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# Research Article
# *Syzygium samarangense* Volatile Oil Inhibited Bacteria Growth and Extracellular Protease of *Salmonella typhimurium*

[1]O.S. Folorunso, [2]S. Adeola and [3]A.O. Giwa Ajeniya

[1]Department of Microbial, Biochemical and Food Biotechnology, Faculty of Natural and Agricultural Sciences, University of the Free State, 9301 Bloemfontein, South Africa
[2]Department of Biochemistry, Faculty of Science, Lagos State University, P.O. Box 0001, Lasu Post Office, Ojo, Lagos, Nigeria
[3]Department of Chemistry, Faculty of Science, Lagos State University, P.O. Box 0001, Lasu Post Office, Ojo, Lagos, Nigeria

## Abstract

**Background and Objective:** Medicinal plants are fast becoming essential pharmaceuticals for disease and infection management. The vast antimicrobial properties of these plants reside in the inhibitory properties of their endogenous secondary metabolites. Therefore, this study aimed to assess if the volatile oil of *Syzygium samarangense* inhibits enteric bacteria growth and its effect against the caseinolytic activity of the extracellular protease of *Salmonella typhimurium.* **Materials and Methods:** The volatile oil was extracted by hydrodistillation, while the antimicrobial assay was assessed with the microdilution method. The extracellular protease was partially purified by salting out, followed by size-exclusion chromatography. The mode of inhibition of this enzyme was deduced from the enzyme-substrate kinetics using a line-weaver burke plot. **Results:** The antimicrobial properties of the oil were reported against ten enteric bacteria. *Proteus vulgaris* has the highest $IC_{50}$ value of 0.75±0.004% v/v, while *S. typhimurium,* the most sensitive bacterium, showed the lowest $IC_{50}$ value of 0.17±0.005% v/v. The extracellular protease of *S. typhimurium* was partially purified to achieve 3.73 purification fold and 314.2 µmol $min^{-1}$ $mg^{-1}$ protein. The optimal caseinolytic activity of this enzyme was found at pH 7.5 and 40 °C. The protease showed significantly higher activity in the presence of $Zn^{2+}$ (9.3±0.33 U $min^{-1}$) as compared to the control (7.0±0.58 U $min^{-1}$) ($p<0.05$), however, $K^{+}$, $Ca^{2+}$, $Co^{2+}$, $Ba^{2+}$, $Pb^{2+}$ and $Hg^{2+}$ considerably reduced the enzyme activity. The activity of this enzyme was competitively inhibited by the volatile oil as an inhibitor. **Conclusion:** The volatile oil of *S. samarangense* inhibited a wide range of enteric bacteria and, therefore proposed as a potential antimicrobial agent. Inhibiting the extracellular protease of *S. typhimurium* may be one of its modes of action against these pathogens.

Key words: Volatile oil, antimicrobial, *Syzygium samarangense*, *Salmonella typhimurium*, extracellular protease, inhibition

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Corresponding Author: O.S. Folorunso, Department of Microbial, Biochemical and Food Biotechnology, Faculty of Natural and Agricultural Sciences, University of the Free State, 9301 Bloemfontein, South Africa Tel: +27 (0)60 8264 711



Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

The antimicrobial potentials of the volatile oil (VO) from medicinal plants are of great interest in ayurvedic and alternative medicines. The VO is naturally produced as secondary metabolites and has been explored for a wide range of applications in pharmaceutical, preservation and aromatherapy[1,2]. The choice of the VO as a complementary antimicrobial agent is a reminiscence of the significant health advantages resided in the natural medicinal plants[3].

*S. samarangense* (Myrtaceae) has been observed as a medicinal plant of momentous value. It has edible fruits rich in phenols, flavonols and antioxidants suitable to cure hypertension and several inflammatory conditions when consumed[4-6]. The ethnopharmaceutical advantages of the secondary metabolites extracted from different species of *Syzygium* have been described and recently reviewed by Chua *et al.*[7]. The VO of the *Syzygium* fruits has been analyzed to contain hexanal, (Z)-hex-3-enal, hexan-1-ol and trans-caryophyllene[8] while the oil from the leaves contained γ-terpinene, α-pinene and p-cymene as significant components[9,10]. Though a lot had been reported about the physiological evaluation and the ethnobotanical importance of this plant, however, no efforts have been directed towards the antimicrobial effect of its volatile oil (VO)[8,11].

*S. typhimurium* (Enterobacteriaceae) is a causative agent of salmonellosis and typhoid infections with a current mortality rate of 144, 500 in 15.5 million cases[12-14]. This Gram-negative peritrichous flagellate bacillus contaminates foods and water and exclusively causes systemic infection in human[15,16]. Together with other enteric pathogens, *S. typhimurium* possesses a type III secretion effector proteins, which allow for the release of self-defence proteases[17-19]. The strategic release of extracellular peptidases and proteases to digest the host extracellular matrix proteins facilitates the pathogenicity of these bacteria[20,21]. Therefore, targeting the activities of these proteases will be of great benefit in antimicrobial chemotherapy, especially against the emerging MDR pathogens[22,23].

In this study, the potential of *S. samarangense* VO to inhibit microbial growth from a range of pathogenic enteric and nosocomial bacteria was evaluated. Furthermore, enzyme-substrate kinetics of partially purified extracellular protease of *S. typhimurium* was monitored in the presence of the VO as a potential inhibitor. The model adopted in this study enables a prediction of the mode of inhibition the VO imposed on the extracellular protease activity and may give insight into the general antimicrobial effect of this oil against the pathogens.

## MATERIALS AND METHODS

This study spanned through a period of nine months starting from January, 2019 and the analysis was jointly carried out at the Lagos State University, Ojo, Lagos, Nigeria and the University of the Free State, Bloemfontein, South Africa.

**Collection of *S. samarangense* plant:** The *S. samarangense* foliage was located and collected at Ojo Local Government Area of Lagos State (Coordinates N 6° 28' 6" and E 3° 10' 59"). The sample was authenticated at the departmental herbarium, Department of Botany, Lagos State University Ojo Lagos (Voucher No: LASU_BOT/2019/02/13/01_010). The leaves were air-dried for seven days, chopped and weighed.

**Collection and culturing of bacteria:** Ten different enteric and nosocomial pathogenic Gram-negative bacteria were obtained from the Department of Microbiology of the Lagos State University Teaching Hospital (LASUTH) and the Nigerian Institute of Medical Research (NIMR) Lagos Nigeria. Each of the bacterial isolates was revived on Nutrient agar (Merck) but used on Mueller-Hinton Agar (MHA) (Merck) for optimum growth of the bacteria during susceptibility tests.

**Extraction of the VO of *S. samarangense* by hydrodistillation:** The VO was extracted under the optimum operating conditions[24]. Briefly, 500 g of dried leaves was packed into the 34/35 Quick-Fit 5 L flask containing 2.0 L of distilled water with fitted Clevenger condenser. The VO was collected over 2 mL n-hexane (Merck) for 3 h at 80°C, dried over anhydrous copper II tetraoxosulphate VI (Merck) and stored at 4°C until it was used.

**Microbial growth inhibition by the VO of *S. samarangense*:** The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the VO were analysed using the micro-broth dilution method with little modification[25]. Briefly, Mueller-Hinton broth (MHB) was prepared with 0.5% v/v Tween-80 (Sigma) as a dissolving medium for the VO. Each of the microwell plates received 200 µL broth. Also, 200 µL of the VO was added to the first microwell in each row of the microplate (21.5×17 cm) to prepare two-fold serial dilution of the oil in each row. At 4°C, each of the microwells was quickly inoculated with $5.0\times10^5$ CFU $mL^{-1}$ bacterial suspension[26].

The initial $OD_{620nm}$ was taken with a microplate reader (EZ Read 800, Biochrom) as $A_0$. The plate was covered and incubated at 37°C for 18 h in a shaker at 350 rpm and another $OD_{620nm}$ was read as $A_1$. About 3 µL of each of the cultures in

the microwell was streaked on the freshly prepared MHA and incubated for 24 h at 37°C. The growth pattern was observed in the microplate and the agar plate accordingly. Each experiment was repeated twice. The MIC was considered as the VO dilution factor that prevents visible growth of the bacteria on the agar plate, while the MBC was regarded as the VO dilution factor that ultimately kills the bacteria and prevents any observable growth. The percentage growth inhibition of the bacteria was estimated as:

$$\text{Inhibition}(\%) = \frac{A_0 - A_1}{A_1} \times 100$$

This fraction represents the efficiency of growth inhibition by the VO[27].

**Preparation of the crude extracellular protease from *S. typhimurium*:** A-25.0 mL MHB culture of *S. typhimurium* incubated for 24 h on a shaker at 37°C was centrifuged (Heal Force® Neofuge 13) at 13, 000×g for 10 min at 4°C. The bacterial-free clarified supernatant was decanted, stored at 4°C and used as crude enzyme extract (CEE).

**Determination of the total protein and caseinolytic activity of the enzyme:** The total protein was quantified using 2.0 mg $mL^{-1}$ of Bovine Serum Albumin (BSA) (Sigma) as a standard purified protein. Furthermore, 100 µL of CEE was assayed with casein (Sigma) (2.0-10.0 mg $mL^{-1}$) in 0.05 mM Tris-HCl buffer pH 7.2 using 0.2 mg $mL^{-1}$ of *L. tyrosine* (Sigma) as the standard for enzyme activity. Both were measured at 750 nm (EZ Read 800, Biochrom) using Lowry protein assay containing Folin-Ciocalteu's phenolic reagent[28,29]. The caseinolytic activity was defined as the amount of enzyme required to liberate 1.0 µmol of *L. tyrosine* in 60 sec at 37°C, while the specific activity was expressed in a unit of enzyme activity/mg protein. The 1.0 mL enzyme reaction containing substrate and CEE in ratio 5:1 by volume was monitored for 5 min at 37°C and measured spectrophotometrically at 750 nm.

**Purification by dialysis of the CEE:** The CEE was dialysed (Sigma Cat# D9402, $\geq$12 kDa) in 0.05mM Tris-HCl buffer (pH 7.2) for 48 h with a 55% saturated solution of ammonium tetraoxosulphate (VI) (Sigma). The dialysed sample was centrifuged at 5 000 x g for 5 min at 4°C. The sediment was reconstituted in 1.0 mL of 0.05 mM Tris-HCl pH 8.0 buffer. The total protein was estimated from the reconstituted sample, while the caseinolytic assay was carried out with a 0.6% w/v casein solution prepared with 0.05 mM Tris-HCl buffer, pH 7.2.

**Purification by gel filtration of the dialysed sample:** A chromatographic column (28 cm×1.5 cm) containing 72 h pre-soaked Sephadex G-100 (Sigma) at 30 mg $mL^{-1}$ was set up with a flow-rate of 100 µL $min^{-1}$. After the equilibration of the column with 0.05 mM Tris-HCl buffer pH 7.2, 1.0 mL of dialysed reconstituted sample was loaded on the column and 36 eluents were collected. In each eluent, total protein and caseinolytic assay were carried out.

**Determination of the optimal pH and temperature for the enzyme activity:** The partially purified extracellular protease of *S. typhimurium* was assayed with a casein solution prepared with 0.05 mM Tris-HCl, pH 6.0-8.5, to determine the optimum pH at 37°C. Similarly, the assay was repeated with the observed optimum pH at varying temperatures (25-60°C).

**Caseinolytic activity of the enzyme in the presence of 1 mM of chloride solutions:** This assay was conducted to determine the metallic chlorides that may probably increase the caseinolytic activity. The assay was carried out in the presence of 1 mM chloride solution of the following metallic salts $Ba^{2+}$, $Ca^{2+}$, $Cu^{2+}$, $Co^{2+}$, $Fe^{3+}$, $Hg^{2+}$, $K^{+}$, $Mn^{2+}$, $Pb^{2+}$ and $Zn^{2+}$.

**Enzyme inhibition by the VO of *S. samarangense*:** With 3.5% v/v of the VO as a potential inhibitor, the partially purified extracellular protease was assayed with a casein solution at the optimal pH and temperature. The VO was prepared with 0.5% v/v Tween-80. The assay contained an equal volume of the substrate and the inhibitor.

**Statistical analysis:** Data obtained in this study were analysed with GraphPad Prism version 8.0.2.263 for Windows, GraphPad Software, San Diego, California USA (www.graphpad.com) and MS Window Excel Spreadsheet 2019. The percentage growth inhibition by the VO against each of the susceptible pathogenic bacteria was analysed using the sigmoidal nonlinear regression curve. The $IC_{50}$ and inhibition gradients were estimated from each of the curves. The magnitude of the hillslope/variable slope was directly

related to the efficiency of growth inhibition by the VO and this was used to estimate the rate of inhibition per h of incubation. The data means were compared using the nonparametric unpaired t-test for the rate of inhibition and $IC_{50}$. Dunnett multiple comparisons of the mean activity of the enzyme, when inhibited by 1 mM chloride salts, was done with one-way ANOVA analysis. All statistical data are presented as either Mean or Mean±SEM. The test of significant difference was at $p<0.05$.

## RESULTS

**Extraction of the VO by hydrodistillation:** As shown in Table 1, the VO was extracted at a constant temperature of 80°C for 3 h with 0.80 v/w yield/dried weight sample. The hydrophobic VO extracted from *S. samarangense* appeared as a colourless volatile liquid at room temperature with a strong fragrance odour when perceived.

**Antimicrobial activities of the VO against ten selected pathogenic bacteria:** Each of the nonlinear curves shown in Fig. 1 represented the % inhibition by the VO against the bacteria growth. The $IC_{50}$ of the VO was estimated to determine the inhibitory concentration that could kill 50% of the bacterial population (Fig. 2). Furthermore, the rate of growth inhibition per h was estimated from the Hillslope of each of the curves as shown in Fig. 2.

Each of the bacteria responded differently to the inhibitory effect of the VO. *P. vulgaris* has the highest $IC_{50}$ value of 0.75±0.004% v/v, while *S. typhimurium* showed the lowest $IC_{50}$ value of 0.17±0.005% v/v (Fig. 2). From Fig. 1, *P. vulgaris* has the steepest sigmoidal curve-a demonstration of the relative resistance to the VO as compared to other bacteria. *E. coli, K. oxytoca, K. pneumoniae, P. aeruginosa, S. enteritidis* and *S. paratyphi,* showed $IC_{50}$ within 0.2-0.4% v/v while *S. dysentariae* and *S. sonnei* showed $IC_{50}$> 0.4% v/v but <0.6% v/v (Fig. 2). The growth inhibition rate for *K. oxytoca, K. pneumoniae*, *P. vulgaris*, *P. aeruginosa*, *S. paratyphi, S. typhimurium* and *S. dysentariae* are within 0.1-0.2 $h^{-1}$ while *E. coli* and *S. enteritidis* showed inhibition rate within 0.2-0.3 $h^{-1}$. From this result, *P. vulgaris* showed a relatively high resistance in terms of $IC_{50}$, while the most susceptible bacterium is the *S. typhimurium*. Likewise, *S. sonnei* showed the highest inhibition rate as more bacteria colonies were killed per h than any other bacterium tested, however, *S. paratyphi* depicted the lowest inhibition rate as fewer bacteria colonies were killed per h as compared to other bacteria tested.

Regarding the diarrhoea-causing bacteria, the average $IC_{50}$ and growth inhibition rate of the VO against the *Salmonella* species vs. *Shigella* species are 0.26±0.06% v/v vs. 0.53±0.04% v/v and 0.18±0.03 vs. 0.23±0.08 $h^{-1}$,` respectively. It could be stated that the VO was significantly more effective against the *Salmonella* species than the *Shigella* species ($p<0.05$) because a two-fold increase in the VO concentration will be needed to exert the same inhibitory effect on the latter as compared to the former.

**Partial purification and characterisation of the extracellular protease of *S. typhimurium*:** The extracellular protease of *S. typhimurium* was partially purified to achieve 45.6% yield, 3.73 purification fold and 5, 341.4 µmol $min^{-1}$ total activity as compared to the CEE (Table 2). The highest specific caseinolytic activity was 314.2 µmol $min^{-1}$ $mg^{-1}$ protein, which was more than a 3.5-fold increase as compared to the CEE (Table 2).

Table 1: Extraction of *S. samarangense* VO by hydrodistillation

| Features | Parameters |
|---|---|
| Plant | Leaves of *S. samarangense* |
| Green foliage | 3.0 kg |
| Dried leaves used | 0.5 kg |
| Water used | 2.0 L |
| n-hexane used | 2 mL |
| Recovery VO | 0.4 mL |
| Operating temperature | 80°C |
| Cooling system | Liebig condenser system |
| Total time of extraction | 3 h |
| VO yield $kg^{-1}$ fresh leaves (%) | 0.13 v/w |
| VO yield $kg^{-1}$ dried weight (%) | 0.80 v/w |

Table 2: Partial purification profile of the extracellular protease of *S. typhimurium*

| Purification step | Total protein (mg) | Total activity (µmol $min^{-1}$) | Specific activity (µmol $min^{-1}$ $mg^{-1}$ protein) | Yield (%) | Purification fold |
|---|---|---|---|---|---|
| CEE | 139 | 11717.7 | 84.3 | 100.0 | 1.00 |
| $(NH_4)_2SO_4$ precipitation | 79 | 11557.7 | 146.3 | 98.6 | 1.74 |
| Gel filtration (Sephadex G-100) | 17 | 5341.4 | 314.2 | 45.6 | 3.73 |

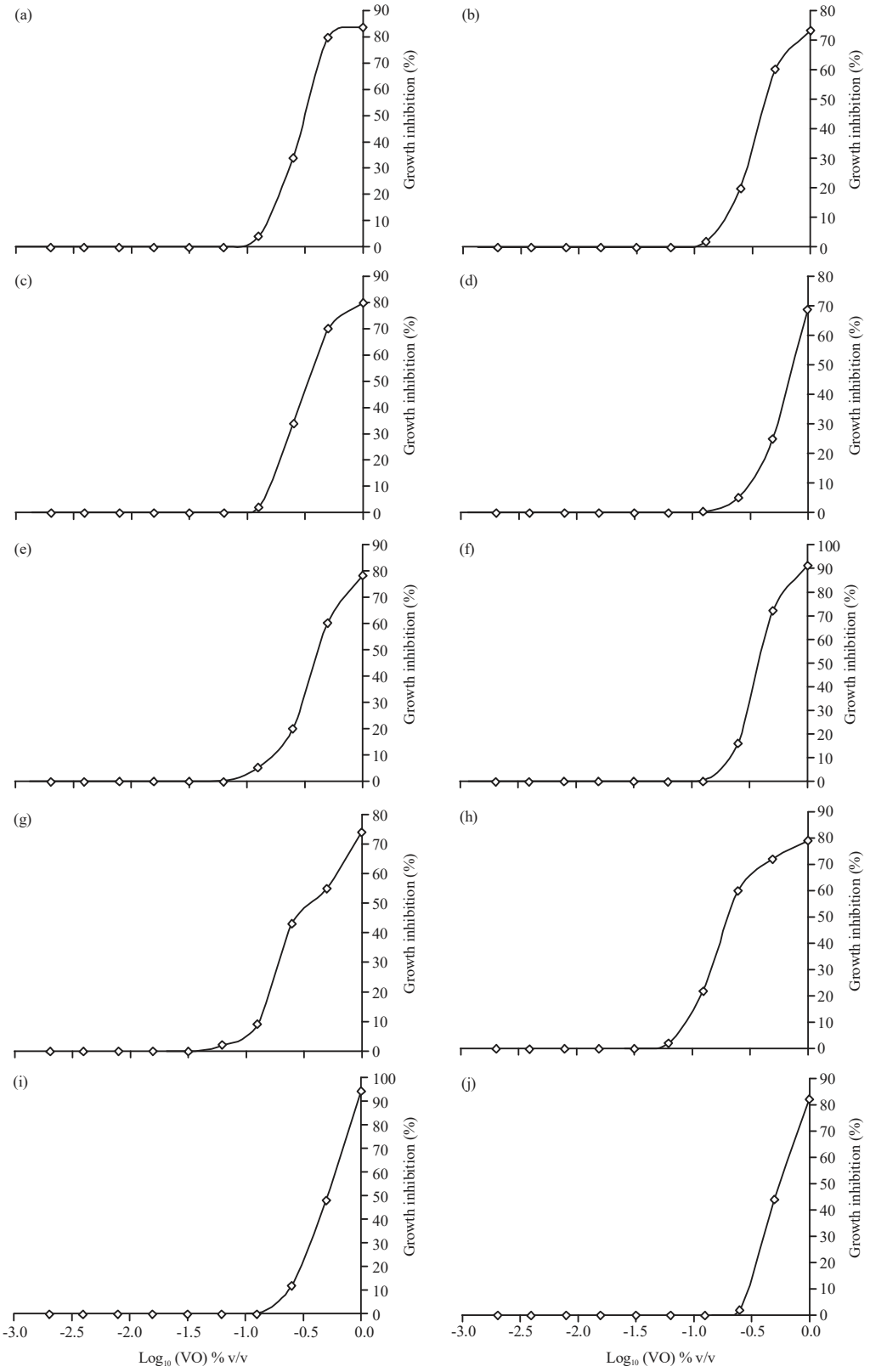


Fig. 1(a-j): Growth inhibition curve for each bacterium susceptible to the VO of *S. samarangense*, (a) *Escherichia coli*, (b) *Klebsiella oxytoca*, (c) *Klebsiella pneumoniae*, (d) *Proteus vulgaris*, (e) *Pseudomonas aeruginosa*, (f) *Salmonella enteritidis*, (g) *Salmonella paratyphi*, (h) *Salmonella typhimurium*, (i) *Shigella dysenteriae* and (j) *Shigella sonnei*

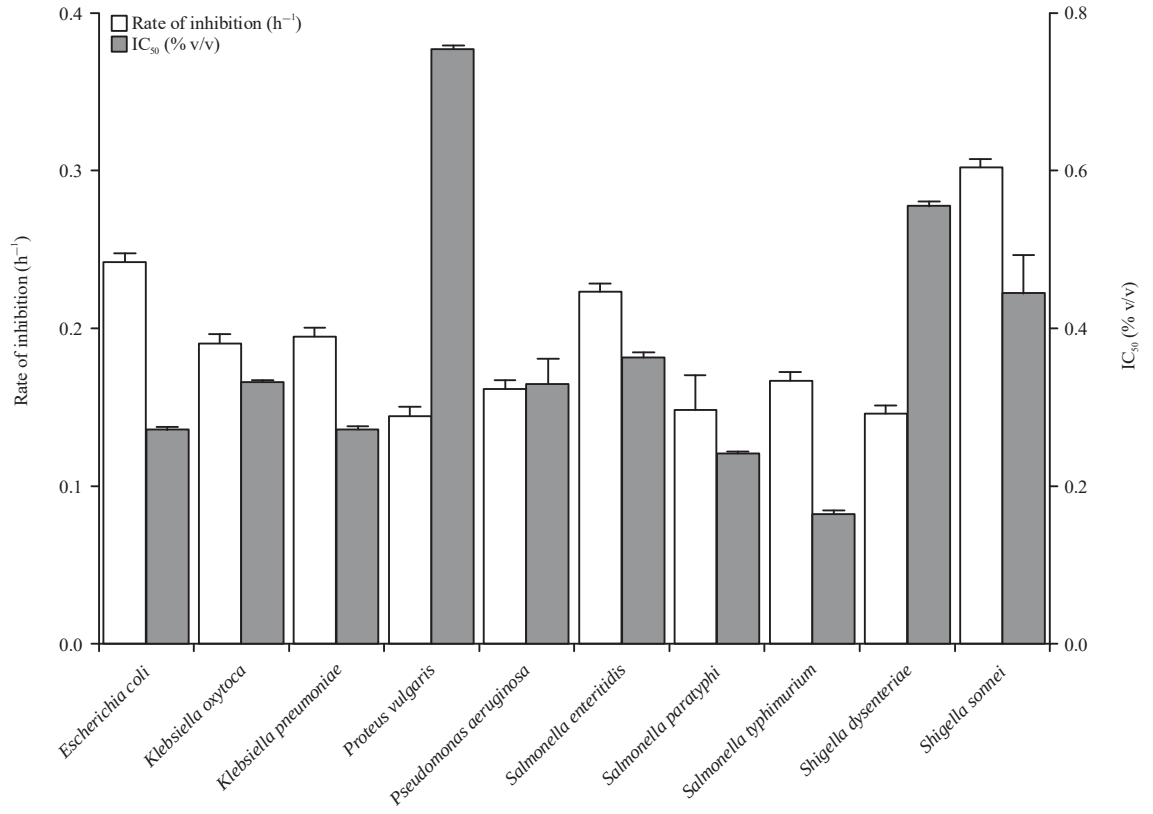


Fig. 2: Growth inhibition and $IC_{50}$ of the VO against the pathogenic bacteria (n = 2)

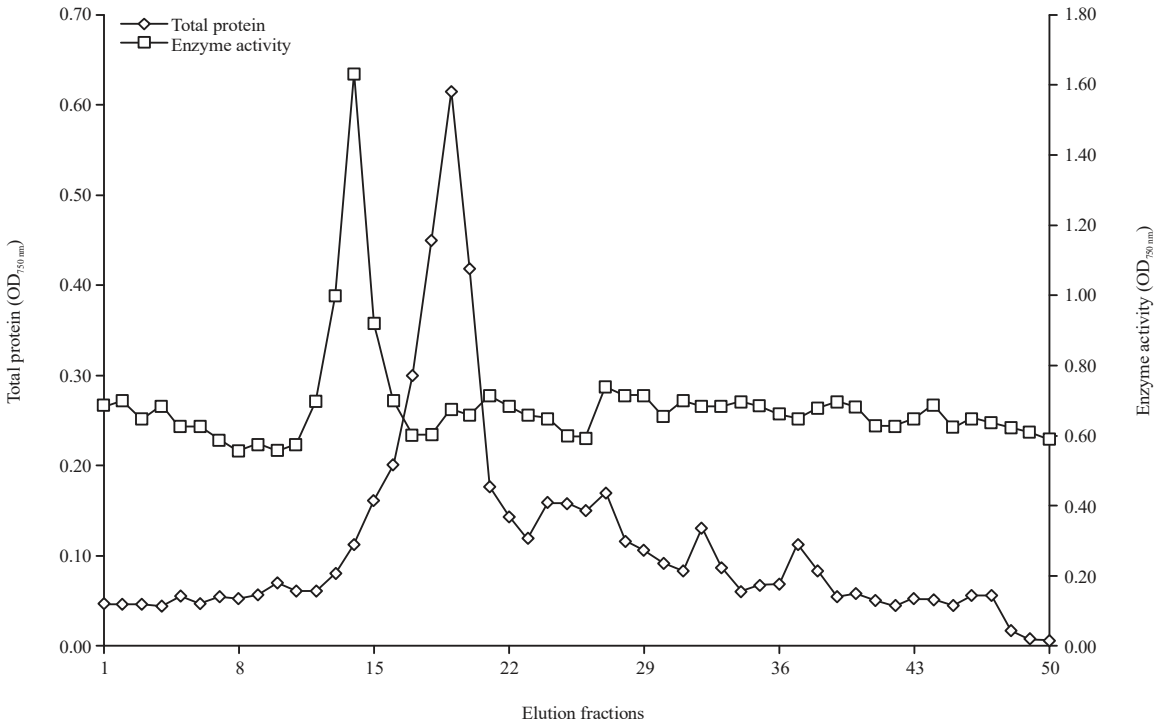


Fig. 3: Elution profile for the partial purification of the extracellular protease of *S. typhimurium*
Fractions 12-16 and 15-21 are the most probable eluents with high enzyme activity and protein content, respectively

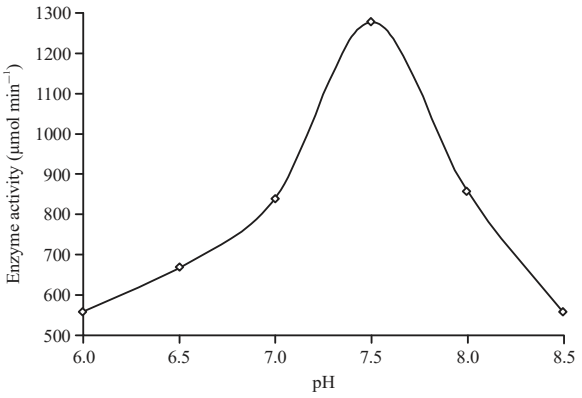


Fig. 4: Different pH affected the activity of the partially purified extracellular protease of *S. typhimurium*
Optimum enzyme activity was found at pH 7.5, a slight alkali medium

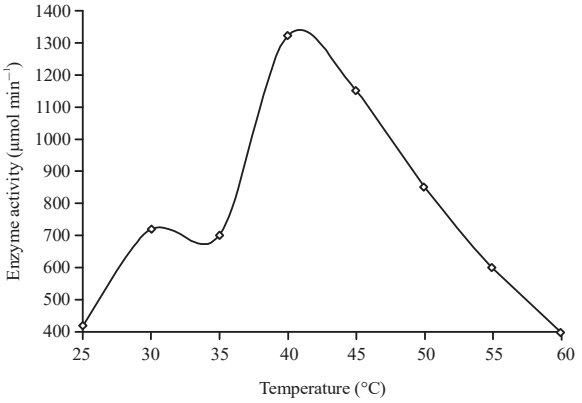


Fig. 5: Different temperatures affected the activity of the partially purified extracellular protease of *S. typhimurium*
Optimum enzyme activity was found at 40°C

The elution fractions 12-16 (Fig. 3) were pooled to characterise the properties of the enzyme. The optimum caseinolytic activity was found at pH 7.5 (a slight alkali medium) (Fig. 4). The enzyme activity was monitored over a range of temperatures (25-60°C) at pH 7.5 and found to show optimum activity at 40°C after when the enzyme activity decreased steadily (Fig. 5).

**Inhibition kinetics of the partially purified extracellular protease of *S. typhimurium*:** Competitive inhibition of the enzyme was observed with casein as a substrate with the VO of *S. samarangense* as a co-substrate (Fig. 6). About a two-fold increase in the substrate affinity was estimated to normalise the enzyme catalysis in the presence of the inhibiting VO. Lastly, the caseinolytic assay was carried out in the presence of 1.0 mM of different metallic chlorides (Fig. 7). There was

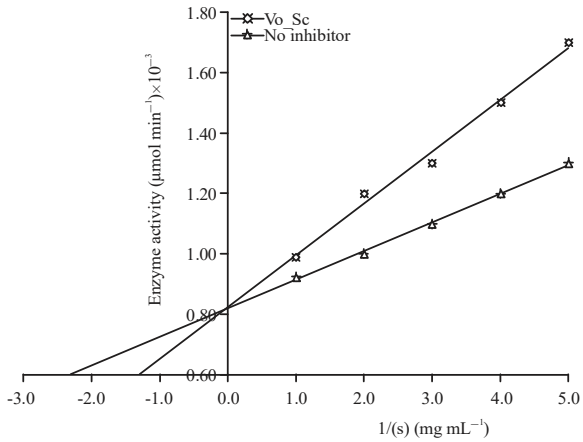


Fig. 6: Line-weaver burke plot showing inhibition of the partially purified extracellular protease of *S. typhimurium*
Line with the triangle is the enzyme activity without inhibitor, while a line with the asterisk is the enzyme activity in the presence of the VO of *S. samarangense* (VO_Sc). The $V_{max}$ = $1.2\times10^3$ µmol $min^{-1}$, the substrate-affinity constant ($K_m$) in the absence of inhibitor = 0.45 mg $mL^{-1}$ and in the presence of inhibitor, $K'_m$ = 0.81 mg $mL^{-1}$

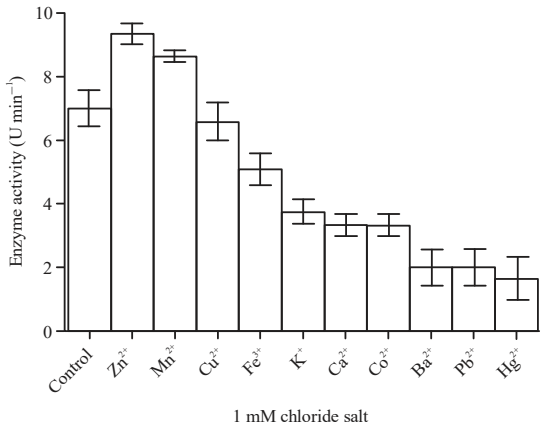


Fig. 7: 1 mM metallic chlorides affected the activity of the partially purified extracellular protease of *S. typhimurium* (n = 3)
There was a general stepwise reduction in the activity of the enzyme from transitional metallic chloride salts to heavy metal salts

no significant difference in the activities of the enzyme when the control, without any chloride salt (7.0±0.58 U $min^{-1}$) was compared with the chlorides of $Mn^{2+}$ (8.6±0.19 U $min^{-1}$), $Cu^{2+}$ (6.6±0.58 U $min^{-1}$) and $Fe^{3+}$ (5.1±0.51 U $min^{-1}$). However, the activity of the enzyme was statistically higher in the $Zn^{2+}$ (9.3±0.33 U $min^{-1}$) as compared to the control ($p<0.05$). Chloride salts of $Ba^{2+}$, $Pb^{2+}$ and $Hg^{2+}$drastically reduced the enzyme activity (Fig. 7).

## DISCUSSION

This study demonstrated a wide range of microbial growth inhibition by the VO of *S. samarangense.* More importantly, there was a significant growth inhibition in all the diarrhoea-associated bacteria, which confirmed the use of this plant against diarrhoea-related infections. Competitive mode of inhibition as revealed in the enzyme-substrate-inhibitor kinetics (Fig. 6), maybe one of the modes by which the VO inhibited the growth of these bacteria.

Few studies have shown the antimicrobial activities of the oils and organic solvent extracts from different species of *Syzygium* plants[30-33], however, there was speculation of more active antimicrobial effects in the Gram-negative than the Gram-positive bacteria perhaps as a result of a more intricate mesh of peptidoglycan layers in the latter[32]. In this study, all the Gram-negative bacteria tested were considered susceptible to the VO of *S. samarangense* and this finding supported the outcome of Raj *et al.*[30] and De Oliveira *et al.*[33] who worked respectively on the VO of *S. gardneri* and the organic extract of *S. cumini* and observed almost equal antimicrobial effects of the two plants against the Gram-negative and Gram-positive bacteria. Terpenes, chalcones, quercetins, flavonoids, tannins and their derivatives are the main secondary phytoconstituents of *Syzygium* plants[30,31,34-36]. Djoukeng *et al.*[31] identified 10 different triterpenes from the leaf extract of *S. guineense* and discovered the most significant antibacterial activity against *S. sonnei* but in this study, though there was no attempt to identify the active components of the VO used, however, it is very significant that *S. sonnei* has the highest growth inhibition per h as compared to other bacteria (Fig. 2). This study confirmed a significant microbial growth inhibition against the diarrhoea-causing *Salmonella* and *Shigella* species. This result complements the use of this plant in diarrhoea and this therapeutic effect had been traced to the presence of calcium channel blocking flavonoid in this plant[37].

This study also confirmed casein as one of the preferred substrates for the *S. typhimurium* extracellular protease because more than a 3.5-fold increase in specific enzyme activity was obtained during the partial purification as compared to the CEE (Table 2). The optimal conditions of enzyme activity obtained in this study further re-iterate the survival, colon colonisation, extracellular matrix protein degradation, invasion and pathogenesis of this bacterium in their hosts[15,38-40].

Competitive inhibition of the extracellular protease of *S. typhimurium* for casein was observed with the VO of *S. samarangense*, which indicates a possible potential drug target. About a 2-fold increase in the substrate concentration will be needed to normalise the caseinolytic activity of the enzyme, which suggests a stronger affinity binding of such component of the VO (Fig. 6). From the extensive work of Amor *et al.*[41], *S. samarangense* possessed intractable secondary metabolites that selectively inhibited serine protease. Likewise, Tezuka *et al.*[42] screened several medicinal plant extracts and identified catechol and quinone as potential inhibitors of prolyl endopeptidase from *Flavobacterium*. By considering the phytoconstituents enrichment of *S. samarangense* VO, it is, therefore, evident that this plant contains microbial protease inhibitor(s). Regarding this study, identification and characterisation of these specific extracellular proteases will enhance further investigation into the type of protease and the exact mechanism of inhibition[43-49].

The present result also indicates a metallic requirement for the activity of extracellular protease released by *S. typhimurium*. Chlorides of $Zn^{2+}$, $Mn^{2+}$, $Cu^{2+}$ and to some extent, $Fe^{3+}$ may be needed for the elevated caseinolytic activity of this protease. However, $Ba^{2+}$, $Pb^{2+}$ and $Hg^{2+}$ are inhibitors because they are toxic heavy metals that can quickly alter the biological function of proteins[50,51].

In this study, the specific extracellular protease of *S. typhimurium* was not identified and this calls for further studies to identify and classify this specific extracellular protease and their preference for casein. Furthermore, there is a need to identify the specific active component of the oil, which exhibited this antimicrobial function and this could be done by wide-range isolation and screening of these phytoconstituents.

## CONCLUSION

The reports from this study show the promising benefits of the VO extracted from the dried leaves of *S. samarangense* as an antimicrobial agent against a wide range of enteric and nosocomial pathogenic bacteria. This VO inhibited the growth of susceptible bacteria and competitively inhibited the extracellular protease of *S. typhimurium*. Further identification and isolation of these bactericidal components of this VO will be of immense benefit in antimicrobial chemotherapy.

## SIGNIFICANCE STATEMENT

This study discovered that the VO extracted from the leaves of *S. samarangense* possessed antimicrobial effect by preventing the growth of a wide-range enteric and nosocomial Gram-negative bacteria and competitively

hindered the caseinolytic activity of the extracellular protease of *S. typhimurium*. This can be of benefit for antimicrobial development in alternative and Ayurvedic medicine. This study will help the researcher to uncover the critical areas of enzyme-substrate-inhibitor kinetics that many researchers were not able to explore. Thus, a new theory on the mode and mechanisms of drug interaction with enzyme catalysis may be arrived at.

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