

Research Article



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 albumin denaturation, Red Blood Cells membrane stabilization and proteinase inhibitory effects with 85 ± 1.46 , 89 ± 1.24 and $93 \pm 0.93\%$ 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 quercetin 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, quercetin.

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 debris at the site of inflammation and promotes the renewal or regeneration of normal tissue. Medicinal plants provides immense benefits with new remedies⁹. 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 activity^{26,27}.

Metabolite products of plants include terpenoids, phenolic compounds such as flavonoids, phenolic acids and tannins which could be the active lead responsible for biological activities¹⁰.

Antioxidants play an important role in neutralizing free radical species which leads to oxidative stress in the system^{15,17}.

This finally leads to damage to macromolecules including DNA, lipids, and proteins and results in inflammation^{4,5}.

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 effects^{7,8}.

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 diabetes^{11,24}. The plant have also shown bactericidal activity against strains like *Escherchia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*^{3,16}. 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 weighed^{1,14}.

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

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.

Dragendroff's Test

Individual filtrates were treated with Dragendroff'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 method²⁵. 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 method¹⁹. 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^{2,20}. 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 saline²¹.

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 aluminium 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 nm²³. 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:

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

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*

| Extracts | Yield (gram) | Yield % |
|---------------|---------------|---------|
| Hexane | 1.5 ± 1.455* | 1.5 |
| DCM | 0.5 ± 0.975* | 0.5 |
| Ethyl Acetate | 7.8 ± 0.955* | 7.8 |
| Methanol | 14.5 ± 2.237* | 14.5 |

*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 methanol⁶. DCM contains only trace amounts of any majority of bioactive constituents (Table 2).

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

| | Hexane | DCM | Ethyl Acetate | Methanol |
|---------------|--------|-----|---------------|----------|
| Alkaloids | + | * | + | ++ |
| Carbohydrates | + | + | + | ++ |
| Saponins | + | + | + | ++ |
| Phytosterols | + | + | + | ++ |
| Phenols | + | * | + | ++ |
| Tannins | - | - | - | - |
| Flavonoids | - | - | + | ++ |

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

| S. No. | Extracts | Total Phenolic | Total Flavonoid |
|--------|---------------|------------------|------------------|
| 1 | Hexane | 18.5 \pm 0.951 | 1.4 \pm 0.865 |
| 2 | DCM | 8.26 \pm 1.54 | 0.86 \pm 0.954 |
| 3 | Ethyl Acetate | 57 \pm 1.745 | 30 \pm 0.875 |
| 4 | Methanol | 66.4 \pm 1.65 | 49.4 \pm 1.325 |

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

| Albumin Denaturation Assay | | | | | |
|----------------------------|----------------|---------------|---------------|---------------|---------------|
| Concentration in μ g | % Inhibition | | | | |
| | Hexane | DCM | EA | MET | Diclofenac |
| 31.25 | 16 \pm 0.095 | 24 \pm 1.22 | 64 \pm 1.45 | 64 \pm 1.33 | 75 \pm 0.92 |
| 62.5 | 19 \pm 0.56 | 27 \pm 2.24 | 68 \pm 0.46 | 64 \pm 0.84 | 80 \pm 0.56 |
| 125 | 23 \pm 0.75 | 35 \pm 1.15 | 74 \pm 0.82 | 75 \pm 0.75 | 84 \pm 1.45 |
| 250 | 23 \pm 2.23 | 38 \pm 0.88 | 76 \pm 0.56 | 76 \pm 0.34 | 88 \pm 1.67 |
| 500 | 42 \pm 0.42 | 54 \pm 0.77 | 85 \pm 0.95 | 83 \pm 1.78 | 88 \pm 1.34 |
| 1000 | 48 \pm 1.78 | 58 \pm 0.99 | 86 \pm 0.75 | 85 \pm 1.46 | 87 \pm 1.22 |

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

| HRBC Stabilization Assay | | | | | |
|--------------------------|-----------------|----------------|---------------|---------------|---------------|
| Concentration in μ g | % Stabilization | | | | |
| | Hexane | DCM | EA | MET | Diclofenac |
| 31.25 | 22 \pm 1.04 | 32 \pm 0.099 | 65 \pm 0.55 | 62 \pm 1.44 | 75 \pm 2.33 |
| 62.5 | 36 \pm 1.30 | 44 \pm 1.34 | 67 \pm 0.62 | 66 \pm 0.97 | 86 \pm 1.33 |
| 125 | 38 \pm 1.32 | 47 \pm 1.42 | 72 \pm 0.88 | 73 \pm 0.07 | 86 \pm 0.92 |
| 250 | 41 \pm 0.072 | 50 \pm 2.34 | 75 \pm 1.77 | 81 \pm 0.07 | 88 \pm 1.23 |
| 500 | 50 \pm 0.091 | 56 \pm 1.66 | 82 \pm 1.34 | 88 \pm 1.77 | 90 \pm 1.45 |
| 1000 | 52 \pm 0.095 | 58 \pm 2.33 | 85 \pm 1.45 | 89 \pm 1.24 | 91 \pm 0.05 |

Table 6: % Inhibition of extracts in proteinase inhibition, each value represents average of three analysis \pm of standard deviation

| Proteinase Inhibition Assay | | | | | |
|--------------------------------|---------------|---------------|---------------|---------------|----------------|
| Concentration in μg | % Inhibition | | | | |
| | Hexane | DCM | EA | MET | Diclofenac |
| 31.25 | 24 \pm 1.34 | 33 \pm 0.95 | 65 \pm | 70 \pm 0.75 | 85 \pm 0.95 |
| 62.5 | 33 \pm 1.76 | 38 \pm 0.87 | 67 \pm | 73 \pm 0.65 | 86 \pm 1.87 |
| 125 | 36 \pm 2.44 | 47 \pm 1.45 | 72 \pm 1.87 | 79 \pm 0.97 | 86 \pm 1.56 |
| 250 | 45 \pm 1.87 | 52 \pm 1.33 | 75 \pm | 80 \pm 1.35 | 88 \pm 1.45 |
| 500 | 46 \pm 1.67 | 58 \pm 1.67 | 82 \pm | 85 \pm 1.87 | 95 \pm 0.92 |
| 1000 | 46 \pm 1.77 | 62 \pm 0.87 | 85 \pm | 93 \pm 0.93 | 97 \pm 0.075 |

Quantification of Total Phenolics and Flavonoids

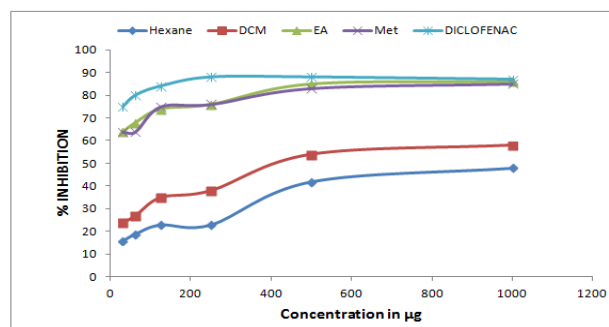
The total phenolic contents in the various extracts examined ranged from 18.5 to 66.4 mg GA/g. The highest concentration of phenols was measured in methanolic and ethyl acetate extracts. Hexane and DCM extracts contains considerably smaller concentration of phenols. The total phenolic contents in plant 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 contains 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 proteins². The process of denaturation is envisaged in inflammation and its related disorders and needs to be closely monitored¹³. 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 \pm 1.46 at 1000 $\mu\text{g}/\text{ml}$. Aspirin, a standard anti-inflammatory drug showed the maximum inhibition, 87 \pm 1.22 at the concentration of 1000 $\mu\text{g}/\text{ml}$.

**Figure 1:** Graphical Representation of % Inhibition of Extracts in Albumin Denaturation.

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 damage²³. 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 $\mu\text{g}/\text{ml}$) showed a possible membrane stabilization potency of RBCs to varying degree as shown in Table 5. Methanolic extract showed the maximum stabilization, 89 \pm 1.24 at 1000 $\mu\text{g}/\text{ml}$. Aspirin, standard drug showed the maximum inhibition, 91 \pm 0.05 at 1000 $\mu\text{g}/\text{ml}$. Thus, it is possible to

say that the *C.pictus* produced this effect which could be brought about by difference in hypotonocytivity which lead to the shrinkage of cell membrane¹².

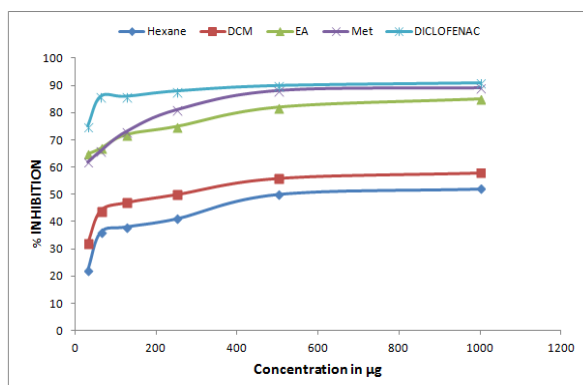


Figure 2: Graphical Representation of % HRBC Stabilization of Extracts.

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 $1000 \mu\text{g/ml}$. Aspirin showed the maximum inhibition 97 ± 0.075 at $1000 \mu\text{g/ml}$.

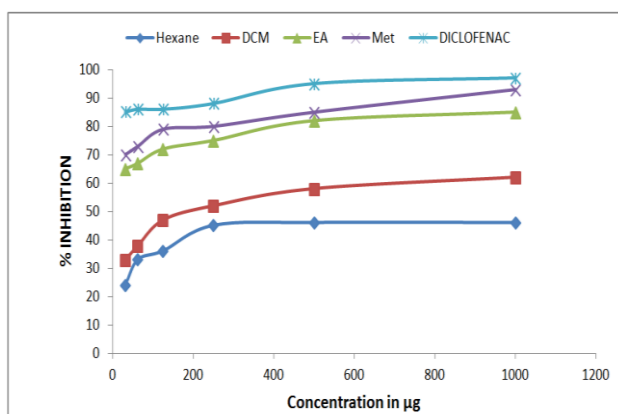


Figure 3: Graphical Representation of % Proteinase Inhibition of Extracts.

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

1. Anokwuru, C.P, Anyasor, G.N, Ajibaye O, Fakoya O and Okebugwu P. Effect of Extraction Solvents on Phenolic, Flavonoid and Antioxidant activities of Three Nigerian Medicinal Plants, Nature and Science, 2011, 9(7).
2. Anti-denaturation and anti-oxidant activity of *Annona cherimola in vitro*, International Journal of Pharma and Bio Sciences, IISSN: Vol: 2, 2, 2001, 0975-6299.
3. Das K, Tiwari RKS, Shrivastava DK. Techniques for evaluation of medicinal plant products as antimicrobial agent: Current methods and future trends. Journal of Medicinal Plants Research, 4(2), 2010, 104-111.
4. E Boaky-Gyasi, E Woode, GK Ainooson, DD Obiri, C Ansah, M Duwejua, A Donkoh. Anti-Inflammatory and antipyretic effects of an ethanolic extract of *Palisota hirsuta* K. Schum roots, Afr. J. Pharm. Pharmacol. 2, 2008, 191-199.
5. Gunjegaonkar Shivshankar M and T. S. Shanmugarajan. *In vitro* potential of plant stress hormone Methyl Jasmonate for anti arthritis, anti-inflammatory and free radical scavenging activity, 2015, International Journal of Pharm Tech Research, ISSN : 0974-4304, Vol.8, No.7, 2014, 161-167.
6. Handa SS, Khanuja SPS, Longo G, Rakesh DD. Extraction Technologies for Medicinal and Aromatic Plants, International centre for science and high technology, Trieste, 2008, 21-25.
7. Jasmine R, Rajasulochana Mand Rutabana Aude, Evaluation of *in vitro* antioxidant and anti-inflammatory property exhibited by silver nanoparticles stabilized by *Adathoda vasica*, Journal of Chemical and Pharmaceutical Research, 8(5), 2016, 128-137.
8. M Bichra, C El-Modafar, A El-Abbassi, H Bouamama, and F Benkhalti. *In Vitro* Assessment of Cytotoxicity, Antioxidant, and Anti-Inflammatory Activities of *Ricinus communis* (Euphorbiaceae) Leaf Extracts, Journal of Microbiology, Biotechnology and Food Sciences. 2(4), 2013, 2320-2338.
9. M Mueller, S Hobiger, A Jungbauer. Antioxidant, α -glucosidase inhibitory and anti-inflammatory effects of aerial parts extract from Korean crowsberry (*Empetrum nigrum* var. *japonicum*), Food Chem. 122, 2010, 987-996.
10. M Yonathan, K Asres, A Assefa and F Bucar. *In vivo* anti-inflammatory and anti-nociceptive activities of *Cheilanthes farinosa*, Journal of Ethnopharmacol, 108, 2006, 462-470.
11. MA Jayasri S Gunasekaran, A Radha and TL Mathew. Anti-diabetic effect of *Costus pictus* leaves in normal and streptozotocin-induced diabetic rats, International Journal of Diabetes & Metabolism, Vol: 16, 2008, 117-122.
12. Membrane stabilization as a mechanism of the anti-inflammatory activity of methanol extract of garden egg (*Solanum aethiopicum*), Chioma A Anosike, Onyechi

- Obidoa, and Lawrence US Ezeanyika, Daru journal of pharmaceutical sciences. 20(1), 2012, 76.
13. Mizushima Y and Kobayashi, Interaction of anti-inflammatory drugs with serum preteins, especially with some biologically active proteins, Journal of Pharmaceutical and Pharmacology, 20, 1968, 169-173.
 14. Mohamed El Hasan Shayoub, Azza Dawoud and Hussien Dawoud. Phytochemical analysis of leaves extract of Eucalyptus camaldulensis Dehnh, Omdurman Journal of Pharmaceutical Science, ISSN: 1858-506X, Volume 2(1), 2015, 64-72.
 15. N Huda-Faujan, A Noriham, AS Norrakiah, and AS Babji. Antioxidant activity of plants methanolic extracts containing phenolic compounds, African Journal of Biotechnology, Vol. 8, No. 3, 2009, 484–489.
 16. Ncube NS, Afolayan AJ and Okoh AI. Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends, African Journal of Biotechnology, 7(12), 2008, 1797-1806.
 17. P Masoko and J N Eloff. Screening of Twenty-Four South African Combretum and Six Terminalia Species (Combretaceae) for Antioxidant Activities, African Journal of Traditional, Complementary and Alternative Medicines, 4(2), 2007, 231–239.
 18. Prashant Tiwari, Bimlesh Kumar, Mandeep Kaur, Gurpreet Kaur and Harleen Kaur. Phytochemical screening and Extraction: A Review, Internatinal pharmaceutica sciencia, Vol 1, Issue 1, 2011.
 19. Quettier, D.C Gressier, Vauueur, J. Dine, T. Brunnet, Luyckx M.C, cayin J.C, Bailleul F and Trotin F. Phenolic compounds and antioxidant activities of buckwheat (*Fagopyrum esculentum* Moench) hulls and flour, Journal of Ethnopharmacol, 72, 2000, 35-42.
 20. S Sakat, A R Juvekar, M N Gambhir. *In vitro* antioxidant and anti-inflammatory activity of methanol extract of oxalis cornicula linn, International Journal of Pharma and Pharmacological Sciences. 2(1), 2010, 146-155.
 21. Sadique J, Al-Rqobahs WA, Bughaith and El-Gindi AR. The bioactivity of certain medicinal plants on the stabilization of RBC membrane system, Fitoterapia, 60, 1989, 525-532.
 22. Sewon Kang, Soyun Cho, Jin Ho Chung, Craig Hammerberg, Gary J. Fisher and John J. Voorhees. Inflammation and Extracellular Matrix Degradation Mediated by Activated Transcription Factors Nuclear Factor- κ B and Activator Protein-1 in Inflammatory Acne Lesions *In Vivo*, American journal of Pathology, 166(6), 2005, 1691–1699.
 23. Shinde UA, Phadke AS, Nari AM, Mungantiwar AA, Dikshit VJ and Saraf MN. Membrane stabilization activity-a possible mechanism of action for the anti-inflammatory activity of Cedrus deodara wood oil, Fitoterapia, 70, 1999, 251-257.
 24. Shiny C.T, Anuj Saxena and Sharad Prakash Gupta. Phytochemical and hypoglycaemic activity investigation of *Costus pictus* plants from Kerala and Tamilnadu, International Journal of Pharmaceutical Science Invention, ISSN : 2319 – 6718, Volume 2 Issue 5, 2013, 11-18.
 25. Singleton V.I, Orthofer R, Lamuela-Raventos R.M. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent, Methods in Enzymology, 299, 1999, 152-178.
 26. Suresh Madduluri, B.Sitaram, Chandra balasekharan. In vitro Evaluation of Anti -Inflammatory activity of Methanolic and Ethanolic leaf extracts of five Indigenous Plants in South India, International Journal of PharmTech Research, ISSN : 0974-4304, Vol.6, No.2, 2014, 569-574.
 27. VR Patel, PP Patel and SS Kujal. Antioxidant Activity of Some Selected Medicinal Plants in Western Region of India, Advances in Biological Research, Vol. 4, No. 1, 2010, 23-26.

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