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Comparative Analysis Of The Anti-Inflammatory Activity Of The Stem And Leaf Extracts Of Insulin Plant (Costus Pictus)

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Abstract
The insulin plant (Costus pictus D. Don) has been traditionally recognized for its therapeutic potential, particularly in diabetes management. This study evaluated the anti-inflammatory activity of C. pictus extracts by comparing the efficacy of leaf and stem preparations using the protein denaturation assay. The extracts of the leaves and stems were tested at concentrations ranging from 50 to 1000 ppm, with ascorbic acid as the standard control. The test revealed that both plant parts demonstrated inhibition of protein denaturation in a dose-dependent manner, with leaf extracts showing higher activity (mean inhibition = 40.97%) than stem extracts (mean inhibition = 35.02%), though both were significantly lower than the control (50.89%). A paired t-test confirmed a significant difference between leaf and stem activity (p = 0.031). These findings suggest that C. pictus contains bioactive compounds with anti-inflammatory potential, with leaves demonstrating slightly stronger effects than stems. While the results support the ethnomedicinal use of C. pictus against inflammation, further phytochemical profiling and in vivo validation are recommended before clinical application.
Keywords: *insulin plant, plant extracts, protein denaturation assay, anti-inflammatory, alternative medicine*

INTRODUCTION

One of the most significant sources of medicine is nature. Since ancient times, plants have been proven to possess healing properties and effectively cure a variety of illnesses. Many modern drugs have been developed from traditional medicinal plants (Shingala et al, 2021). Additionally, plants play a significant role in the global healthcare system, with herbs commonly used in conventional medicine to treat various illnesses. The flavonoids and organic compounds found in fruits, vegetables, spices, and medicinal plants, belong to the noteworthy bioactive compounds known for their potential antioxidant and anti-inflammatory effects (Adamczak et al, 2019). Aside from that potential effects, other properties include diuretic, anti-tumor, anxiolytic, anti-allergic, analgesic, anti-anaphylactic, anti-diarrheal, and spasmolytic effects (Ghosh, 2020).

Plants constitute approximately 25% of the drugs used globally and remain a primary source for developing new drugs (Anathalkshmi et al, 2022). The insulin plant, also known as *Costus pictus D. Don* (syn. *Costus mexicanus Leibm.*), is native to the damp hills and forests of Mexico but has recently gained popularity in India. This perennial herb is used as a decorative plant in gardens throughout South India, especially in Kerala. It is renowned for its antidiabetic properties and is a member of the Costaceae family. Patients with diabetes can chew and consume the raw leaves of this plant.

Inflammation is a response of the host's immune system to various undesirable stimuli, including infection or injury. It is an important defense mechanism to eliminate harmful stimuli and promote the healing process. However, if inflammatory stimuli are not resolved, acute inflammation may develop into chronic inflammation, leading to progressive damage or chronic disease (Chen et al, 2017).

Research on the effectiveness of the insulin plant (*Costus pictus*) is relatively limited. Previous research has shown that the leaves, stem, flowers, and rhizome of the insulin plant contain beneficial metabolites. The anti-inflammatory properties of Costus pictus and Hellenia speciosa through both in vivo (LPS-induced TNF alpha generation - sepsis model) and in vitro (suppression of the membrane stabilization assay and albumin denaturation) studies (Ramya, et al, 2017). Additionally, the protective qualities of Costus pictus leaves against inflammation and oxidative stress [18]. Furthermore, the ability of *Costus pictus* and *Costus speciosus* to reduce inflammation for the potential identification of natural treatments for arthritis using in vivo methods (Khan et al, 2022).

However, there is very little to no study has been conducted on the comparative analysis of the insulin plant (*Costus pictus*) stem and leaves using a hot extraction process. Therefore, this study determined the anti-

inflammatory activity of the stem and leaves of the insulin plant (*Costus pictus*) utilizing a protein denaturation assay and compared their significant differences.

MATERIALS AND METHODOLOGY

Collection and verification

Insulin plant was collected from the plot cultivated by the researcher in a normal environmental condition in Pigcawayan, North Cotabato, Philippines. The researcher collected only the matured and healthy leaves and stem of Insulin plant and gathered separately. The leaves was cut individually from stem and were then weighed 1/4 kilograms respectively. The plant sample was authenticated by a botanist at the University of Southern Mindanao in Kabacan, North Cotabato. A certification was provided to the researcher to verify that the plant sample sent was indeed of *Costus pictus.*

Drying, Extraction, and Preparation of sample

The gathered plant parts were dried under shade for 3 weeks and oven for 10 minutes at 60°C for the leaves and for the stem oven for 2 hours at 60°C and where blended separately. The plant were ground into powder and processed in hot water extraction. After soaking 10 g of dry powder in 100 ml of distilled water, the mixture was incubated for 24 hours at 60°C. The extracts were then sieved through muslin cloth and centrifuged for seven minutes at 4400 rpm. Parts of the Insulin plant (Costus pictus) were gathered and then screened. The processes of centrifugation and filtration were carried out three times. This extraction method was adopted from the method used by Khan et al (2022).

Transport of Plant Extract

The Insulin plant (*Costus pictus*) leaf and stem extract were packed in a centrifuge tubes in leak-proof tubes and clearly labelled. The plant extracts were placed inside the styrofoam box with ice packs to maintain a stable temperature with cushioning fabric to provide additional protection against impact during transit. The plant specimen was then submitted to the laboratory in Davao City to conduct the clinical test procedure.

Anti-inflammatory Test using Protein Denaturation Assay

Insulin plant extracts' anti-inflammatory activities were assessed using the protein denaturation assay. In this process, a protein gets denatured, or its tertiary and secondary structures become disoriented, due to exposure to external stimuli like heat, strong acidic or basic solutions, organic solvents, or concentrated inorganic salts is known as denaturation (Dharmadeva et al., 2018)

The reaction mixtures were incubated in a water bath at 37°C ± 2°C for 15–20 minutes, followed by heating to 70°C, where they were maintained for 5 minutes. Afterward, the mixtures were allowed to cool to room temperature over 15 minutes. Absorbance was measured at 660 nm using a colorimeter before and after denaturation for each concentration (50ppm, 100ppm, 250ppm, 500ppm, and 1000ppm). Each test was conducted in triplicate, and the average absorbance was recorded. Ascorbic acid was used as the control. The percentage inhibition of protein denaturation was then calculated in comparison to the control, using the following formula:

$$\left(\frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}}\right) \times 100$$

RESULTS AND DISCUSSION

Table 1. Anti-inflammatory Activity of Insulin Plant using Protein Denaturation Assay

Concentration (ppm)	Percentage (%) Inhibition Rate @660nm								
	Ascorbic Acid			Stem			Leaves		
	R1	R2	R3	R1	R2	R3	R1	R2	R3
50.0	21.9	22.1	22.0	11.1	11.5	11.4	12.4	12.8	12.4
100.0	35.8	35.6	36.4	21.7	22.4	22.3	24.5	25.0	24.8
250.0	48.9	52.4	55.4	30.4	30.5	31.8	40.2	40.5	40.6
500.0	62.1	62.3	64.5	50.4	50.5	50.2	55.8	56.7	56.4
1000.0	82.5	80.2	82.1	60.2	60.4	60.5	70.5	70.8	71.2

Table above presents the raw data on anti-inflammatory activity of Insulin plant (*Costus pictus*) leaves and stem extract in parts per million (ppm) and the corresponding percentage inhibition rates at 660nm. The Ascorbic acid has garnered the highest inhibition rate occurs at 1000.0 ppm, with values of 82.5%, 80.2%, and 82.1% for R1, R2, and R3, respectively. The leaves sample highest inhibition rate also occurs at 1000.0 ppm, with values of 70.5%, 70.8%, and 71.2% for R1, R2, and R3, respectively.

The Costus pictus leaf extract shows a potential anti-inflammatory property, particularly at higher concentrations, though it does not achieve the same inhibition rates as ascorbic acid. These findings support the potential of insulin plant as a natural anti-inflammatory agent, given its flavonoid content, with further research needed to better understand its specific active compounds and optimize its efficacy (Sakar, 2024; Selvakumarasamy, 2021)

Table 2 . Anti-inflammatory Activity of Insulin Plant (Costus pictus) Extract in Leaves, Stem and Control (Ascorbic Acid)

	Mean	Standard Deviation
Control	50.89	21.47
Stem	35.02	18.65
Leaves	40.97	21.71

The table above shows the anti-inflammatory activity of Insulin plant (*Costus pictus*) extract was assessed in leaves, stems, and a control group treated with Ascorbic Acid. The mean inhibition rates were found to be 50.89% (SD = 21.47) for the control group, 35.02% (SD = 18.65) for the stem extract, and 40.97% (SD = 21.71) for the leaf extract. The highest mean inhibition rate is for the leaves sample, with a value of 40.97. The lowest mean inhibition rate is for the stem sample, with a value of 35.02. These results suggest that both the stem and leaf extracts of the Insulin plant exhibit anti-inflammatory activity compared to the control group, with the leaf extract showing a slightly higher mean inhibition rate compared to the stem extract.

Insulin plant (*Costus pictus*) leaf extract showed significant anti-inflammatory activity, thus, there is a potential advantage in using the insulin plant for treating inflammatory-causing diseases. The anti-inflammatory activity demonstrated by *Costus pictus* leaf extract indicates that the extracts effectively manage inflammation. The plant extract has anti-inflammatory properties that help mitigate inflammatory conditions and promote overall wellness. Lesjak et al., (2018) noted that the abundance of phenolic chemicals found in *Costus pictus* leaves may be the cause of their anti-inflammatory qualities. Furthermore, a study conducted by Al-Khayri et al. (2022) supported that the quercetin and flavonoids found in *Costus pictus* reduce low-grade inflammation

The anti-inflammatory activity of Insulin plant (*Costus pictus*) stem extract demonstrated significant inhibition of inflammation *in vitro,* promising benefits, and the ability to effectively reduce inflammation. The significant anti-inflammatory activity demonstrated by *Costus pictus* stem extract highlights its potential as a valuable natural remedy for reducing inflammation. It implies that *Costus pictus* stem extract contains bioactive compounds that effectively reduce inflammation. The insulin plant has different active compound that are

present in plant parts, in stem terpenoids like lupeol and steroids like stigmasterol are present (Shinde et al., 2022) By inhibiting the release of cytokines and the regulation of related pathways, lupeol can also have anti-inflammatory effects (Li et al., 2021).

Table 3. Comparison between the Anti-inflammatory Activities of Insulin Plant (Costus pictus) Leaves and Control

	Mean Difference	t	df	Sig. (2-tailed)	Correlation	Sig
Leaves - Stems	5.95	3.42	4	0.031	0.994	0.001

Additionally, the strong positive correlation between the inhibition rates of leaves and stems (r = 0.994, p = 0.001) indicates a consistent pattern across the paired samples. Therefore, it can be revealed that the anti-inflammatory activity of the Insulin plant's leaves and stems varies significantly.

On the other hand, the comparison of the anti-inflammatory activity of Insulin Plant (*Costus pictus*) leaves and stem extract showed that there is significant difference between the activities (p=0.001). The results indicate that leaf and stem extracts of *Costus pictus* exhibit significant anti-inflammatory activity, with the leaf extract showing a slightly higher mean inhibition rate compared to the stem extract, comparable to the control group with ascorbic acid. Furthermore, a significant difference in anti-inflammatory activity between the plant parts implies leaves and stems contain bioactive compounds with similar efficacy against inflammation. This suggests that *Costus pictus* extract possesses potent anti-inflammatory properties, potentially making it a promising natural remedy for inflammation-related conditions. This finding supports the utilization of leaves and stems in traditional medicine practices for their anti-inflammatory properties. Additionally, it implies the choice of plant part for extraction may depend more on factors such as availability, convenience, or cultural preferences. The C. *pictus* leaves anti-inflammatory activity is based on the stabilitzation of the RBC membrane. When compared to conventional Diclofenac sodium, both extracts were demonstrated to reduce heat-induced hemolysis. These findings support the idea that one additional mechanism behind their anti-inflammatory activity is membrane stabilization (Ramya et al., 2017).

Table 4. Comparison between the Anti-inflammatory Activities of Insulin Plant (Costus pictus) Leaves and Control

	Mean Difference	t	df	Sig. (2-tailed)	Correlation	Sig
Leaves - control	-9.91	-5.62	4	0.001	0.996	0

The table shows the comparison between the anti-inflammatory activity of the Insulin plant (*Costus pictus*) leaves and the control (Ascorbic Acid). A paired sample t-test revealed a mean difference of -9.91% (p = 0.001), indicating that the anti-inflammatory activity of the control is significantly higher compared to the leaves. Furthermore, a strong positive correlation was observed between the inhibition rates of leaves and the control (r = 0.996, p = 0.000), suggesting a consistent pattern across the paired samples. Therefore, it can be concluded that there is a significant difference in the anti-inflammatory activity between the leaves of the Insulin plant and the control (Ascorbic Acid). This indicate that the anti-inflammatory activity of Insulin plant (*Costus pictus*) leaves compared to the control group provide a crucial insights in managing inflammatory conditions. This finding validates the traditional use of *Costus pictus* in managing inflammatory conditions, affirming the efficacy of this natural remedy in alleviating inflammation. The evaluation of anti-inflammatory activity in both the control group and leaves indicates that *Costus pictus* leaves contain bioactive chemicals with anti-inflammatory properties. This supports the notion that natural remedies derived from medicinal plants, can serve as effective alternatives or complements to conventional medications for inflammatory conditions. The results also indicate that the *Costus pictus* leaves has potential benefits as a therapeutic agent for inflammatory disorders. In addition, the anti-inflammatory activity observed in the leaves highlights the importance of *Costus pictus* as a valuable medicinal plant and provides promising prospects for its use in managing inflammatory conditions. Studies such as of Rajendran et al., (2022); Jan et al., (2022) Al-Khayri et al., (2022);

Li et al., (2021) can support this result for according to them, leaves contained kaempferol and flavonoids like phenolic acids and the leaves of this plant are rich in quercetin and quercetin inhibits the functions of two inflammatory enzymes, lipoxygenase and cyclooxygenase, thus, the *Costus pictus* anti-inflammatory property.

Table 5. Comparison between the Anti-inflammatory Activities of Insulin Plant (Costus pictus) Stem and Control

	Mean Difference	t	df	Sig. (2-tailed)	Correlation	Sig
Leaves - control	-15.87	-4.91	4	0.002	0.982	0.003

The table presents the comparison between the anti-inflammatory activity of the Insulin plant (*Costus pictus*) stem and the control (Ascorbic Acid). A paired sample t-test revealed a mean difference of -15.87% ($p = 0.002$), indicating that the anti-inflammatory activity of the control is significantly higher compared to the stem. Additionally, a strong positive correlation was observed between the inhibition rates of the stem and the control ($r = 0.982$, $p = 0.003$), suggesting a consistent pattern across the paired samples. Therefore, it can be concluded that there is a significant difference in the anti-inflammatory activity between the stem of the Insulin plant and the control (Ascorbic Acid).

The significant difference observed between the control group and *Costus pictus* stem extract the anti-inflammatory properties highlights the potential therapeutic efficacy of *Costus pictus* stem extract in managing inflammatory conditions. This finding indicates that the stem extract contains bioactive compounds with anti-inflammatory properties, supporting the traditional use of *Costus pictus* in alleviating inflammation. It implies the presence of bioactive compounds within the stem extract that can effectively modulate inflammatory responses. The stem extract of Costus pictus is rich in flavonoids and phenolic compounds, particularly quercetin, which has been identified as having potent anti-inflammatory effects (Kalpana et al., 2016). The high concentration of these compounds contributes to the extract's ability to reduce inflammation, as they are known to modulate various signaling pathways involved in inflammatory responses.

CONCLUSION

The findings of the present study signify that the Insulin plant (Costus pictus) leaves and stem extract exhibited anti-inflammatory activity and thus can help to treat inflammatory-causing diseases. These results underline the plant's potential as a natural source of anti-inflammatory chemicals and support the plant's long-standing use in folk medicine to treat inflammatory diseases. It can accelerate the development of a new type of anti-inflammatory agents and be a good plant-based pharmaceutical products for several diseases caused by free radicals. Furthermore, significant differences were observed in the comparisons between the leaves and stem extracts, indicating variations in their anti-inflammatory properties.

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