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Original Research Article

In-vitro Anti-Inflammatory Potential of Leaf, Stem, and Fruit Extracts of *Ficus capensis*

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ABSRTACT

Chronic inflammation is a major contributor to various diseases, including arthritis, diabetes, and cancer. Natural products, particularly plant extracts, have been recognized as valuable sources of anti-inflammatory agents. *Ficus capensis*, a plant species widely used traditionally, has been reported to possess various medicinal properties. This study aimed to investigate the anti-inflammatory potential of *Ficus capensis* leaf, stem, and fruit extracts and to explore their potential as novel phytopharmaceuticals. The anti-inflammatory activity was evaluated *in-vitro* using inhibition of albumin denaturation, inhibition of proteinase activity, and membrane stabilization tests at 0.625-10.00 mg/ml. Diclophenac and aspirin served as reference drugs. Standard biochemical methods were used. From the results, the extracts of *Ficus capensis* (0.625-10.00 mg/ml) demonstrated concentration-dependent anti-inflammatory activity. The percentage inhibition of albumin denaturation ranged from 2.78-90.28%, with the leaf extract exhibiting the most potent activity (90.28% inhibition, IC_{50} = 3.80 ± 0.11 mg/ml). Similarly, the leaf extract showed the highest anti-proteinase activity (67.11% inhibition, IC_{50} = 4.19 ± 0.04 mg/ml). Inhibition of heat and hypotonicity-induced hemolysis ranged from 1.80-50.74% and 2.08-42.78%, respectively, with the leaf extract exhibiting the most potent activity in both assays (IC_{50} = 8.27 ± 0.10 and 11.09 ± 0.08 mg/ml, respectively). While the leaf extract demonstrated the most significant anti-inflammatory activity, the stem and fruit extracts also showed moderate activity. This study provides evidence for the anti-inflammatory properties of *Ficus capensis* extracts, supporting their potential as novel phytopharmaceuticals for the management of inflammatory disorders.

Keywords: *Ficus Capensis*, Anti-Inflammation, Proteinase, Albumin, Denaturation, Hemolysis.

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Introduction

Inflammation is a dynamic and intricate physiological response that enables the body to respond to and mitigate the effects of various injuries or stresses, including infections, physical harm, and chemical or biological threats.[1] Although inflammation is a normal physiological response, dysregulation of this process can lead to devastating consequences, exacerbating major diseases and precipitating severe pathological complications. The conventional treatment for inflammatory conditions relies heavily on non-steroidal anti-inflammatory drugs (NSAIDs), which, despite their effectiveness, are associated with significant adverse effects, including gastric irritation and ulceration.[2] While conventional medicines have shown promise in managing inflammatory conditions, the quest for more cost-effective, potent, and safe remedies with reduced gastrointestinal side effects remains an important area of research and development. Natural products play a pivotal part in the advancement of modern medicine, with numerous plant-derived compounds being discovered, developed, and utilized as therapeutic agents. Recent years have witnessed a renewed focus on natural product research.[3]

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Notably, the World Health Organization (WHO) reports that about 40% of pharmaceuticals in use presently originated from organic compounds, underscoring the vital importance of conserving biological diversity as well as adopting sustainable practices.[4] In this context, medicinal plants and herbal remedies have been extensively utilized in Complementary and Alternative Medicine (CAM) to manage inflammation and related disorders. *Ficus capensis* (Thunb.), or the 'bush fig tree', is a species within the Moraceae family. It is widely recognized in Nigeria, where it is called Akokoro in Igbo, Opoto in Yoruba, and Uwaraya in Hausa.[5] Characterized by its broad, greenish leaves and all-year-round fruit production[6]. *Ficus capensis* has significant potential. However, this plant has not been fully exploited and is considered underutilized. In southeastern Nigeria, *Ficus capensis* leaves are used as a vegetable in traditional dishes like soups and yam pottage.[5] In addition to its culinary value, the plant has been traditionally utilized for the treatment of a variety of health conditions like wounds, dysentery,[7] leprosy, circumcision wounds, rickets, epilepsy, infertility, edema, respiratory disorders, and gonorrhea.[8] Scientific investigations have validated the medicinal properties of *Ficus capensis*, revealing its potential in treating azoospermia,[9] anti-diabetic,[10] exhibiting antibacterial activity,[11] and possessing antidiarrheal,[12] immune-stimulatory, and anti-trypanosomal,[13] hematopoietic properties[5] and antioxidant properties.[14] In an attempt to expand the repertoire of anti-inflammatory agents derived from natural products, this research examined the *in-vitro* anti-inflammatory potential of the extracts from the leaves, stems, and fruits of *Ficus capensis*.

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Materials and Methods

Sample Collection

Collection of Leaf, stem, and fruit samples of *Ficus capensis* was from the premises of Enugu State University of Science and Technology (ESUT) in June 2024. The plant specimens were identified and confirmed by Prof. Eze, C.S., of the Department of Applied Biology and Biotechnology, ESUT, Agbani, Nigeria.

Sample Preparation

The samples were thoroughly sorted, rinsed, and dried under shade. They were crushed into a powder and kept in a dry, airtight container. Approximately 250 g of the each of the powdered samples was accurately weighed using an electronic balance (Adam AFP800L model). The sample (250 g) was extracted using 80% ethanol (1000 ml) and dried at 50 ºC in a water bath.

In-vitro Anti-inflammatory Assay

This was performed using three *in-vitro* assays: Anti-proteinase, albumin denaturation inhibition and membrane stabilization assays.

Anti-Proteinase Activity Assay

The anti-proteinase activity was evaluated using a method adapted from the method of Sakat et al.[15] One millimeter of the sample of concentration 0.625-10 mg/ml was mixed with trypsin (0.06 mg) and 1 ml of 20 mM Tris-HCl buffer of pH 7.4. After incubation for 5 minutes at 37°C, casein (1 ml of 0.8% (w/v) was added, and the mixture was kept in the dark for 20 minutes. Addition of 70% perchloric acid (2 ml) stopped the reaction, giving rise to a cloudy solution that was spun using a centrifuge (4000rpm for 15 mins). The upper layer absorbance of each sample was read at 210 nm against a buffer blank. Diclofenac sodium (100 µg/ml) served as the reference compound. The percentage Antiproteinase activity was calculated using the formula:

$$\text{Percentage inhibition} = \frac{Abs\ control - Abs\ sample}{Abs\ control}\ X\ 100$$

Inhibition of Albumin Denaturation Assay

This was performed using the method described by Sakat et al.[15] Aqueous solution of bovine albumin (500 µl, 1%) was added to the extract (100 µl), followed by adjustment of the pH (5.3) with a pinch of Hydrochloric acid (1N). The mixture was kept in the dark for 20 minutes at 37°C and then heated at 51°C for an extra 20 minutes. The mixture was cooled to room temperature, followed by measurement of the absorbance of the turbidity at 660 nm. The reference compound used was diclofenac. The percentage inhibition was calculated thus:

$$\%\ \text{inhibition} = \frac{Abs\ control - Abs\ sample}{Abs\ control}\ X\ 100$$

Membrane Stabilization Assay

Assessment of the membrane-stabilizing activity can be performed using heat-induced hemolysis and hypotonicity-induced hemolysis assays, with erythrocytes from humans[16] or rats[17] serving as the test model. In this study, we used erythrocytes obtained from rats. The inhibition of heat-induced hemolysis of red blood cells was assessed using the method of Sakat et al.,[15] while the inhibition of hypotonicity-induced hemolysis assay was done using to the method of Leelaprakash and Mohan.[18]

Red Blood Cell Suspension Preparation

The blood samples were obtained from albino rats that had not been administered any non-steroidal anti-inflammatory drugs for 14 days before the assay. Centrifugation of the blood samples was done for 10 minutes at 3000 rpm. It was rinsed trice using normal saline, and re-suspended at a concentration of 10% volume/volume solution using normal saline.

Assessment of Inhibition of Heat-Induced Hemolysis

The extract's ability to prevent heat-induced hemolysis was evaluated by mixing 1 ml of the extract at doses of 0.625, 1.25, 2.50, 5.00, and 10.00 mg/ml with 1 ml of RBC suspension in centrifuge tubes. A control tube containing the standard, RBC suspension, and normal saline was also prepared, with aspirin serving as the reference. The tubes were kept in the dark at 56°C for 30 minutes, cooled, and then centrifuged for 5 minutes at 2500 rpm. Absorbance of the supernatant was measured at 560 nm, and the % inhibition of hemolysis was calculated:

$$\%\ \text{inhibition} = \frac{Abs\ control - Abs\ sample}{Abs\ control}\ X\ 100$$

Hypotonicity-Induced Hemolysis Inhibition Assay

Various concentrations of the extract (0-10 mg/ml), control, and reference sample were combined separately with phosphate buffer (1 ml), hyposaline (2 ml), and RBC solution (0.5 ml). The reference drug was diclofenac sodium (100 µg/ml). Incubation of the mixtures was done for 30 minutes at 37°C, followed by centrifugation at 3000 rpm. Measurement of the hemoglobin concentration in the upper layer was one at 560 nm. The % RBC membrane protection/stabilization was calculated thus:

$$\text{Percent protection} = \frac{100 - OD\ sample}{OD\ control}\ X\ 100$$

Statistical Analysis

The results were presented as mean ± SD of duplicate analyses. Linear equations derived from plots of anti-inflammatory activity versus extract concentration were used to calculate the IC_{50} values. Statistical analysis was done using SPSS version 22. ANOVA was used to determine significant differences between the means of the three samples, while Tukey's post-hoc test was used to the specific sample that is significantly different ($\alpha < 0.05$).

Results and Discussion

The anti-inflammatory properties of the leaves, stem, and fruits of *Ficus capensis* were assessed using three in-vitro tests. The results were compared to standard anti-inflammatory medications, aspirin and diclofenac. *In-vitro* studies provide a controlled environment to examine cellular reactions, which is essential for understanding the anti-inflammatory mechanisms of natural medicinal products.[19]

Inhibition of Albumin Denaturation

Ficus capensis leaves, stem, and fruit extracts demonstrated a concentration-dependent protein denaturation inhibition. Figure 1 shows the inhibitory effects of each extract at concentrations of 0.625-10.0 mg/ml.

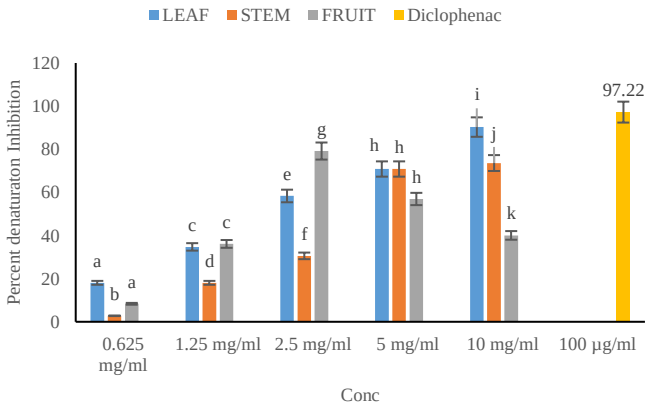

Figure 1: Inhibition of albumin denaturation activity of the leaves, stem and fruits extracts of *Ficus capensis*, and the standard; diclofenac. Data represent mean ± SD of duplicate determinations. Bars labeled with different letters (a-k) indicate significant variations ($p < 0.05$), while bars with the same letter represent non-significant variations ($p > 0.05$).

The highest inhibition was seen in the ethanol leaf extract, with an inhibition percentage of 90.28±1.96% at 10 mg/ml. The stem extract followed, with an inhibition percentage of 72.22 ± 1.96%. In contrast, the fruit extract gave a maximum inhibition percentage of 56.94 ± 9.72% at 5 mg/ml, with a decline in activity to 40.28 ± 4.17% at 10

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mg/ml. Diclofenac, used as a standard, gave an inhibition percentage of 97.22 ± 2.78% at 100 µg/ml. Furthermore, a significant variation ($p < 0.05$) in albumin denaturation inhibition was seen. The leaves gave the maximum inhibition, with an IC_{50} of 3.80 ± 0.11 mg/ml, followed by the stem (IC_{50} = 5.43 ± 0.35 mg/ml) and fruit (IC_{50} = 7.80 ± 2.29 mg/ml). Protein denaturation, a consequence of inflammation in diseases such as arthritis[16] is a widely studied phenomenon. It is a key feature of inflammatory and arthritic conditions, such as those described by Russo *et al*.[20] Denaturation of intercellular material or cellular protein components can give rise to tissue injury.[21] Therefore, the capacity of a substance to prevent protein denaturation is a strong indicator of its anti-inflammatory properties. According to the report by Mizushima[22], preventing protein denaturation among the main mechanisms through which NSAIDs exert their therapeutic effects. Previous works have investigated the impact of various plant parts on protein denaturation, such as *Psychotria densinervia* leaf,[23] *Calotropis gigantea* extract,[24] and *Tamilnadia uliginosa*,[25] which have shown promise in preventing protein denaturation. This study demonstrated that *Ficus capensis* leaves, stem, and fruit extracts effectively inhibited heat-induced denaturation of bovine albumin, with the leaf extract showing optimal activity at 90.8%. This was comparable to that of the standard, which gave activity of 97.22% at 100 µg/ml. The observed inhibitory effects are likely due to the interaction and modulation of membrane proteins, suggesting potential anti-rheumatoid properties.

Anti-proteinase Activity

The proteinase inhibitory activity is shown in Figure 2. The leaves and stem demonstrated a concentration-dependent anti-proteinase activity, with higher inhibition percentages as sample concentrations increased. Similarly, the fruit showed a concentration-dependent increase, although activity declined at 10 mg/ml.

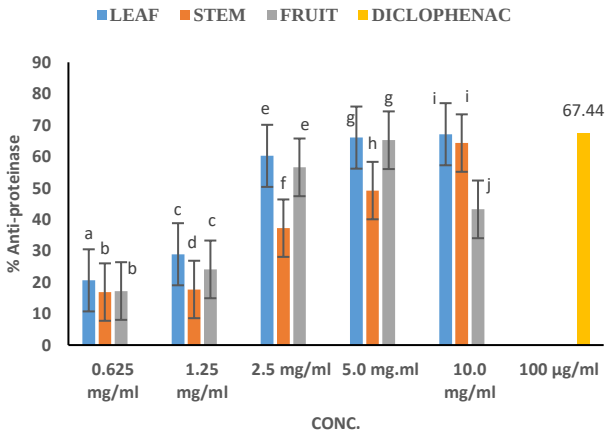

Figure 2: Anti-proteinase Activity of the leaves, stem and fruits of *Ficus capensis*, and the standard; diclofenac. Data represent mean ± SD of duplicate determinations. Bars labeled with different letters (a-j) indicate significant variations ($p < 0.05$), while bars with the same letter represent non-significant variations ($p > 0.05$)

The inhibition levels ranged from 16.86-67.11%. At 0.625 mg/ml, the ethanol extracts of leaves, stem, and fruit demonstrated 20.59 ± 0.23%, 16.86 ± 0.25%, and 17.19 ± 0.12% anti-proteinase activity, respectively, while at 10 mg/ml, the activities were 67.44 ± 0.47%, 63.95 ± 0.49%, and 43.19 ± 0.47% respectively. The standard drug was tested at 100 µg and exhibited 67.44 ± 0.47% activity. Notably, the leaves exhibited significantly higher ($p < 0.05$) proteinase inhibition levels with an IC_{50} of 4.19 ± 0.04 mg/ml, compared to the stem and fruit with IC_{50} of 6.42±0.16 and 7.49±0.19 mg/ml, respectively. Proteinases are associated with arthritic reactions, with neutrophils containing serine proteinases in their lysosomal granules.[24] Leukocyte proteinases contribute significantly to tissue damage during inflammation. Notably, proteinase inhibitors have been shown to offer substantial protection.[26] Flavonoids have been identified as key contributors to anti-inflammatory and antioxidant properties of various plants.[27, 28] Specifically, Flavonoids have been shown to inhibit certain proteases that perform a vital function in the inflammatory process.[29] Bioactive compounds in these extracts may, therefore, underlie their anti-inflammatory activity. As studies have demonstrated, *Ficus capensis* extracts contain high levels of polyphenols, flavonoids, and carotenoids.[30, 31] This study demonstrated that the extracts displayed concentration-dependent inhibition of proteinase activity, with a maximal inhibition of 63.95% at 10 mg/ml by the leaf. This inhibitory effect is comparable to that of diclofenac, which achieved 67.11% inhibition at 100 µg/ml. These findings suggest that the extract may help reduce inflammatory reactions mediated by proteinase activity.

Membrane Stabilization

Figure 3 illustrates the % inhibition of heat-induced hemolysis of red blood cells at various concentrations (0.625-10 mg/m) of each extract. Extracts of *Ficus capensis* leaves and stem demonstrated concentration-dependent inhibition activity.

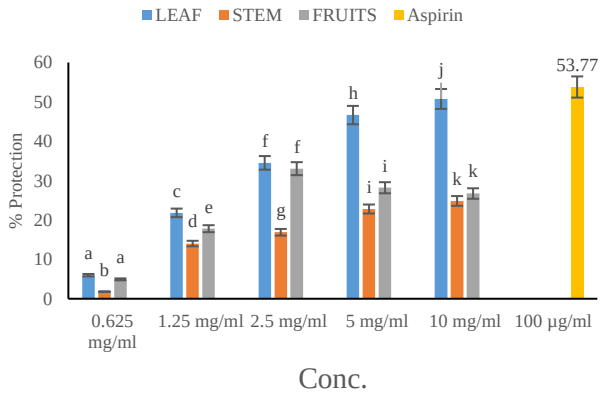

Figure 3: Inhibition of heat-induced hemolysis activity of the leaves, stem and fruits extracts of *Ficus capensis*, and the standard; aspirin. Data represent mean ± SD of duplicate determinations. Bars labeled with different letters (a-k) show significant variations ($p < 0.05$), while bars with the same letter represent non-significant variations ($p > 0.05$).

The fruits equally demonstrated concentration-dependent increase in activity, but with a decline in activity at 5 and 10 mg/ml. Inhibition percentages ranged from 1.80-50.74% at concentrations of 0.625-10 mg/ml. The leaves gave significantly the highest ($p < 0.05$) inhibition levels with IC_{50} value of 8.27 ± 0.10 mg/ml, while the fruits exhibited the least inhibition with IC_{50} value of 22.50±1.65 mg/ml but was non-significantly ($p<0.05$) lower than that of the stem (IC_{50}= 21.51 mg/ml). The extracts of leaves, stem, and fruits of *Ficus capensis* demonstrated a concentration-dependent inhibition of hypotonicity-induced hemolysis activity, as shown in Figure 4. The protection percentage increased with increasing concentrations, ranging from 2.08 to 42.78%. The fruit extract showed a decline in activity at 10 mg/ml. The leaf extract was the most potent (IC_{50} value =11.09 ± 0.08 mg/ml), then the stem (IC50 = 15.79 ± 0.01 mg/ml), and the fruit was the least (IC50 = 17.84 ± 0.33 mg/ml). Stabilizing lysosomal membranes is critical in controlling inflammation by preventing the release of damaging enzymes and proteases from activated neutrophils.[32] The resemblance of the erythrocyte membrane and the lysosomal membrane implies that the extract's membrane-stabilizing properties may also apply to lysosomal membranes,[33] thereby mitigating the inflammatory response by hindering the liberation of proteases and lysosomal enzymes. While the membrane stabilization mechanism of the extracts remains unclear, hypotonicity-induced hemolysis is thought to arise from the osmotic loss of intracellular electrolytes and fluids, resulting in cellular shrinkage.

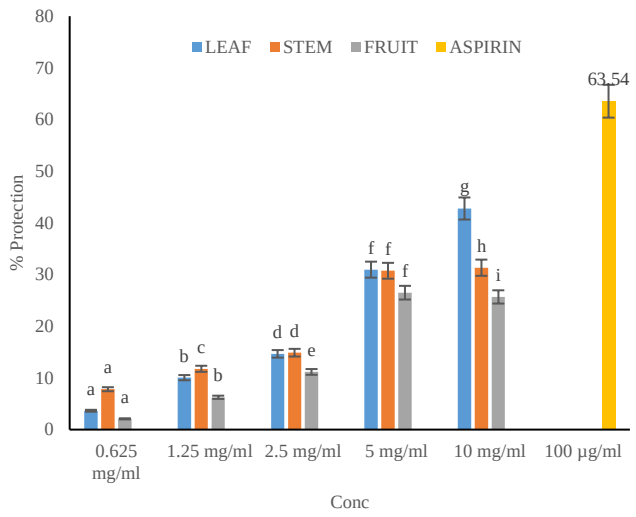

Figure 4: Inhibition of hypotoncity-induced hemolysis activity of the leaves, stem and fruits extracts of *Ficus capensis*, and the standard; aspirin. Data represent mean ± SD of duplicate determinations. Bars labeled with different letters (a-i) show significant variations ($p < 0.05$), while bars with the same letter represent non-significant variations ($p > 0.05$).

The extracts may have hindered the processes that promote the release of these intracellular components, thereby exerting its membrane-stabilizing effect.[34] In numerous previous studies, extracts of *Ficus capensis* have exhibited significant anti-inflammatory activity in vivo.[35] The extract's anti-inflammatory activity may be due to its ability to reduce the synthesis and release of pro-inflammatory cytokines, as suggested by Nisar et al.[36] Additionally, the polyphenols and flavonoids present in the extract may exert anti-inflammatory effects by targeting the NF-κB pathway, a key regulator of inflammation, and inhibiting the expression of pro-inflammatory cytokine-induced chemokines.[37]

Conclusion

This study provides *in-vitro* evidence for the anti-inflammatory properties of *Ficus capensis*, particularly the leaf extract. The findings corroborate its traditional use in the management of inflammatory conditions and suggest its potential as a source of natural anti-inflammatory agents.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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