Insights on Anti-inflammatory Effect of Sequentially Extracted Fractions of Costus pictus: An In vitro Study.

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ABSTRACT

Extracts varying from low to high polarity (Hexane, Dichloromethane (DCM), Ethylacetate (EA) and Methanol) of leaves of Costus pictus Linn (Family: Costaceae) was assessed for anti-inflammatory activity by in vitro methods. Quantitative analysis of anti-inflammatory components like total amount of phenolics and flavonoids were estimated using spectrophotometric method. In-vitro anti-inflammatory activity was evaluated using albumin denaturation assay, membrane stabilization assay and proteinase inhibitory activity at different concentrations. Aspirin (Diclofenac) was used as a standard drug for the study of anti-inflammatory activity. Results showed that, the yield was the maximum for the methanolic extract which was about 14.5% and substantially all bioactive phytochemical constituents were at a greater concentration in methanolic extract. It also exhibited the most and significant inhibition of the heat induced albumen denaturation, Red Blood Cells membrane stabilization and proteinase inhibitory effects with 85±1.46, 89±1.24 and 93±0.93% for 250 μg/ml respectively. Total phenol content and flavonoid was the highest for methanolic extract and was estimated as 66.4±1.65 mg of gallic acid equivalents of dry extract and 49.4±1.325 mg of quercitin equivalents per gram of dry extract respectively. The above results help us to substantially conclude that bioactive components are extracted well in high polar solvents which mainly include flavonoids and related polyphenols. These compounds present in C.pictus may be an active lead for potent anti-inflammatory activities.

Keywords: C. pictus, Diclofenac, Hexane, DCM, EA, Methanol, gallic acid, quercitin.

INTRODUCTION

Inflammation is the initial response caused by harmful stimuli and is achieved by the increased flow of plasma and leucocytes from the body into the injured site or tissue. A sequence of cellular and biochemical signals propels and propagates the inflammatory response. This involves the debris at the injured site, vascular network and immune cells. Inflammation leads to a complete debriment at the site of inflammation and promotes the renewal or regeneration of normal tissue. Medicinal plants provide immense benefits with new remedies1. Medicinal plants are readily available and have very low side effects and efficacy compared to other commercial drugs. Active lead compounds from plants possess several biological activities including antioxidant and anti-inflammatory activity26,27.

Metabolite products of plants include terpenoids, phenolic compounds such as flavonoids, phenolic acids and tannins which could be the active lead responsible for biological activities20.

Antioxidants play an important role in neutralizing free radical species which leads to oxidative stress in the system15,17.

This finally leads to damage to macromolecules including DNA, lipids, and proteins and results in inflammation4,5.

A persistent and chronic inflammation may act as an underlying definition for many of the long term illness such as Rheumatoid arthritis, diabetic foot ulcer, alzheimers disease etc. The aggravation of mediators mainly cytokines and growth factors may cause prolonging of the process of inflammation and cause degradation of tissues which is otherwise a normal process. At present, both steroidal anti-inflammatory drugs and Non-Steroidal Anti-inflammatory Drugs (NSAIDS) are used in medicine as a remedy for inflammation. Steroids and NSAID have a greater impact in the cure for inflammatory diseases, but due to their toxicity, they can be used only over short periods. Therefore there is a call for new anti-inflammatory agents with minimum side effects7,8.

C. pictus a member of Costaceae family is found in various parts of India and is commonly called as a insulin plant. This may provide an inexpensive remedial measure for the people affected with diabetes11,24. The plant have also shown bactericidal activity against strains like Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae and Pseudomonas aeruginosa3,14. As there is a availability of geographical variables on phytochemicals and hypoglycaemic effect of the plant, It is par with the inflammatory modulation efficacy of the plant as well11. Our present interest is to evaluate the in vitro anti-inflammatory potency of various extractions of C.pictus using simple lab techniques.

Plant Material

Costus pictus namely the Insulin plant, leaves were collected, washed in distilled water and allowed to shade dry, the dried material was homogenized in domestic mixture into fine powder, stored in a dry plastic container at room temperature.
Preparation of Plant Extract
The dried leaf powder (100g) of Costus pictus was extracted sequentially using various solvents in increasing order of polarity (hexane, dichloromethane, ethyl acetate and methanol) in their respective boiling point and was concentrated using reduced pressure under vacuum. Dried extracts were used for the further study.

Drugs and Chemicals
The chemicals and solvents utilized for the study were obtained from SIGMA-ALDRICH and were of analytical grade.

Yield of Various Solvent Extracts
The various extracts obtained from sequential extraction method in the increasing order of polarity of solvents were dried under reduced pressure and weighed11,14.

\[ \text{Percentage Yield} = \frac{\text{Weight of the sample extract obtained (g)}}{\text{Weight of the powdered sample used (g)}} \times 100 \]

Phytochemical Analysis
All phytochemical Analysis was carried out using standardized protocol18.

Detection of alkaloids
Various extracts were separately dissolved in Dilute Hydrochloric acid and filtered. Well mixed, 1 ml filtrate was used as a sample for further experimentation analysis.

Mayer’s Test
Individual filtrates were treated with Mayer’s reagent (Potassium mercuric iodide).

Formation of a yellow coloured precipitate by the addition of reagent along the side walls of the test tube indicates the presence of alkaloids.

Wagner’s Test
Individual filtrates were treated with Wagner’s reagent (Iodine in potassium iodide). Formation of brown or reddish precipitate along the rim of the tubes indicates the presence of alkaloids.

Dragendorff’s Test
Individual filtrates were treated with Dragendorff’s reagent (solution of Potassium Bismuth iodide). Formation of red precipitate inside the tube immediately after the addition of reagent indicates the presence of alkaloids.

Detection of Carbohydrates
5mg of the dried extracts was dissolved individually in 5 ml distilled water and filtered. Out of this 1 ml of filtrates were used to test for the presence of carbohydrates.

Molisch’s Test
Individual filtrates were treated with drops of alcoholic α-naphthol solution along the sides of a test tube. Formation of a violet ring at the interfacial junction indicates the presence of Carbohydrates.

Benedict’s Test
Individual filtrates were treated with Benedict’s reagent and heated gently. Formation of orange red precipitate indicates the presence of reducing sugars like aldehydes.

Detection of Saponins

Froth Test
Individual extracts were diluted with 20ml of distilled water and shaken in measuring cylinder for 15 minutes, formation of a thick layer of foam between 0.5-1 cm indicates the presence of saponins.

Detection of Phytosterols

Salkowski’s Test
Individual filtrates were treated with chloroform and filtered. The filtrates were treated with few drops of concentrated sulphuric acid, shaken and allowed to settle. A golden yellow colour formation indicates the presence of triterpenes.

Detection of Phenols

Ferric Chloride Test
Individual filtrates were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenolic compounds.

Detection of Tannins

Gelatin Test
Individual extracts were added with 1% gelatin solution containing sodium chloride. Formation of white precipitate indicates the presence of tannins.

Detection of Flavonoids

Alkaline Reagent Test
To about 5mg/ml concentration of extracts a few drops of sodium hydroxide solution were added. Formation of yellow colour precipitate, which turns colourless on addition of dilute acid, shows the indication of flavonoids.

Lead Acetate Test
To about 5mg/ml concentration of extracts a few drops of lead acetate solution were added. Formation of yellow colour precipitate indicates the presence of flavonoids.

Quantification of Flavonoids and Total Phenolics

Determination of Total Phenolic Contents in the Plant Extracts
The concentration of phenolics in various plant extracts was determined using spectrophotometric method15. Methanolic solution of various extracts in the concentration of 1 mg/ml was used in the analysis. The reaction mixture was prepared by mixing 0.5 ml of methanolic solution of various extracts, 2.5 ml of 10%
Folin-Ciocalteu’s reagent dissolved in water and 2.5 ml 7.5% NaHCO₃, Blank contains 0.5 ml methanol 2.5 ml 10% Folin-Ciocalteu’s reagent dissolved in water and 2.5 ml of 7.5% of NaHCO₃. All samples were kept in water bath at 45°C for 45 min. The absorbance was determined using a spectrophotometer at an absorbance of 765 nm.

All samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of gallic acid and the calibration line was constructed. The content of total phenolics in extracts was expressed in terms of gallic acid equivalent (mg of GA/g of extract).

**Determination of Flavonoid Concentrations in the Plant Extracts**

The concentration of flavonoids in various plant extracts was determined using spectrophotometric method. The sample contained 1 ml of methanol solution of various extract in the concentration of 1 mg/ml and 1 ml of 2% AlCl₃ solution dissolved in methanol. The samples were incubated for an hour at room temperature. The absorbance was determined using spectrophotometer at 415 nm.

The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of quercetin and the calibration line was constructed. The content of flavonoids in extracts was expressed in terms of quercetin equivalent (mg of quercetin/g of extract).

**In vitro Anti-inflammatory Assays**

**Inhibition of Albumin Denaturation**

The reaction mixture consisted of 0.2 ml of hen’s egg albumin, 2.8 ml of phosphate buffered saline and 2 ml of varying concentrations of various extracts of Costus pictus was added so that final concentrations become 31.25, 62.5, 125, 250, 500, 1000 μg/mL. Similar volume of distilled water was taken as control.

The samples were incubated at 37°C for 20 min and then again heated at 70°C for 20 min. After cooling the samples in running water, the turbidity was measured spectrophotometrically at 660 nm. Diclofenac sodium at the final concentration as that of the extracts and was used as reference drug. The experiment was performed in triplicate. Percent inhibition of protein denaturation was calculated as follows:

\[
\text{Percentage Inhibition} = \left( \frac{\text{Absorbance of Control}}{\text{Absorbance of Sample}} - 1 \right) \times 100
\]

**Membrane Stabilization Test**

**Preparation of Red Blood Cells (RBCS) Suspension**

Roughly about 10 ml of whole human blood was collected and transferred to the heparinized centrifuged tubes. The tubes were then subjected to centrifugation at 3000 rpm for 10 min and were washed three times with equal volume of normal saline. The volume of the RBC was measured and reconstituted as 10% v/v suspension with normal saline.

**Heat Induced Hemolysis HRBC Membrane Stabilization Assay**

The sample consisted of 1 ml of various extracts of Costus pictus and 1 ml of RBC suspension was prepared. The control tube consisted of saline and 1 ml of RBC suspension. Diclofenac sodium/Aspirin was taken as a standard drug. The entire set of centrifuge tubes containing reaction mixture was incubated at 56°C for 30 min under aluminum foil wrapping. This was then cooled under running tap water. The reaction mixture was subjected to centrifugation at 2500 rpm for 5 min and the absorbance of the supernatants was measured at 560 nm. The experiment was performed in triplicates.

\[
\% \text{ Membrane Stabilization Activity} = \left( \frac{\text{Absorbance of Test}}{\text{Absorbance of Control}} \right) \times 100
\]

\[
\% \text{ Hemolysis} = \left( \frac{\text{Absorbance of Test}}{\text{Absorbance of Control}} \right) \times 100
\]

**Proteinase Inhibitory Action**

The reaction mixture consisted of 0.06 mg trypsin, 1 ml 20 mM Tris HCl buffer at a pH 7.4 and 1 ml various extracts of Costus pictus at different concentrations. The mixture was incubated at 37°C for 5 min and then 1 ml of 0.8% (w/v) casein was added. This reaction mixture was again incubated at room temperature for about 20 minutes. Finally, termination was carried out by adding 2 ml of 70% perchloric acid. This mixture was vortexed and the absorbance of the supernatant was measured at 210 nm. The experiment was performed in triplicate. The percentage inhibition of proteinase inhibitory activity was calculated using the formula below:

\[
\text{Percentage Inhibition} = \left( \frac{\text{Absorbance of Control}}{\text{Absorbance of Test}} - 1 \right) \times 100
\]

**RESULTS AND DISCUSSION**

**Extraction of Plant Material**

The extracts yielded by sequential extraction have shown considerable differences (Table 1). In the extraction, maximum yield was obtained with methanol solvent and it was followed by ethyl acetate and hexane (least polar solvent).

DCM did not show any considerable yield comparatively.

**Table 1: Yield % of Various Extracts of C. pictus**

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Yield (gram)</th>
<th>Yield %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexane</td>
<td>1.5 ± 1.455*</td>
<td>1.5</td>
</tr>
<tr>
<td>DCM</td>
<td>0.5 ± 0.975*</td>
<td>0.5</td>
</tr>
<tr>
<td>Ethyl Acetate</td>
<td>7.8 ± 0.955*</td>
<td>7.8</td>
</tr>
<tr>
<td>Methanol</td>
<td>14.5 ± 2.237*</td>
<td>14.5</td>
</tr>
</tbody>
</table>

*Each value represents average of three analysis ± of standard deviation.
Phytochemical Screening

The preliminary phytochemical tests indicated the presence of phytosterols, carbohydrates, alkaloids, phenols, saponins, tannins and flavonoids in various extracts of *C. pictus*. All extracts showed variation in concentration levels. Among seven phytochemical tests conducted, five tests gave positive results in respect of all extracts. The tannins were lacking in all the extracts obtained.

The majority of the bioactive compounds were present in methanolic extracts in a higher concentration and comparatively lower in that of ethyl acetate. Flavonoids were present only in extractions with high polar solvents like ethyl acetate and methanol. DCM contains only trace amounts of any majority of bioactive constituents (Table 2).

### Table 2: Presence (+), high concentration (++), Absence (-) and trace amount (*) of phytochemicals.

<table>
<thead>
<tr>
<th></th>
<th>Hexane</th>
<th>DCM</th>
<th>Ethyl Acetate</th>
<th>Methanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td></td>
<td></td>
<td></td>
<td>++</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td></td>
<td></td>
<td></td>
<td>++</td>
</tr>
<tr>
<td>Saponins</td>
<td></td>
<td></td>
<td></td>
<td>++</td>
</tr>
<tr>
<td>Phytosterols</td>
<td></td>
<td></td>
<td></td>
<td>++</td>
</tr>
<tr>
<td>Phenols</td>
<td></td>
<td></td>
<td></td>
<td>++</td>
</tr>
<tr>
<td>Tannins</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flavonoids</td>
<td>-</td>
<td></td>
<td></td>
<td>++</td>
</tr>
</tbody>
</table>

### Table 3: Quantification of Total phenolics and Flavonoids in various extracts of *C. pictus*, each value represents average of three analysis ± of standard deviation.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Extracts</th>
<th>Total Phenolic</th>
<th>Total Flavonoid</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Hexane</td>
<td>18.5 ± 0.951</td>
<td>1.4 ± 0.865</td>
</tr>
<tr>
<td>2</td>
<td>DCM</td>
<td>8.26 ± 1.54</td>
<td>0.86 ± 0.954</td>
</tr>
<tr>
<td>3</td>
<td>Ethyl Acetate</td>
<td>57 ± 1.745</td>
<td>30 ± 0.875</td>
</tr>
<tr>
<td>4</td>
<td>Methanol</td>
<td>66.4 ± 1.65</td>
<td>49.4 ± 1.325</td>
</tr>
</tbody>
</table>

### Table 4: % Inhibition of extracts in albumin denaturation, each value represents average of three analysis ± of standard deviation

<table>
<thead>
<tr>
<th>Concentration in µg</th>
<th>Hexane</th>
<th>DCM</th>
<th>Ethyl Acetate (EA)</th>
<th>MET</th>
<th>Diclofenac</th>
</tr>
</thead>
<tbody>
<tr>
<td>31.25</td>
<td>16 ± 0.095</td>
<td>24 ± 1.22</td>
<td>64 ± 1.45</td>
<td>64 ± 1.33</td>
<td>75 ± 0.92</td>
</tr>
<tr>
<td>62.5</td>
<td>19 ± 0.56</td>
<td>27 ± 2.24</td>
<td>68 ± 0.46</td>
<td>64 ± 0.84</td>
<td>80 ± 0.56</td>
</tr>
<tr>
<td>125</td>
<td>23 ± 0.75</td>
<td>35 ± 1.15</td>
<td>74 ± 0.82</td>
<td>75 ± 0.75</td>
<td>84 ± 1.45</td>
</tr>
<tr>
<td>250</td>
<td>23 ± 2.23</td>
<td>38 ± 0.88</td>
<td>76 ± 0.56</td>
<td>76 ± 0.34</td>
<td>88 ± 1.67</td>
</tr>
<tr>
<td>500</td>
<td>42 ± 0.42</td>
<td>54 ± 0.77</td>
<td>85 ± 0.95</td>
<td>83 ± 1.78</td>
<td>88 ± 1.34</td>
</tr>
<tr>
<td>1000</td>
<td>48 ± 1.78</td>
<td>58 ± 0.99</td>
<td>86 ± 0.75</td>
<td>85 ± 1.46</td>
<td>87 ± 1.22</td>
</tr>
</tbody>
</table>

### Table 5: % HRBC Stabilization of Extracts, each value represents average of three analysis ± of standard deviation

<table>
<thead>
<tr>
<th>Concentration in µg</th>
<th>Hexane</th>
<th>DCM</th>
<th>Ethyl Acetate (EA)</th>
<th>MET</th>
<th>Diclofenac</th>
</tr>
</thead>
<tbody>
<tr>
<td>31.25</td>
<td>22 ± 1.04</td>
<td>32 ± 0.099</td>
<td>65 ± 0.55</td>
<td>62 ± 1.44</td>
<td>75 ± 2.33</td>
</tr>
<tr>
<td>62.5</td>
<td>36 ± 1.30</td>
<td>44 ± 1.34</td>
<td>67 ± 0.62</td>
<td>66 ± 0.97</td>
<td>86 ± 1.33</td>
</tr>
<tr>
<td>125</td>
<td>38 ± 1.32</td>
<td>47 ± 1.42</td>
<td>72 ± 0.88</td>
<td>73 ± 0.07</td>
<td>86 ± 0.92</td>
</tr>
<tr>
<td>250</td>
<td>41 ± 0.072</td>
<td>50 ± 2.34</td>
<td>75 ± 1.77</td>
<td>81 ± 0.07</td>
<td>88 ± 1.23</td>
</tr>
<tr>
<td>500</td>
<td>50 ± 0.091</td>
<td>56 ± 1.66</td>
<td>82 ± 1.34</td>
<td>88 ± 1.77</td>
<td>90 ± 1.45</td>
</tr>
<tr>
<td>1000</td>
<td>52 ± 0.095</td>
<td>58 ± 2.33</td>
<td>85 ± 1.45</td>
<td>89 ± 1.24</td>
<td>91 ± 0.05</td>
</tr>
</tbody>
</table>
Table 6: % Inhibition of extracts in proteinase inhibition, each value represents average of three analysis ± of standard deviation

<table>
<thead>
<tr>
<th>Concentration in µg</th>
<th>Hexane</th>
<th>DCM</th>
<th>EA</th>
<th>MET</th>
<th>Diclofenac</th>
</tr>
</thead>
<tbody>
<tr>
<td>31.25</td>
<td>24 ± 1.34</td>
<td>33 ± 0.95</td>
<td>65±</td>
<td>70 ± 0.75</td>
<td>85 ± 0.95</td>
</tr>
<tr>
<td>62.5</td>
<td>33 ± 1.76</td>
<td>38 ± 0.87</td>
<td>67±</td>
<td>73 ± 0.65</td>
<td>86 ± 1.87</td>
</tr>
<tr>
<td>125</td>
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<td>47 ± 1.45</td>
<td>72 ± 1.87</td>
<td>79 ± 0.97</td>
<td>86 ± 1.56</td>
</tr>
<tr>
<td>250</td>
<td>45 ± 1.87</td>
<td>52 ± 1.33</td>
<td>75±</td>
<td>80 ± 1.35</td>
<td>88 ± 1.45</td>
</tr>
<tr>
<td>500</td>
<td>46 ± 1.67</td>
<td>58 ± 1.67</td>
<td>82±</td>
<td>85 ± 1.87</td>
<td>95 ± 0.92</td>
</tr>
<tr>
<td>1000</td>
<td>46 ± 1.77</td>
<td>62 ± 0.87</td>
<td>85±</td>
<td>93 ± 0.93</td>
<td>97 ± 0.075</td>
</tr>
</tbody>
</table>

Quantification of Total Phenolics and Flavonoids

The total phenolic contents in the various extracts examined ranged from 18.5 to 66.4 mg GA/g. The highest concentration of phenols was measured in methanolic and ethyl acetate extracts. Hexane and DCM extracts contain considerably smaller concentration of phenols. The total phenolic contents in plant extracts of the species C.pictus depends on the type of solvent being employed for the extraction process i.e. the polarity of solvent used in extraction. High solubility of phenols in polar solvents provides high concentration of these compounds in the extracts obtained using polar solvents for the extraction (Table 3). The concentration of flavonoids in various plant extracts of the species C.pictus was determined using spectrophotometric method with aluminium chloride. The content of flavonoids was expressed in terms of Quercetin equivalent. The concentration of flavonoids in plant extracts from C.pictus ranged from 1.4 to 49.4 mg/g. Methanolic, and ethyl acetate extracts contain the highest flavonoid concentration. The concentration of flavonoids in methanol extract was 49.4 mg Q/g and that of ethyl acetate extract was 30 Q/g.

The lowest flavonoid concentration was measured in Hexane and DCM extracts. The concentration of flavonoids in various plant extracts depends on type of solvent being employed for the extraction process i.e. the polarity solvent used in extraction. (Table 3)

Albumin Denaturation Assay

Structural and functional damage to proteins could either alter or denature the proteins. The process of denaturation is envisaged in inflammation and its related disorders and needs to be closely monitored. Diclofenac sodium or aspirin is a widely accepted anti-inflammatory drug which have shown dose dependent efficacy to stabilize thermally induced protein denaturation. As a part of the investigation on the mechanism of the anti-inflammatory activity, ability of various extracts of Costus pictus to inhibit protein denaturation was studied. Methanolic extract was the most effective in inhibiting heat induced albumin denaturation at different concentrations as shown in Table 4. Maximum inhibition was observed in methanolic extract, 85±1.46 at 1000µg/ml. Aspirin, a standard anti-inflammatory drug showed the maximum inhibition, 87±1.22 at the concentration of 1000µg/ml.

![Figure 1: Graphical Representation of % Inhibition of Extracts in Albumin Denaturation.](image)

Membrane Stabilization Test

Phagocytic effect of neutrophil is due to neutrophil lysosomal constituents include suicidal enzymes for bacteria such as proteases, which upon extracellular release into the environment can cause further tissue inflammation and damage. RBCs membranes are documented to be similar in chemical constituent with that of neutrophils, therefore stabilization of the RBC membrane was studied to further establish the mechanism of anti-inflammatory action of various extracts of Costus pictus. Methanolic extract was the most effective in inhibiting the heat induced haemolysis at different concentrations. The membrane stabilization thus compromised to be an effective mechanism of their anti-inflammatory effect. This extract may possibly inhibit or keep the membrane intact in order to prevent the release of lysosomal content of neutrophils at the site of inflammation. Test extract (31.25 to 1000 µg /ml) showed a possible membrane stabilization potency of RBCs to varying degree as shown in Table 5. Methanolic extract showed the maximum stabilization, 89±1.24 at 1000µg/ml. Aspirin, standard drug showed the maximum inhibition, 91±0.05 at 1000 µg/ml. Thus, it is possible to...
say that the *C. pictus* produced this effect which could be brought about by difference in hypotonocity which lead to the shrinkage of cell membrane12.

![Graphical Representation of % HRBC Stabilization of Extracts.](image)

**Figure 2:** Graphical Representation of % HRBC Stabilization of Extracts.

**Proteinase Inhibitory Activity**

Tissue damage during injury in any pathophysiological condition like diabetes could lead to necrosis and finally amputation.

This could be due to prolonged inflammation which is mainly done by proteinases which are responsible for the degradation of the extracellular matrix22.

Proteinase inhibitors may depict a counter part of the effective plant extract which can combat the proteolytic enzymes that could develop tissue damage during inflammatory reactions. Neutrophils are known to be a rich source of proteinase which carries the serine proteases in their lysosomal granules.

Various extracts of *C. pictus* exhibited significant antiproteinase activity at different concentrations as shown in (Table 6). Methanolic extract showed maximum inhibition 93±0.93 at 1000μg/ml. Aspirin showed the maximum inhibition 97±0.075 at 1000μg/ml.

![Graphical Representation of % Proteinase Inhibition of Extracts.](image)

**Figure 3:** Graphical Representation of % Proteinase Inhibition of Extracts.

**CONCLUSION**

In conclusion, present study revealed the *in-vitro* anti-inflammatory activity of various extracts of *Costus pictus* Linn.

The presence of secondary metabolites like flavonoids and related polyphenols may be responsible for the activity.

Further investigations are required to find active component of the active extract and to confirm the mechanism of action.

**REFERENCES**


12. Membrane stabilization as a mechanism of the anti-inflammatory activity of methanol extract of garden egg (Solanum aethipicum), Chioma A Anosike, Onyechi


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