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RESEARCH ARTICLE

The volatile oil of *Chromolaena Odorata*: Its Antimicrobial and Inhibitory Effects on Partially Purified and Characterized Extracellular Protease of *Pseudomonas Aeruginosa*

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ABSTRACT

Context: The recent development in the antimicrobial therapy to meet the challenges of resistant strains of clinical pathogenic organisms has led to the insight of exploring the phytomedical properties of the volatile oils of medicinal plants.

Purpose: This work was designed to assess the antimicrobial activity of the volatile oils extracted from the leaf and stem of *Chromolaena odorata* on nine different types of enteric human pathogenic bacteria and to compare this effect with commonly used antibiotics. More importantly, the mode of inhibition of these oils on the extracellular protease of *Pseudomonas aeruginosa* was determined using double reciprocal plot.

Procedures: The volatile oils were extracted by hydrodistillation from air-dried leaves and stems of *Chromolaena odorata*. Antibacterial activity of these oils was tested against nine different types of both gram negative and positive pathogenic bacteria under favourable conditions and the results were compared with commonly used antibiotic drugs. In addition, the mode of inhibition of these volatile oils against partially purified and characterized extracellular protease of *Pseudomonas aeruginosa* was determined from Lineweaver Burke plot (double reciprocal plot).

Findings: The antimicrobial activity of the essential oils of *Chromolaena odorata* showed inhibition zones ranging from 13.0±1.0mm to 43.5±2.5mm in *Salmonella paratyphimurium* and *Shigella dysenteriae* respectively. Ceftriaxone, among other antibiotics, has the highest inhibition of 26.0±2.0mm against *Salmonella paratyphimurium*. There was a significant difference *($p<0.05$)* between the total average inhibition of the antibiotics, 5.0±0.82 mm, and the volatile oils, 18.0±4.0mm. Each of the microbes was either sensitive to both types of the oils or at least one of the oils. *Enterohaemorrhagic Escherichia coli (EHEC)* and *Escherichia coli* have the same lowest possible minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of 0.09%v/v and ≥0.18%v/v of the oils, hence the most sensitive among the pathogens. The extracellular protease of *Pseudomonas aeruginosa* had optimal activities at pH 7.5 and 35^{o}C. The volatile oils displayed a competitive inhibition against the extracellular protease of *Pseudomonas aeruginosa* with V_{max} of 0.91µmol/min and K_m of 0.48mg/ml in the absence of the volatile oils but the K'_m was increased to 0.93mg/ml and 1.25mg/ml in the presence of the volatile oils of the leaf and stem of this plant. The highest purification fold of 2.35 corresponding to 6.92µmol/min/mg protein was achieved from the crude enzyme with DEAE cellulose ion exchange chromatography. The successive purification profile revealed oligomeric nature of this protein.

Conclusion: Therefore, the volatile oil of the stems and leaves of *Chromolaena odorata* possessed antimicrobial activity with higher significant impact. In addition, it possessed ability to inhibit extracellular protease of *Pseudomonas aeruginosa*. This may probably suggest *Chromolaena odorata* as a possible source of nutraceuticals for clinical purpose.

KEYWORDS: volatile oil, *Chromolaena odorata,* antimicrobial, *Pseudomonas aeruginosa,* inhibition, extracellular protease

INTRODUCTION:

Medicinal plants are plants with proven chemical properties, which have been used for centuries as remedies for human diseases because they contain chemical components of therapeutic values (1). Chemically, essential/volatile oils, as one of the phytoconstituents of medicinal plants, are extremely complex mixtures containing compounds of every major functional group like ether, alkoxide, enol, polyaromaphenolic compounds and many others. Most of these phytoactive components have been reported to possess antibacterial, antifungal, antiviral antiprotozoans (2, 3, 4). Asides these, the essential oils from medicinal plants have displayed some credible effects as antidiarrheal, antihypertensive, antidiabetic, anti-inflammatory and also as immunomodulatory and antioxidant (5, 6). They are hydrophobic liquids containing volatile fragrant aroma (7). The oils are isolated by steam distillation, hydrodistillation, solvent extraction or mechanical expression of the plant material and the plant parts often used are the roots, buds, leaves, stems and

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flower parts. *Chromolaena Odorata* is herbaceous perennial shrubs belonging to the plant family *Asteraceae.* It occurs naturally in South America and Central America and has been introduced into the tropical regions of Asia, Africa and the pacific where it served as invasive weeds (8). The volatile oil from its leaves has been shown to have antimicrobial and antiseptic properties (9), as well as enhancing homeostatic activity and stimulating the re-epithelization process thus promoting wound healing (10).

Pseudomonas aeruginosa is a gram-negative aerobic bacillus belonging to the bacterial family *Pseudomonadaceae.* Although members of its genera are well-known plant pathogens, *Pseudomonas aeruginosa* have become increasingly recognized as an emerging opportunistic pathogen of clinical relevance (11). *Pseudomonas aeruginosa* is one of the major causes of nosocomial pneumonia and spread mainly through hospital equipment and health care workers than from person-to-person (12). Their frequent contamination of ventilators and hospital equipment is attributed to the fact that they are resistant to extreme temperature and drying. *Pseudomonas aeruginosa* is the fourth most commonly isolated nosocomial pathogen accounting for 10.1% of all hospital acquired infections (13). It is found on the skin of healthy persons and has been isolated from the throat and stool of 3 - 5% of non-hospitalized persons (13). *Pseudomonas aeruginosa* is involved in the aetiology of many diseases including endocarditis, meningitis, bronchopneumonia, burns and wound infections; wound infection is one of the major causes of limb amputations in Nigerian children (12). One of the most worrisome characteristics of *Pseudomonas aeruginosa* is its high antibiotic resistance, which is attributable to a concerted action of multidrug efflux pumps, chromosomally encoded antibiotic resistance genes and low permeability of the bacterial cellular envelopes (14).

Most pathogenic organisms secrete both intracellular and extracellular proteases with which they accomplish their pathogenic activities. These extracellular proteases also lend to the virulent nature of the pathogens (15). *Pseudomonas aeruginosa* secretes quite a lot of extracellular proteases some of which include elastase, alkaline protease, exotoxin A and exoenzyme S as well as some soluble cytotoxic proteins. These extracellular proteases make it a very virulent organism with high level of antibiotic resistance thus indicating the need for a more novel approach towards fighting this microbe (16). The prominence gained by most extracellularly produced proteases in the virulence of microorganisms has led to the purification and characterization of these proteases in order to design drugs, which could effectively prevent the infections caused by such pathogen.

The *in vitro* activity of a range of essential oils against *Pseudomonas aeruginosa* has been examined with some medicinal plants (17, 18). There were promising results of antibacterial activities of both aqueous and organic solvents extraction of medicinal plants (19). In this work, assessment of the antimicrobial property of the volatile oils from the leaf and stem of *Chromolaena odorata* comparing with the standard antibiotics was carried out. In addition, the inhibition of these oils on the partially purified and characterized extracellular proteases of *Pseudomonas aeruginosa* was determined.

MATERIALS AND METHODS:

Plants materials: *Chromolaena odorata* plants were obtained at Amuwo Odofin Local Government Area of Lagos State. The sample was gotten as green foliage and air-dried for four days. The green sample was taken to Botany Department, Faculty of Science, Lagos State University, Ojo Lagos for proper identification and authentication.

Microorganisms: The microorganisms used in this work were obtained from the Nigeria Institute of Medical Research (NIMR), Yaba, Lagos Nigeria and maintained on nutrient agar petri-dishes at 4^{o}C. These microbes were *Staphylococcus aureus* (a gram positive bacterium), while others were gram negative bacteria: *Enterohaemorrhagic Escherichia coli (EHEC), Escherichia coli, Pseudomonas aeruginosa, Salmonella paratyphimurium, Salmonella typhimurium, Shigella flexneri, Salmonella enteritis,* and *Shigella dysenteriae*.

Susceptible antibiotic drugs: A susceptible antimicrobial sensitivity discs was purchased from a Pharmaceutical store in Ojo Local Government of Lagos 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.

Extraction of volatile oils: The volatile/essential oil of *Chromolaena odorata* was extracted by the method described by Lawrence and Reynolds (20). Briefly, the five-day-air-dried *Chromolaena odorata* plant was separated into leaves and stems and each part was cut into pieces and packed into the 5 L 34/35 Quick fit round bottom flask containing 2.0 L distilled water with fixed Clevenger. The oil

was extracted at a steady temperature of 80^{o}C for 3 hours and the oil was collected over 2 ml *n*-hexane. The resulting extract was run through the tap and stored in a tightly sealed sample bottle and kept inside the refrigerator at 4^{o}C.

Antimicrobial susceptibility tests of antibiotics and volatile oils: The volatile oil of *Chromolaena odorata* was tested for antimicrobial sensitivity against nine microorganisms using a diffusion technique method (21) on different nutrient agar. A 5mm diameter paper disc paper was soaked into the volatile oil, picked with a sterilized tong and placed on the media which has been surface spread with each of the colony of the nine used. The plates were `inoculated in their appropriate media for 24 hours at 37 °C. The results were recorded by measuring the zones of inhibition surrounding the paper disc.

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC): The MIC and MBC of the volatile oil of *Chromolaena odorata* were carried out using microbroth dilution method (22) with little modification. A colony of each organism was added to 200 µl of susceptible test Muller Hinton broth containing two-fold serial dilution of the volatile oil using Tween 80 (0.5 %v/v) as diluent in a microtitre plate (21.5 x 17 cm^2). The plates were covered and incubated at 37^{o}C for 24 hours. Each of the microwell was inoculated on a freshly prepared Muller Hinton agar where MIC and MBC were determined.

Extraction of crude enzyme: In the medium, microbes utilize the nutrient by secreting some extracellular proteases which could be gotten through centrifugation. The proteases are responsible for growth, pathogenesis and invasion of host cells. The extracellular protease of *Pseudomonas aeruginosa* was extracted by the method described by Makino et al, (23). A colony of the microbe was inoculated into the Muller Hinton broth. It was then incubated for 24 hours at 37^{o}C. The broth was centrifuged (Kendros PicoBiofuge, Heraeus) at 9000 rpm for 10 minutes at room temperature. The cell-free supernatant was decanted and stored in a sample bottle at 4°C until it was used.

Determination of total protein and protease activity: The total protein of the crude enzyme extract was determined using a method of Lowry et al, (24), with casein as substrate. Total protein was determined 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) and 1ml of solution B (5g cupper II tetraoxosulphate VI pentahydrate and 10g sodium-potassium tartrate in 1 L) to 0.1ml of crude enzyme extract and mixed. The reaction solution 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 (using Spectronic-21, Bausch and Lomb) after 30 minutes. Bovine serum albumin (BSA) was used as standard protein (0.20mg/ml)

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 10min at 37^{o}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 (using Spectronic-21, Bausch and Lomb) after 30 min. L-tyrosine solution (0.20 mg/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 1.0 minute at 37 ^{o}C*.

Determination of optimum pH: The method adopted was described by Makino et al, (23) with little modification. Protease activity was assayed using 0.6% casein solution in 0.05 M Tris buffer solution (pH 6.0 - 9.0) at 37^{o}C.

Determination of optimum temperature: As described by Makino et al, (23), protease activity was assayed under varying temperature conditions (30 - 70 ^{o}C) using 0.6% casein solution in 0.05 M Tris buffer at pH 8.0.

Inhibitory assay: The method used was described by Makino et al, (23) with a slight modification. Briefly, 0.1 ml of the crude protease extract and 0.1 ml of 3.5 %v/v of the volatile oil (as inhibitor) in 0.5 %v/v Tween 80 solutions were added concomitantly to different concentration of casein solution (0.2 - 1.0 %w/v) in 0.05 M Tris buffer, pH 8.0. The reaction mixture was mixed and incubated at 37 ^{o}C for 10 min. The reaction was stopped and the protease activity was assayed with the volatile oils of the three different parts of the plant. The procedure was repeated without an inhibitor.

Dialysis: Salting out technique was carried out on the crude enzyme extract. A 35 % $(NH_4)_2SO_4$ saturated solution of the crude enzyme extract was dialyzed (using SIGMA Dialysis

Tubing Cellulose Membrane, D9402) for 48 hours and thereafter centrifuged (using Kendros PicoBiofuge, Heraeus) at 5000 g for 5 minutes Then, 50 % $(NH_4)_2SO_4$ saturated solution of the sediment was dialyzed for 48 hours. This was followed by the dialysis of 55% and 65% saturated solutions of the sediment for the same hours. In each case both total protein and enzyme assay 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 (28.0 cm by 1.5 cm column) and formed a bed length of 22 cm with a flow rate of 1.5 ml/min and this was used to separate 35% $(NH_4)_2SO_4$ dialysate. A total number of 50 elutions were collected. Each elution contained 3.0 ml of eluent and for each of the eluent; both total protein and enzyme assay were carried out.

Ion exchange chromatography: This was done by soaking 6g of DEAE cellulose powder in 0.05 M Tris buffer of pH 8.0 for 48 hours. It was packed in column of about 22cm length. The flow rate was 0.6ml/min. The column was prepared and equilibrated with Tris buffer (0.05 M, pH 8.0).

Statistical analysis: Comparison of the antimicrobial activities of the volatile oils and antibiotics was carried out using *t-test* analysis and the mean difference was considered significant at $p<0.05$.

RESULTS:

The volatile oils obtained from the stems, leaves of Chromolaena odorata plants were tested for antimicrobial activity on nine microorganisms using disc diffusion method, and its inhibition on the extracellular protease of *Pseudomonas aeruginosa* was carried out. This protease was partially purified by ammonium sulphate precipitation, gel filtration and ion exchange chromatography.

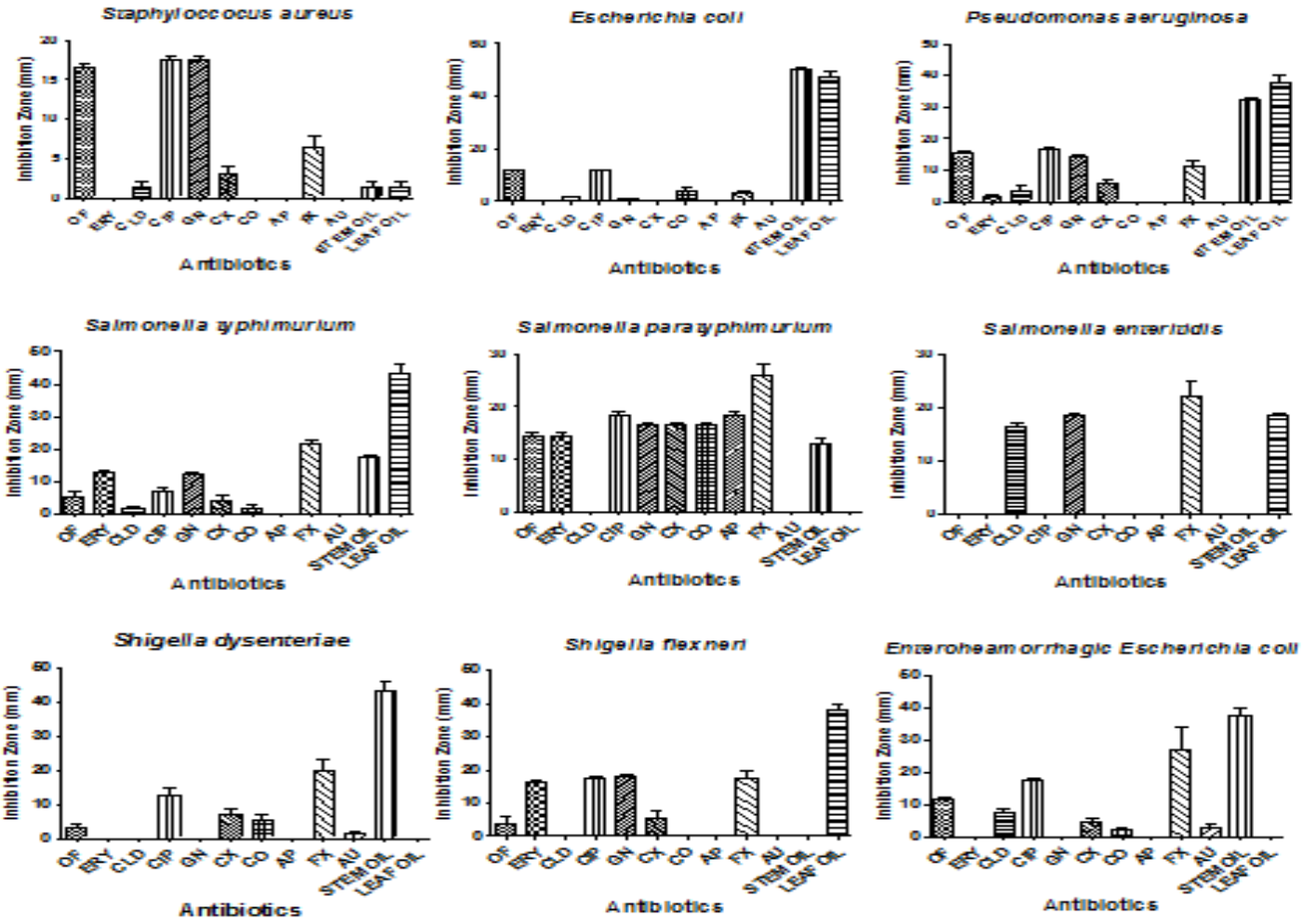

Figure 1: Antimicrobial screening of the volatile oils of *Chromolaena odorata* as compared with the clinical antibiotics

OF - Ofloxacin, ERY - Erythromycin, CLD - Clindamycin, CIP - Ciprofloxacin, GN - Gentamicin, CO - Cotrimoxazole, AP - Ampicillin, FX - Ceftriaxone, AU - Augmentin.

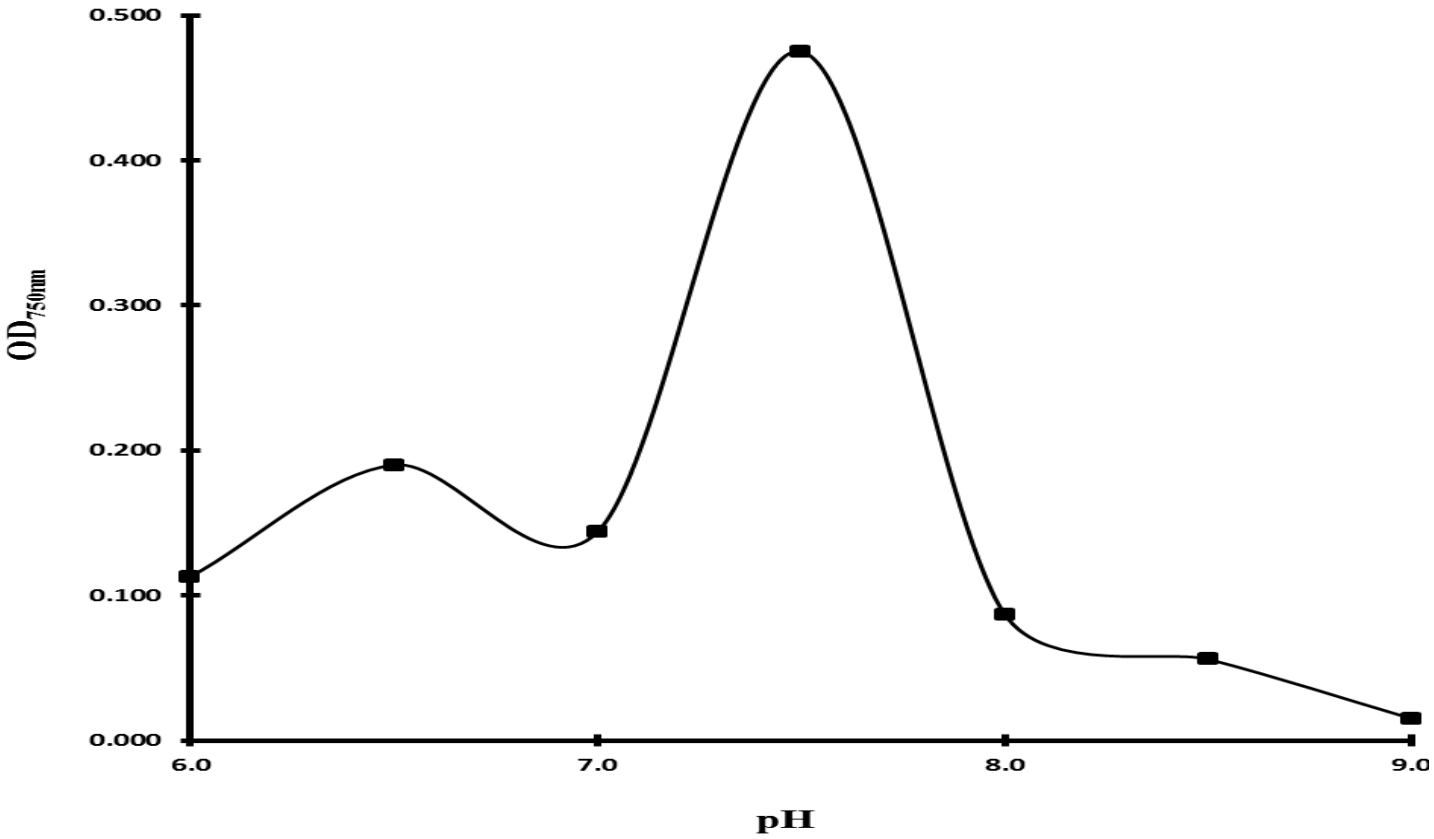

Figure 2: Effect of pH on the enzymatic activity of extracellular protease of *Pseudomonas aeruginosa*. The enzyme exhibited highest activity at pH 7.5

Table 1: The MIC and MBC of the volatile oils of *Chromolaena odorata against* nine pathogenic enteric bacteria.

Micro-organisms	Stem volatile oil		Leaf volatile oil	
	MIC (%v/v)	MBC (%v/v)	MIC (%v/v)	MBC (%v/v)
Staphylococcus aureus†	100	≥ 100	100	≥ 100
*Escherichia coli**	0.09	≥ 0.18	0.09	≥ 0.18
Pseudomonas aeruginosa	0.18	≥ 0.39	0.18	≥ 0.39
Salmonella typhimurium	6.25	≥ 12.50	0.18	≥ 0.39
Salmonella paratyphimurium	0.18	≥ 0.39	100	≥ 100
Salmonella enteritidis	100	≥ 100	6.25	≥ 12.50
Shigella dysenteriae	0.18	≥ 0.39	100	≥ 100
Shigella flexneri	100	≥ 100	0.18	≥ 0.39
*Enterohaemorrhagic Escherichia coli**	0.09	≥ 0.18	0.09	≥ 0.18

† Gram positive bacteria and most insensitive microorganism
**** Most sensitive organism to the volatile oils***
MIC (minimum inhibitory concentration); MBC (minimum bactericidal concentration)

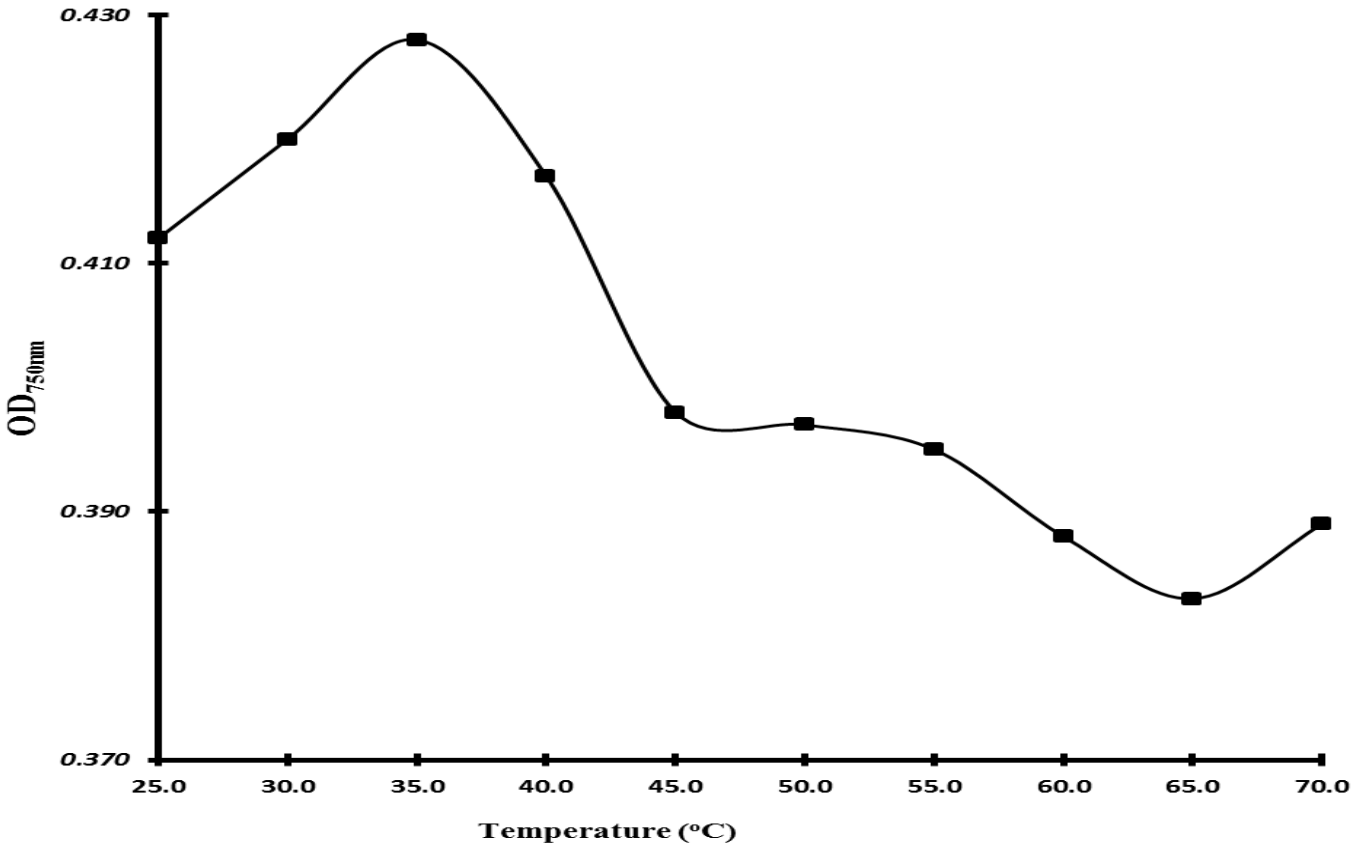

Figure 3: Effect of temperature on the enzymatic activity of extracellular protease of *Pseudomonas aeruginosa*. The enzyme exhibited highest activity at 35 °C

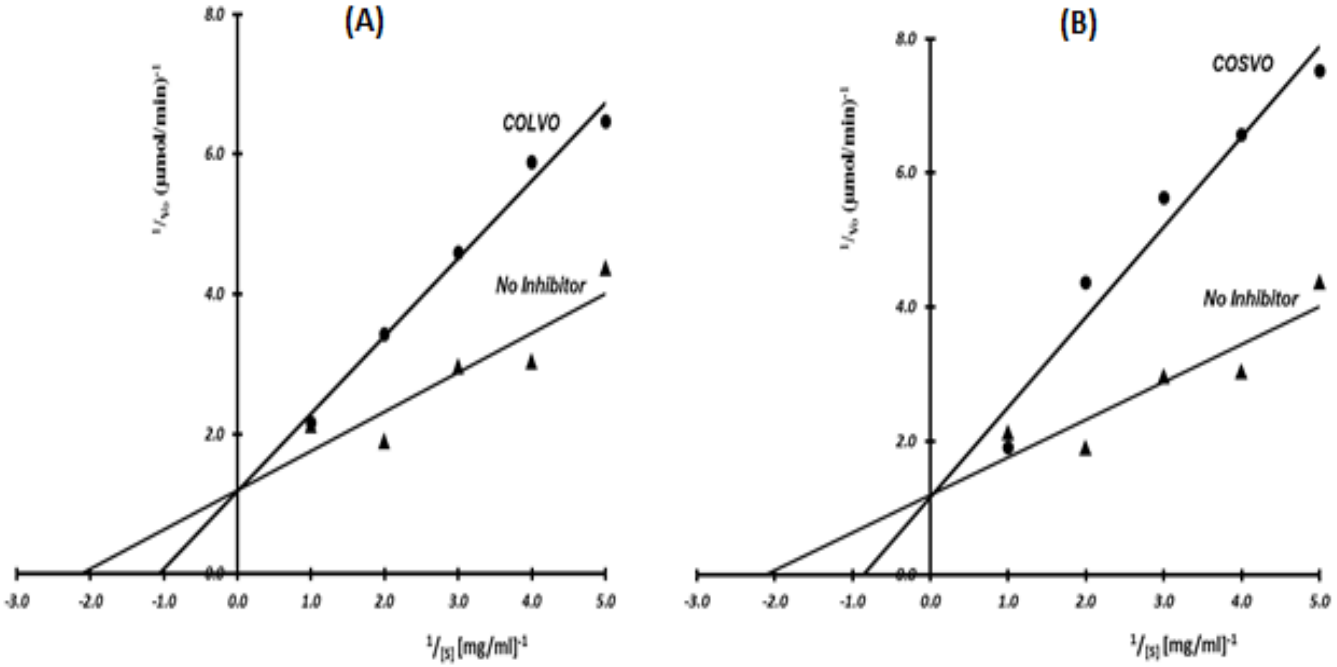

Figure 4: Lineweaver Burke plot of enzyme activity

(a) COLVO - *Chromolaena odorata* leaf volatile oil and (b) COSVO - *Chromolaena odorata* stem volatile oil have the same V_{max} (0.91 µmolmin^{-1}) but different K_m values (K_m in the absence of inhibitor = 0.48 mg/ml, K_{mCOLVO} = 0.93 mg/ml and K_{mCOSVO} = 1.25 mg/ml).

Table 2: Purification table of the extracellular protease of *Pseudomonas aeruginosa*

Purification Steps	*Total Protein (mg)*	*Total Activity (µmol/min)*	*Specific Activity (µmol/min/mg protein)*	*Percentage Yield*	*Purification Fold*
Crude Enzyme Extract	3.163	9.310	2.94	100	1.00
65% $(NH_4)_2SO_4$ precipitation	0.033	0.104	3.15	1.12	1.07
55% $(NH_4)_2SO_4$ precipitation	0.028	0.098	3.50	1.05	1.19
50% $(NH_4)_2SO_4$ precipitation	0.021	0.094	4.47	1.00	1.52
35% $(NH_4)_2SO_4$ precipitation	0.020	0.102	5.08	1.10	1.73
Sephadex G-100	0.0170	0.093	5.47	1.00	1.86
Sephadex G-75	0.016	0.092	5.75	0.99	1.95
DEAE Cellulose Ion Exchange	0.013	0.09	6.92	0.97	2.35

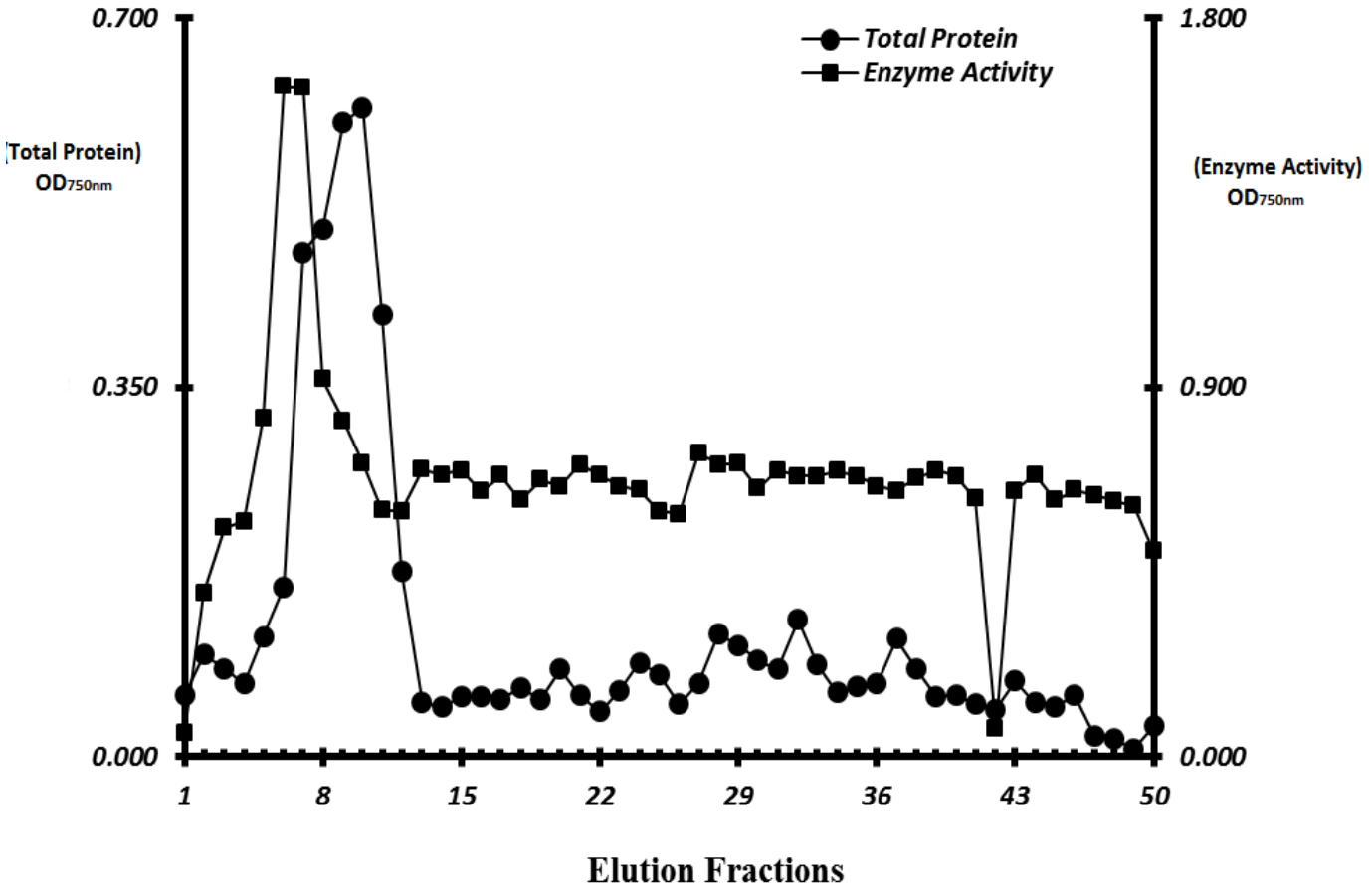

Figure 5: Elution fractions obtained from Sephadex G-100 gel filtration

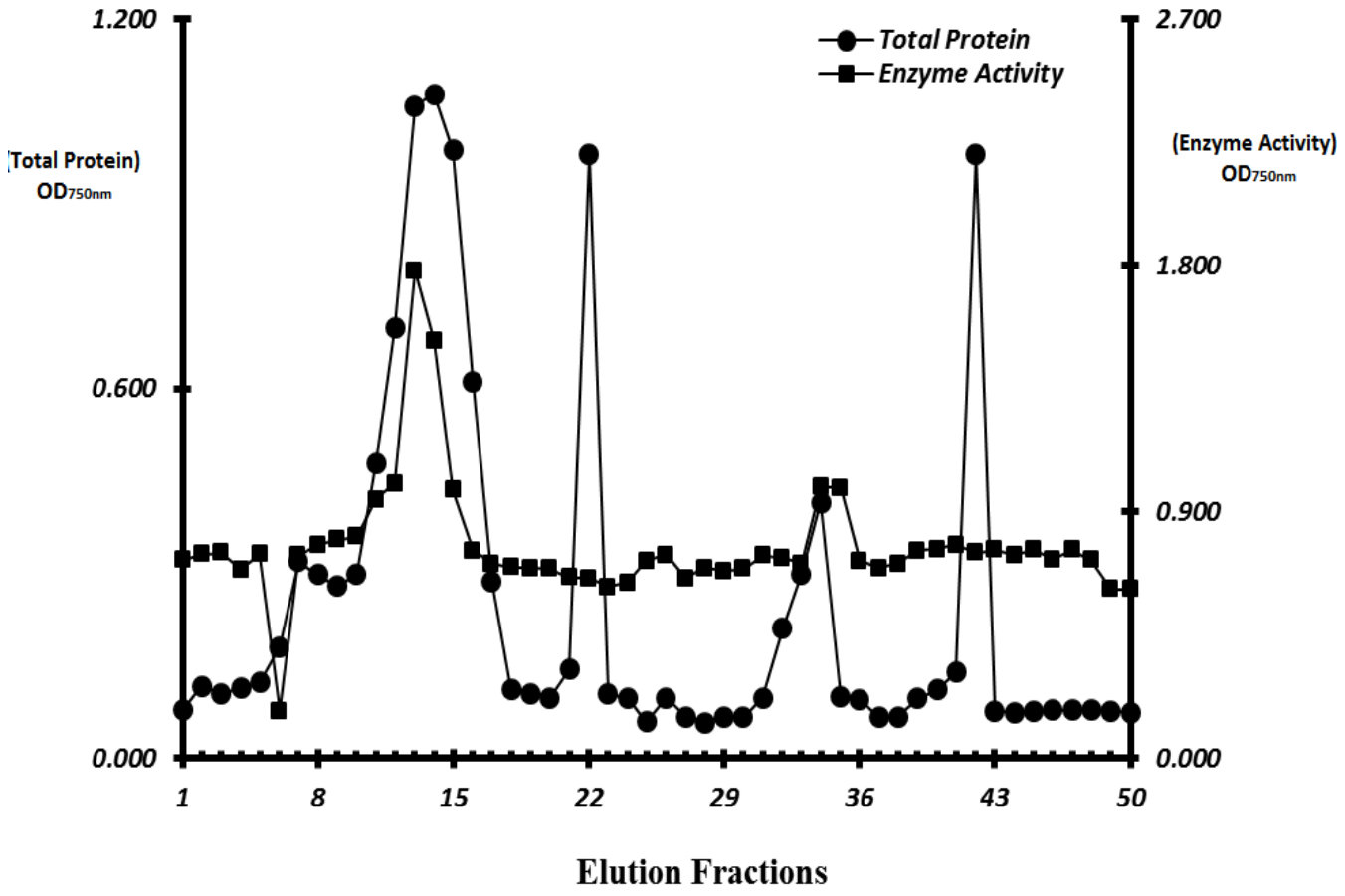

Figure 6: Elution fractions obtained from Sephadex G-75 gel filtration

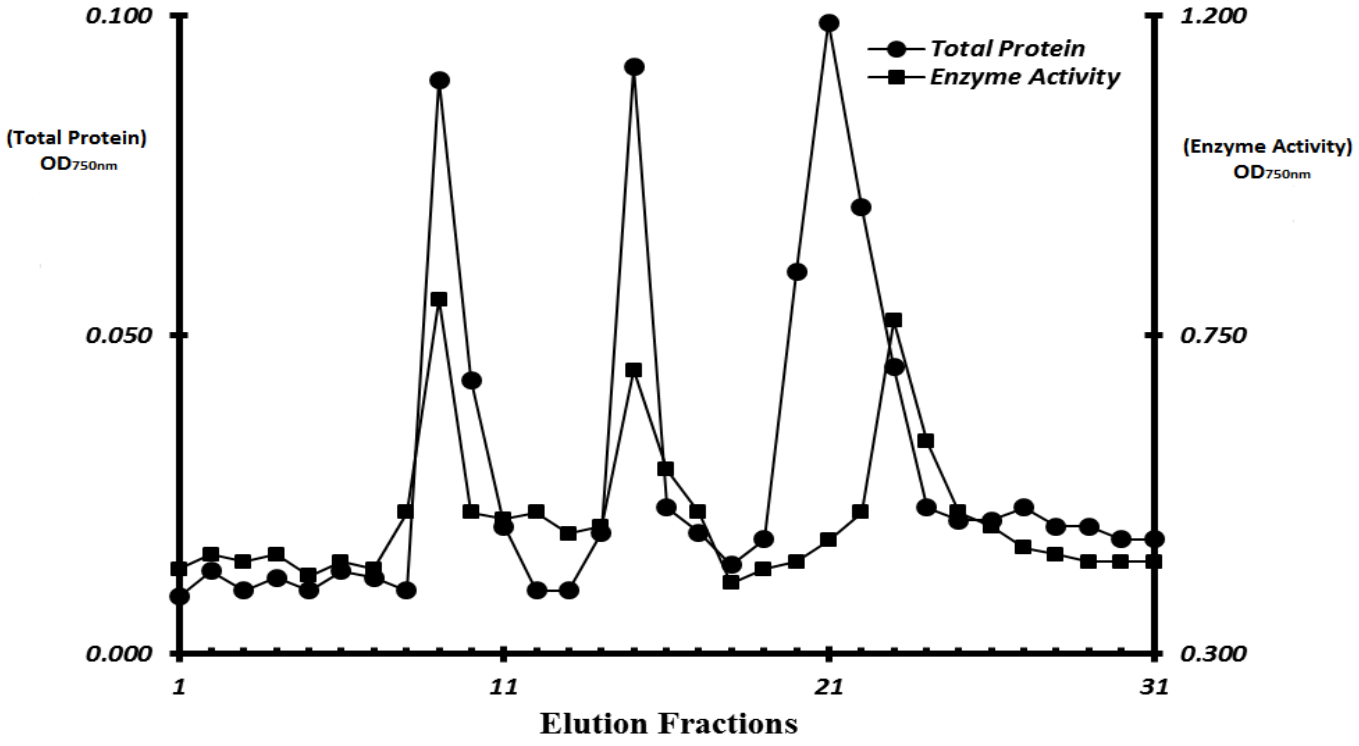

Figure 7: Elution fractions obtained from DEAE- cellulose ion exchange chromatography. Formation of multiple peaks indicated the presence of trimetric protein.

Figure 1 shows the antimicrobial sensitivity of the volatile oils of the leaves and stems of *Chromolaena odorata* on nine microorganisms. The zones of inhibition obtained ranged from 13.0±1.0 mm to 43.5±2.5 mm with *Salmonella paratyphimurium* having the lowest zone of inhibition and *Shigella dysenteriae* having the highest. The inhibition zones for *Pseudomonas aeruginosa* were 32.0±1.0 mm and 37.5±2.5 mm for the stems and leaves respectively. The volatile oil from the stem of *Chromolaena odorata* showed higher inhibitions on seven out of nine organisms used while the volatile oil from the leaf inhibited five out of the nine microorganisms tested. The highest inhibition zone of 26.0±2.0 mm was observed in ceftriaxone against *Salmonella paratyphimurium.* Similarly, all antibiotics used had inhibition zones ≥10.0 mm except augmentin, which has no effect. There was a significant difference *($p<0.05$)* between the total average inhibition of by the antibiotics, 5.0±0.82 mm, and the volatile oils, 18.0±4.0 mm. Each of the microbes was either sensitive to both types of the oils or at least one the oils. The sensitivity test of *Chromolaena odorata* volatile oils from the stems and leaves on these nine different micro-organisms showed that the oil from the stem of *Chromolaena odorata* is highly potential than the leaves because of its inhibitory effect on almost all the micro-organisms tested.

Table 1 shows the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) obtained by micro-serial dilution. Most of the organisms were sensitive to relatively low concentrations of the oils except *Staphylococcus aureus*. *Enterohaemorrhagic Escherichia* coli *(EHEC)* and *Escherichia coli* have the same lowest possible MIC and MBC values of 0.09 %v/v and ≥0.18 %v/v of the oils.

Figures 2 and 3 show the effect of pH and temperature on the extracellular protease activity of *Pseudomonas aeruginosa*. The protease had highest activity at 7.5 and 35 °C.

Figure 4 shows the enzyme kinetics of the extracellular protease of *Pseudomonas aeruginosa* under the influence of volatile oils *Chromolaena odorata* as potent inhibitor. The double reciprocal plot shows that inhibition is competitive in both COLVO - *Chromolaena odorata* leaf volatile oil and COSVO - *Chromolaena odorata* stem volatile oil, because they both have the same V_{max} (0.91 µmolmin^{-1}) but different K_m values (K_m in the absence of inhibitor = 0.48 mg/ml). The K_m in the presence of the volatile oil from the leaf (K_{mCOLVO}) increased to 0.93 mg/ml while stem (K_{mCOSVO}) increased to 1.25 mg/ml. This suggests that substrate and one or more component(s) of the oils, as inhibitor, have the same structural/functional similarities and this made them to compete for the active site of the enzyme.

Table 2 shows a summary of the purification profile obtained for the extracellular protease of *Pseudomonas aeruginosa*. Purification by 65% ammonium sulphate precipitation gave a purification fold of 1.07 and percentage yield of 1.12 while 35% ammonium sulphate precipitation gave a purification fold of 1.73 and percentage yield of 1.10. The highest purification recovery of 2.35, as compared to the crude extract, was achieved from DEAE cellulose ion exchange chromatography.

Figures 5, 6 and 7 show the elution fractions obtained from Sephadex G-100, G-75 and DEAE cellulose ion exchange chromatography respectively. The formation of multiple peaks in these orders shows that the extracellular protease of *Pseudomonas aeruginosa* is an oligomeric protein.

DISCUSSION:

The volatile oils of *Chromolaena odorata* has shown to be more potent antimicrobial agent compared to numerous clinical antibiotics. The volatile oil from the stems and leaves of *Chromolaena odorata* inhibited the growth of *Pseudomonas aeruginosa* with an inhibition zone of 32.0±1.0mm and 37.50±2.5mm respectively. This is in line with the finding of Jahan et al, (18), who confirmed that *Azadirachta indica* oil (Neem oil) was highly sensitivity to isolate of *Pseudomonas aeruginosa*. The organism has also been found to be susceptible to cider oil, cinnamon oil, lemon oil and vetiver oil. In the studies carried out by Seenivasan et al, (25), *Pseudomonas aeruginosa* susceptible to different types of essential oils from nineteen medicinal plants. Out of twenty-one plants tested, cinnamon oil had the highest inhibition against *Pseudomonas aeruginosa* with inhibition zones of 33.3mm and the MIC was $> 8.0\%$ while basil oil had the least inhibition of 8.2mm.

The activities of the extracellular protease of *Pseudomonas aeruginosa* under the influence of pH and temperature have probably revealed why this organism can thrive well even in the human gastrointestinal tracts. It was obvious that this organism may not survive the gastric conditions except there were other means of protections adopted by this organism to survive this condition. High *in vivo* abundance of amino acid decarboxylases (GadB and AdiA) and protein disaggregation chaperones (HdeA, HdeB and ClpB) were indicative of a coordinated bacterial survival response and enhancer of pH homeostasis in the cytoplasm of these pathogens to acid stress in human GIT (26). Shahanara et al, (27) in their work to characterization the intracellular protease of *Pseudomonas aeruginosa,* the protease showed highest activities at pH 8.0 and 50^{o}C.

Reports have shown that most enteric and opportunistic pathogenic organisms thrive well in pH range 6 – 9 and temperature range of 30 – 50 ^{o}C.

The volatile oil from the stems and leaves of *Chromolaena odorata* competitively inhibited the extracellular protease indicating that these oils were potentially capable of reducing the catalytic activity of the extracellular protease of *Pseudomonas aeruginosa.* The competitive nature of the inhibition showed that these oils might serve as a template for developing new antimicrobial drug that can be targeted against this protease.

The Sephadex G-100 gel filtration revealed a peak each for both total protein and total enzyme activity while Sephadex G-75 further separated this protein into three peaks for protein and two peaks for enzyme activity. By this separation, it may be inferred that the extracellular protease of this organism may likely to be oligomeric in nature. The DEAE-cellulose anion exchanger revealed multiple peaks, lending credence to the existence of probably more than one extracellular protease or an oligomeric protein. The extracellular protease of this pathogen may likely possess net negative charged. Further work on this is needed to reveal the molecular weight of these subunit proteins.

The volatile oil from the stems and leaves of *Chromolaena odorata* possessed antimicrobial activities against some enteric pathogenic bacteria tested in this work. It has been shown that these oils demonstrated competitive inhibition against the kinetics of the extracellular protease of *Pseudomonas aeruginosa,* a pathogenic microorganism whose virulence is indirectly aided by the secretion of some extracellular proteases.

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