CHARACTERIZATION OF PARTIALLY PURIFIED CYSTEINE PROTEASE INHIBITOR FROM THE FRUITS AND SEEDS OF SOURSORP (ANNONA MURICATA)

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Abstract
Introduction: Soursop (Annona muricata Linn) is an edible lowland tropical fruit-bearing tree that is widely cultivated across regions of the world. It has been extensively researched as a result of its store of acetogenin; a potent anticancer agent. However, there is a dearth of information on the precise mechanism of action of acetogenin. It is therefore imperative to investigate this plant in the hope of discovering a different class of anticancer agent inherent in it. Various studies have demonstrated that cysteine protease inhibitors (CPIs) have considerable therapeutic potential which can be utilized in a variety of disease states including cancer.

Aims: Study was designed to isolate, purify and characterize CPI from the fruits and seeds of Soursop.

Materials and Methods: Isolation and purification of CPI were achieved by simple methods consisting of ammonium sulphate precipitation, anion exchange chromatography and size exclusion chromatography. Mode of inhibition, optimum pH and temperature, as well as the effect of metals on the enzyme activity was determined using spectrophotometry.

Results: The purified CPI from seeds and fruits exhibited competitive and non-competitive inhibition against papain respectively. However, maximal inhibitory activities for both fruit and seed samples were observed at similar optimal pH and temperature of 8 and 40°C respectively. Although, metal cations such as cobalt (Co²⁺), copper (Cu²⁺) and zinc (Zn²⁺) did not impact a considerable decrease on the inhibitory activity of the CPI; Lead (Pb²⁺), Magnesium (Mg²⁺) and manganese (Mn²⁺) significantly inhibited CPI at a very low concentration (1mM).

Conclusion: The antagonistic properties exhibited by the purified CPI certainly indicate its likely suitability for pharmaceutical application in the treatment of some pathological conditions such as cancer, in which uncontrolled proteolytic activities of cysteine proteases are implicated. There is an ample scope for further research on structure elucidation and protein engineering to facilitate its usage in a wide range of application.

Keywords: Annona muricata, cysteine protease inhibitor, enzyme inhibition, papain, purification, characterization

All co-authors agreed to have their names listed as authors.

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1. INTRODUCTION

Soursop (Annona muricata Linn) also known as graviola or guanabana and belonging to the Annonaceae family; is an edible lowland tropical fruit-bearing tree that is widely cultivated across regions of the world [1]. Its rank has risen in the milieu of scientific research in the past three decades as a result of its efficacy in the treatment of a wide range of human pathological conditions including diabetes, hypertension, neurodegenerative diseases and cancer [2].

The name soursop is due to the sour and sweet flavour of its large fruit [2]. Cherimoya (A. cherimola), sugar-apple (A. squamosa) and pawpaw (Asimina triloba) are related species in the family of Annona muricata. This pharmacologically fascinating plant is native to tropical Central and South America and the Caribbean but is also cultivated in tropical areas worldwide, including Nigeria [3]. The crop is ubiquitously distributed in most parts of southern Nigeria, bearing several local names including “Shawashawa”, “Shawa chop” and “Chop chop”[4]. Soursop fruit is widely known for its excellent agronomical potential which is utilized for commercial production of juice, candy, and sherbet [1].

Acetogenin, a phytochemical from Soursop has been extensively researched as a potent anticancer agent against varieties of cancer [5]. Acetogenins are potent inhibitors of NADH oxidase (Nicotinamide Adenine Dinucleotide Phosphate-oxidase) of the plasma membranes of cancer cells [6]. Several researchers have substantiated the antiproliferative effects of acetogenin, however, the precise mechanism of action of soursop as an anticancer agent have not been elucidated [7]. It is therefore imperative to investigate this plant in hopes of discovering a different mode of anticancer activity inherent in it.

Unwanted proteolysis resulting from an imbalance between cysteine proteases and their inhibitors in favour of the cysteine protease plays a critical role in a wide spectrum of pathological processes including cancer [8]. Imperatively, the activities of cysteine proteases in spite of their numerous advantages have to be tightly controlled by peptides that serve as molecular checkpoints, as their excesses can be detrimental to the host organism. This task is performed by a group of endogenous inhibitors called cysteine protease inhibitors (CPIs) [9].

CPIs fall under a group of peptides called Protease inhibitors (PIs). PIs aptly inhibit the operation of proteases by using domains which either block or enter the protease active site thereby preventing substrate access and leaving the enzyme ineffective [10]. They belong to a protein superfamly, the cystatins; comprised of three families: the steffins (family I) with molecular weights of approximately 11 kDa, with no disulfide bridges; the cystatins (family II) of molecular weight of 13 kDa containing two loops formed by disulfide bridges near the carboxyl terminal of the molecule and the third is the kininogen family (family III) of high molecular weights (over 40 kDa) (Shamsie et al., 2016). Kininogens are characterized by the presence of six loops formed by disulfide bridges and which are clearly the result of duplication of genetic material originating from family II inhibitors. Recently, a fourth family has been included which is known as phytocystatins and it includes all cysteine proteinase inhibitors isolated from plant [10]. Each cystatin contain the conserved Gln-Val-Val-Ala-Gly segment known as the ‘cystatin motif’ in the central part of their sequences which is probably involved in complex formation with the enzymes [11].

CPIs thus have considerable chemotherapeutic potential which can be utilized in a variety of disease states [12]. Studies have proved that use of Protein PIs (PPIs) provide considerable safety advantage over chemical PIs, as PPIs are safer and more specific in their targeted action. PPIs are thus considered as attractive candidates for oral administration, with lesser consequences [10]. The aim of this study was to isolate, purify and characterize cysteine protease inhibitor (CPI) from Annona muricata.

2. MATERIAL AND METHODS

2.1. Sample collection

Soursop (Annona muricata) fruits were obtained at Iyana-Iba market in Ojo Local Government Area of Lagos State, South-Western part of Nigeria. The plant sample was identified and authenticated at the Herbarium of the Botany Department, Faculty of Science, Lagos State University, Lagos state.

2.2. Sample preparation

1000g each of the seeds and fruits of Soursop were dried and ground to powder using a grinding machine. The powdered sample was then defatted with n-hexane using the soxhlet apparatus, according to the method of Franz Von Soxhlet [13].

2.3. Extraction and isolation of cysteine protease inhibitor

Isolation of cysteine protease inhibitor was carried out according to the method of Benjakul et al; [14]. 40 g of the defatted sample was suspended in 250ml of 10mM phosphate buffer solution, pH 7.2 containing 0.13mM sodium and 0.1 % (%v/v) β-mercaptoethanol with continuous stirring for 2 h. The mixture was filtered using a clean white piece of cloth. The filtrate was centrifuged at 7000g for 10 min to collect cell debris. Total protein was then precipitated out from the resulting supernatant by ammonium sulphate precipitation according to the method described by Englard and Seifter[15].
2.4. Ammonium sulphate precipitation

Ammonium sulphate required to precipitate the protein was optimized by adding varying concentrations (35, 55, 65, 75 and 90%) to the crude extract independently. After each precipitation, the precipitate was collected by centrifugation at 7000g for 10 min. The precipitate was then re-dissolved in a small volume of buffer and dialyzed overnight against 100 mM Tris buffer pH 7.8 that was changed every 6 h.

2.5. Protein determination

Protein concentration was determined using Bradford method and bovine serum albumin (BSA) as standard [16]. The absorbance was read at 595nm.

2.6. Inhibitory activity

Cysteine protease inhibitory activity of the extracted protein was monitored using papain according to the modified method of Murachi[17], using casein as substrate. Papain (5 mg in 0.1M Tris-HCl buffer, pH 7.8, 0.5 mM cysteine, 0.2 mM EDTA) and the inhibitor extract (50 mg/ml) were pre-incubated at 37°C for 15 min. The reaction mixture (150 ml) was then added to tubes containing 2.0 ml of 0.5% casein (casein prepared in 0.1M Tris-HCl pH 7.8 containing 0.2 mM EDTA and 0.5 mM cysteine) at 37°C. The assay was incubated for 30 min at 37°C and the reaction terminated by the addition of 3.0 ml of 5% trichloroacetic acid (TCA). The absorbance was measured at 280nm after 30 min. One unit of inhibitor activity was defined as the decrease by one unit of absorbance of trichloroacetic acid-soluble casein hydrolysis product liberated by protease action at 280nm at 37°C in a given assay volume. Percentage Inhibition was determined as shown in the equation below:

\[
\% \text{ Inhibition} = \frac{\text{Abs of standard} - \text{Abs of Sample}}{\text{Abs of standard}} \times 100
\]

2.7. Protein purification

The crude protein after dialysis was purified by ion-exchange chromatography as described by Rossomando[18], followed by size exclusion chromatography. 3.0 ml of the dialysate (with highest % inhibitory activity against papain) was loaded on DEAE-cellulose column previously equilibrated with 100 mM Tris-HCl buffer (pH 7.8). 5 ml fractions were collected into 60 test tubes using an increasing linear gradient of NaCl concentration from 0 to 0.3 M in the same buffer (Tris-HCl). Total protein and inhibitor activity were carried out on each fraction as earlier described. Fractions with highest inhibitory activity were pooled together, concentrated and loaded on Sephadex G-75 column previously equilibrated with 100 mM Tris-HCl buffer (pH 7.8) and eluted using the same buffer. The yield of protein and of each fraction during purification is the percentage activity obtained by dividing the total protein content of that fraction with the total protein content of the crude extract. Yield of protease inhibitory activity is the percent activity obtained by dividing the activity of that fraction with the activity of the crude extract. The fold of purification in each step was calculated by dividing the specific activity of the respective fraction with that of crude extract.

2.8. Mechanism of inhibition

Mode of inhibition of the purified CPI was analyzed as described in inhibitory assay above but now varying the concentration of casein. This was carried out both in the absence and presence of the inhibitor respectively.

2.9. Optimum temperature

The optimum temperature for the inhibitor was determined by incubating the reaction mixtures at varying temperatures ranging from 10 -100°C with 10-unit increase.

2.10. Optimum pH

Estimation of the optimum pH was carried out using Tris-HCl buffer with varying pH (6.0 -11.0) differently in the reaction mixtures. Other steps were as described for inhibitory assay.

2.11. Effect of metals on inhibitory activity

Effect of different metal ions on protease inhibitory activity was carried out by incubating the protease inhibitor with different concentrations of various metal ions for 30 min followed by measuring inhibitory activity as described above. The metals that were investigated included Mg$^{2+}$, Mn$^{2+}$, Zn$^{2+}$, Co$^{2+}$, Cu$^{2+}$, Na$^+$ and Pb$^{2+}$. Each with concentrations between 1 to 10 mM.

2.12 Statistical analysis

The statistical package (Graph pad prism 5) was used for data analysis. Results were subjected to linear regression, one-way analysis of variance (ANOVA) and Post Hoc test (Tukey’s honesty significance test; Tukey’s HSD).

3. RESULTS AND DISCUSSION

3.1 Purification of cysteine protease inhibitor

Table 1 show that the purification folds of cysteine protease inhibitor of Annona muricata fruit and seedsfrom ammonium sulphate precipitation, ion-exchange chromatography and gel filtration chromatography increased from 1.09, through 1.13, to 4.13 and 1.05, through 1.29 to 5.0 respectively.
Table 1. Purification table of cysteine protease inhibitor (CPI) from *Annona muricata* fruit.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total protein (mg)</th>
<th>Inhibitory activity (units)</th>
<th>Specific inhibitory activity (unit/mg)</th>
<th>Yield (%)</th>
<th>Activity yield</th>
<th>Purification fold</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude extract 65%</td>
<td>178</td>
<td>82.65</td>
<td>0.46</td>
<td>100</td>
<td>100</td>
<td>1.00</td>
</tr>
<tr>
<td>ammonium sulphate</td>
<td>11</td>
<td>5.5</td>
<td>0.5</td>
<td>6.18</td>
<td>6.65</td>
<td>1.09</td>
</tr>
<tr>
<td>DEAE-Ion exchange</td>
<td>8</td>
<td>4.14</td>
<td>0.52</td>
<td>4.49</td>
<td>5.0</td>
<td>1.13</td>
</tr>
<tr>
<td>Sephadex G-100</td>
<td>5.75</td>
<td>1.9</td>
<td>1.68</td>
<td>6.95</td>
<td>4.13</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Purification table of cysteine protease inhibitor (CPI) from *Annona muricata* seed.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total protein (mg)</th>
<th>Inhibitory activity (units)</th>
<th>Specific inhibitory activity (unit/mg)</th>
<th>Yield (%)</th>
<th>Activity yield</th>
<th>Purification fold</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude extract 65%</td>
<td>197</td>
<td>69.95</td>
<td>0.35</td>
<td>100</td>
<td>100</td>
<td>1.00</td>
</tr>
<tr>
<td>ammonium sulphate</td>
<td>13</td>
<td>4.91</td>
<td>0.37</td>
<td>6.59</td>
<td>7.02</td>
<td>1.05</td>
</tr>
<tr>
<td>DEAE-Ion exchange</td>
<td>11</td>
<td>4.99</td>
<td>0.45</td>
<td>5.58</td>
<td>7.13</td>
<td>1.29</td>
</tr>
<tr>
<td>Sephadex G-100</td>
<td>4</td>
<td>7</td>
<td>1.75</td>
<td>2.03</td>
<td>10</td>
<td>5</td>
</tr>
</tbody>
</table>

Figure 1 shows the elution profile of cysteine protease inhibitor extracted from *Annona muricata* seed following gel filtration chromatography. The elution profile of cysteine protease inhibitor from *Annona muricata* seed (Figure 1a) shows 6 major peaks (fractions 41, 43, 53, 55, 57 and 59) while *Annona muricata* fruit (Figure 1b) shows 3 major peaks (fractions 54, 57 and 59). Fractions which exhibited high inhibitory activity against papain had a lower protein concentration resulting from decreased casein hydrolysis by papain.

Figure 2a shows the elution profile of cysteine protease inhibitor extracted from *Annona muricata* seed following gel filtration chromatography. The elution profile of cysteine protease inhibitor from *Annona muricata* seed (Fig. 2a) shows 3 major peaks while the *Annona muricata* fruit (Fig. 2b) shows 2 major peaks. Fractions which exhibited high inhibitory activity against papain had a lower protein concentration resulting from decreased casein hydrolysis by papain.

Figure 3(a) and 3(b) shows the effect of pH on purified cysteine protease inhibitor from *Annona muricata* fruit and seed respectively. The result shows the purified cysteine protease inhibitor is optimal at pH 8.0. The purified inhibitors were active at a pH range of 7-8 and almost inactive at low pH values (below pH 7). Also, a significant decrease in the inhibitory activity of CPI was observed above pH 8 for both fruit and seed samples.

Figure 4(a) and 4(b) shows the effect of temperature on purified cysteine protease inhibitor from *Annona muricata* fruit and seed respectively. The result shows that the optimal temperature of the cysteine protease inhibitor is around 40°C. Cysteine protease inhibitor was less active at temperatures above 40°C up to 50°C but was almost inactive at temperatures above 50°C. Similarly, a significant decrease in the inhibitory activity of CPI was observed at temperatures below 40°C which peaked below 30°C.

Figure 5 shows the mode of inhibition of CPI isolated from *Annona muricata* fruit and seed. In the absence and presence of purified inhibitor extract, the reciprocal of the varying amount of substrate concentration [1/S] used and the reciprocal of the absorbance (1/V) at 280 nm were plotted against each other. The double reciprocal plot in Fig. 5a shows that inhibition is non-competitive having the same Km of 5.13 μMol and different Vmax values of 0.0486 μmol/min and 0.0362 μmol/min in the absence and presence of inhibitor respectively. [V]i = with inhibitor and [V]o = without inhibitor. The double reciprocal plot in Fig. 5b shows that inhibition is competitive having the same Vmax of 0.0454 μmol/min and different Km. Km (without inhibitor) = 3.412 μMol and km (with inhibitor) = 7.647 μMol. [V]i = with inhibitor and [V]o = without inhibitor.

Figure 6 shows the effect of metal ions such as Mn²⁺, Mg²⁺, Pb²⁺, Cu²⁺, Co²⁺ and Zn²⁺ (contributed by their respective chlorides) on the activities of cysteine protease inhibitor. Varying levels of decrease in the inhibitory activity of the cysteine protease inhibitor was observed at heavy metal ions concentrations ranging from 1 to 10mM for seed and fruit samples. Although, 1mM concentration of Co²⁺ and Cu²⁺ decreased the residual inhibitory activity of the cysteine protease inhibitor of seed samples (up to 30.9 and 29.7% respectively), their concentrations at higher concentration (10mM) significantly decreased the residual inhibitory activity of cysteine protease inhibitor.
up to 45.2 and 42.9% respectively, compared to that of the control. However, Mn$^{2+}$, Zn$^{2+}$, Mg$^{2+}$ and Pb$^{2+}$ at a concentration as low as 1mM considerably decreased the residual inhibitory activity of cysteine protease inhibitor of seed samples up to 48.8, 41.6, 51.1 and 47.6% respectively, compared to that of the control. Metal cations produced a similar pattern of inhibition on the residual cysteine protease inhibitory activity of fruit samples.

Fig.1a. Elution profile of cysteine protease inhibitor extracted from *Annona muricata* seed following DEAE Cellulose anion exchange chromatography using NaCl gradient.

Fig.1b. Elution profile of protease inhibitor extracted from *Annona muricata* fruit following DEAE Cellulose anion exchange chromatography using NaCl gradient.

Fig.2a Elution profile of cysteine protease inhibitor extracted from *Annona muricata* seed following gel filtration chromatography.

Fig.2b Elution profile of cysteine protease inhibitor extracted from *Annona muricata* fruit following gel filtration chromatography.
Fig. 3. Effect of pH on the activity of purified CPI isolated from *Annona muricata*. The figures represent the mean ± SEM of triplicate analysis of the samples (a) activity at optimum pH of purified CPI isolated from *Annona muricata* fruit (b) activity at optimum pH of purified CPI isolated from *Annona muricata* seed.

Fig. 4. Effect of temperature on the activity of purified CPI isolated from *Annona muricata*. The figures represent the mean ± SEM of triplicate analysis of the samples (a) activity at the optimum temperature of purified CPI isolated from *Annona muricata* fruit (b) activity at the optimum temperature of purified CPI isolated from *Annona muricata* seed.
Fig. 5. Lineweaver-Burk plot of purified CPI isolated from (a) *Annona muricata* fruit (b) *Annona muricata* seed.

Fig. 6. Effect of Metal ions on the activity of cysteine protease inhibitor of (a) purified CPI isolated from *Annona muricata’s* seed (b) purified CPI isolated from *Annona muricata’s* fruit; the residual activity of protease inhibitor was analyzed after incubation with different concentrations of Metal ions at 37°C for 30 min; values are presented as mean±SD of three sets of observations. Bars with same superscripts are not statistically different at $P=.05$. 
DISCUSSION

Cysteine protease inhibitors (CPIs) are ubiquitous in plants and represent a member of protease inhibitors that effectively take care of the excesses of cysteine proteases in plants or serve as defense support for the plant against invading insects and microbes. Also, CPIs have been researched and implicated in some disease conditions such as diabetes, cancer and neurodegenerative disorders among many others [19]. Plant cysteine protease inhibitors (CPIs) have therefore been studied extensively [2, 9, 20]. However, this study is the first at attempting to characterize CPI from *A. muricata*.

Activities of cysteine protease inhibitor were enhanced as a consequence of an upsurge in the concentration of protein obtained from its crude extraction. This is an indication of the presence of CPI in the fruit and seeds crude extracts of soursop. It has been reported that 0.1M phosphate buffer at pH 7.6 is most suitable for the maximal extraction of protein from pigeon pea seeds (*Cajanus cajan*) with high protease inhibitor activity [21].

The maximum inhibitory effect observed with 55% and 65% ammonium sulphate precipitation for seed and fruit extracts respectively are in the reported range of ammonium sulphate concentration for salting out CPIs. Several protease inhibitors extracted from plants have been reported to be more effective after precipitating with high ammonium sulphate concentration. Obayomiet al.; [9] reported that 65% ammonium sulphate saturation of CPI exhibited the maximum inhibitory effect. Ashouriet al.; [22], also reported that a 50-70% ammonium sulphate precipitation of protease inhibitors from seeds of *Helianthus annuus* produced the highest inhibitory effect (41.53%) when incubated with larva gut proteases. Similarly, cysteine protease inhibitors from *Brassica napus* L. exhibited an optimal inhibitory effect on digestive protease of Colorado potato beetle at 50-70% ammonium sulphate precipitation [23].

The purification fold after dialysis of extracted protein did not produce a monumental increase in enzyme inhibitory activity when compared to the crude extract. This is an indication that the protein still has some impurities. Further purification of the CPI using DEAE cellulose ion-exchange chromatography yielded multiple peaks. The eluent from DEAE cellulose anion exchange chromatography with the highest inhibitory activity was purified further by gel filtration and a reduction in the number of peaks was observed. Although, gel filtration resulted in an increase in the specific inhibitory activity and produced the highest purification fold for fruit and seed samples; higher values were observed for extracted CPI from *A. muricata* seeds. Activities of partially purified Bromelain from Pineapple (*Ananas comosus*) were reported to be enhanced after purification with DEAE ion exchanger [24]. However, higher degree of purity usually accompanies gel filtration following ion exchange chromatography [24].

Isolated CPI from *A. muricata* seed and fruit was most active at a pH range of 7.0 – 8.0 with an optimum pH observed to be at 8.0. Most protein at extreme levels of pH could be denatured, they unfold, causing a loss of both structure and stability. Under extreme pH conditions, disruption of electrostatic interactions which play an important role in protein stability results in structural perturbations [25]. Extreme pH condition will affect the state of ionization of acidic or basic side chain of amino acid thereby changing the chemical feature (e.g. via covalent bond formation) [26]. Change in pH causes modification in protein folding resulting in the deactivation of the inhibitor, causing irreversible proteolysis [27]. The optimum pH of cysteine protease inhibitors isolated in this study is similar to the pH of 7.0 that was reported for *M. oleifera* leaves [28].

Cysteine protease inhibitor isolated from the *Annona muricata* seeds and fruits exhibited an optimal temperature of 40°C. Most plant’s cysteine protease inhibitors are active at this temperature up to 50°C [29]. Thermal inactivation of the cysteine protease inhibitor at a temperature below or above the optimum temperature resulted in a radical loss of activity. The thermal inactivation could be due to folding and unfolding of protein due to change in temperature resulting in modulation of covalent and non-covalent interaction and consequent loss of inhibitory activity of the protease inhibitor [30].

Metals such as Co$^{2+}$, Cu$^{2+}$ and Zn$^{2+}$ did not affect the inhibitory activity of CPI isolated from both the seeds and fruits of *Annona muricata* at low concentration (1mM) but at higher concentrations, there was a significant decrease in their inhibitory activities. However, at a concentration of 1.0 mM, metal ions (Pb$^{2+}$, Mg$^{2+}$ and Mn$^{2+}$) reduced the residual inhibitory activity of CPI significantly compared to the control. It may be due to alteration of the protease inhibitor leading to deactivation as a result of its sulphur-philic nature. As a result, there is a conformational change in the three-dimensional structure of the protein and is denatured, thereby inhibiting its activity [31].

Purified cysteine protease inhibitor from *Annona muricata* seed and fruit samples demonstrated competitive and noncompetitive inhibition against papain respectively. This suggests that the inhibitor might have altered the rate of cysteine protease reaction by competitively inhibiting the enzyme or probably directs its effect on the substrate as substrate ligand, making it inaccessible to the active site of the protease. The vast majorities of protease inhibitors are competitive inhibitors and bind a critical portion of the inhibitor in the active site in a substrate-like manner [32]. On the other hand, the cysteine protease inhibitor might have reacted by binding to an allosteric site (non-competitive inhibitors) without forming covalent adducts with the enzyme and can be removed by dialysis if the non-covalent binding affinity is not too high [12].
4. CONCLUSION

The cysteine protease inhibitor from *Annona muricata* was successfully isolated, purified and characterized. The experimental results confirmed the antiproteolytic action of the cysteine protease inhibitor. The antagonistic properties exhibited by the purified cysteine protease inhibitor from *Annona muricata* may be responsible for its anticancer activity. This inhibitory potential could be biotechnologically harnessed for use in the treatment of some pathological conditions such as cancer in which uncontrolled and unwanted proteolytic activities of cysteine proteases are implicated. There is an ample scope for further research on structure elucidation and protein engineering to facilitate its usage in a wide range of applications.

COMPETING INTERESTS

The authors have not declared any conflict of interest.

AUTHORS‘ CONTRIBUTIONS

Segun Adeola designed the study and wrote the protocol. Habeeb Bankole performed the statistical analysis. Kanmodi Rahmon and Habeeb Bankole performed the laboratory protocols. Kanmodi Rahmon wrote the first draft of the manuscript and managed the literature searches. All authors read and approved the final manuscript.

REFERENCES


29. Bijina B, Chandrasekaran M. Isolation, purification and characterization of Protease Inhibitor from Moringa oleifera Lam: Cochin University of Science & Technology; 2006.

