinical infections is a paradigm in ethnomedicine. Medicinal plants are ubiquitous and found to possess series of phytochemicals. Some of the clinical properties of these nutraceuticals include anticancer, antibacterial, antifungal, antiviral, anthelminthics and antiparasitic protozoans[5,6].

In this study, the antibacterial effects of the volatile oils from the leaf, stem and root of *Camellia sinensis* plant against nine enteric pathogenic bacteria (*Escherichia coli, Enteroheamorrhagic Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhimurium, Salmonella paratyphimurium, Salmonella enteritidis, Shigella flexneri* and *Shigella dysenteriae*) were compared with ten different antibiotics (ceftriaxone,

erythromycin, clindamycin, ciprofloxacin, gentamicin, cephalexin, cotrimoxazole, ampicillin, ofloxacin and augumentin) commonly used for the treatment of infections caused by these microorganisms. A remarkable difference in the antibacterial effects between the volatile oils and the antibiotics was observed. However, the inhibitory effects of ceftriaxone (standard drug) and ciprofloxacin, gentamicin, ofloxacin, erythromycin, ciprofloxacin, volatile oils from stem and root on the bacteria tested showed no significant difference *($p>0.05$)*. Nevertheless, the average total antimicrobial activity of the volatile oil from the leaf of *Camellia sinensis* (39.44 mm) against all the enteric pathogenic organisms used showed a significant difference *($p<0.05$)* from the standard antibiotics (22.56 mm). The finding of this study is in consonance with a previous study, which reported the inhibitory effect of *Camellia sinensis* on enteric bacteria[4,20]. However, in that study, the aqueous and organic solvents extracted from *Camellia sinensis* exhibited the inhibitory effects and not the volatile oils. The antimicrobial activities of this volatile oil has been attributed to its ability to cross bacteria cell wall and disrupt the macromolecular arrangement of the cell membrane resulting in the death of the cell[21]. Although the minimum inhibitory and bactericidal concentrations (MIC and MBC) of the oils showed that *Escherichia coli* was the most sensitive but a relatively higher concentration is required to achieve similar sensitivity against *Salmonella* and *Shigella species* (Figure 3).

The partially purified extracellular protease of *Shigella dysenteriae*, with a yield and fold of 90.5 and 33.2 respectively from Sephadex G-75 elution chromatography exhibited optimal caseinolytic activities of 6.2 x 10^{-2}µmol/min and 8.2 x 10^{-2} µmol/min at pH 8.0 and 43.4 ^{o}C respectively. The activity of this enzyme, which increased steadily between the pH 7.0 – 8.0, implies that *Shigella dysenteriae* may survive a fairly alkaline environment. In addition, the activity of the enzyme was moderately high between 40 – 50 ^{o}C. However, temperature above 50 ^{o}C reduced the activity of the enzyme in agreement with the report of Louboudy [22] and Adeola[23]. The extracellular protease of pathogenic microbes have been referred to as enzyme virulence factors that facilitate host specification, invasion and colonization [24]. It therefore implies that inhibiting the catalytic activity of the protease will adversely affect the virulence of these organisms.

The competitive inhibition of the volatile oils from the leaf and root and noncompetitive status from the stem with casein (a known substrate for protease) for active site of the protease further demonstrate the mode of action of the oils on *Shigella dysenteriae*. Generally, protease activities have been known to be inhibited by inorganic salts such as Cu^{2+}, Pb^{2+}, Hg^{+}, Ca^{2+}, Mn^{2+}; phosphoryl molecules, chelators and detergent[19,25]. It could be speculated that, there may be some other active ingredients in the oil that may compete for the protease. However, this was beyond the scope of this study. Further studies will be necessary to unfold precisely the mechanism of action of the oil on the protease.

Volatile oils extracted from the leaf, stem and root of *Camellia sinensis* has exhibited antimicrobial activity on many enteric pathogenic bacteria especially *Escherichia coli* and *Shigella dysenteriae.* Inhibitory action of the oil on the partially purified extracellular protease from *Shigella dysenteriae* suggests the possible mode of action. Volatile oil from *Camellia sinensis,* especially the leaf, may be an important source of antibiotic against these organisms with particular reference to *Shigella dysenteriae.*

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