Original research article.

# Preclinical evaluation of the anti-inflammatory properties of plants extracts of Ficusexasperata(Moraceae) on Wistar rat models.


## ABSTRACT

**Introduction:** In view of the rising global death rate associated with inflammatory diseases, a costfriendly, more effective and, safer drug with lesser side effectsis needed. Inflammation is a part of the complex biological response of vascular tissue to harmful stimuli, such as pathogens, damaged cells, or irritants. It is characterized by redness, swollen joints, jointpain, heat, and loss of joint functions. Chronic inflammatory diseases have been recognized as the most significant cause of death in the world today, with more than 50% of all deaths being attributable to inflammation-related diseases.Non-steroidal anti-inflammatorydrugs (NSAIDs) and Steroidal anti-inflammatory (SAIDs) are used throughout the world for the treatment and management of inflammation, pain, and fever. Since many NSAIDsare associated with, side effects such as gastrointestinalbleeding and suppressed function of the immune system, medicinal herbs are new alternatives to search out new chemical substances that would have better therapeutic results with low toxic profile. Recent studies on*Ficusexasperata*suggestthat it might be as effective assome NSAIDs in the treatment of inflammation and related pain.**Objectives** To evaluate the anti-inflammatory properties of the different plants extracts of *Ficusexasperata* in rats of *Wistar* train.**Methods:** The powdered material of leaves of *Ficusexasperata was* extracted by decoction, infusion, aqueous maceration, and hydroethanolic maceration.Phytochemical screening was carried out on the four different extracts: aqueous extract, hydroethanolic extracts, decoction and Infusion. The anti-inflammatory properties of *Ficusexasperata* were evaluated *in vitro* by egg albumin and bovine albumin using the heat induced Protein denaturation assay. **Results:** The leaves of *Ficusexasperata* was effective in inhibiting albumin denaturation. Varying concentrations of the plant extract significantly, ($p < 0.05$) inhibited the denaturation of albumin when compared to the control group. Aspirin and Indomethacin showed a similar trend. The inhibition by the extract is concentration-dependent with 32.25µg/ml having an inhibition of 8.89 for infusion, 9.66% for hydroethanolic, 10.29% for aqueous maceration, and 12.6% for decoction compared to indomethacin and aspirin with inhibition 36.94% and 40.36%, respectively. The highest percentage inhibition was seen at 1000 µg/mL with inhibition of 34.22% for aqueous maceration, 40.77% for infusion, 41.61 % for hydroethanolic and 42.34% for decoction compared to indomethacin and aspirin with 67.06% and 58.45%,respectively. The plants extracts showed anti-inflammatory activity by decreasing the rate of denaturation of protein.**Conclusion:** The studied extract justifies anti-inflammatory properties, whichwereconfirmed by protecting against protein denaturation.



**Keywords:** *Ficusexasperata,* extractinflammation**,** heatprotein denaturationassay Wistar rats




## INTRODUCTION

Medicinal plants continue to be an interesting source of natural products for treating varioushealth conditions. Studies have been carried out globally to verify their efficacy and some of the findings have led to the production of plant-based medicines[1]. It is estimated that more than 150,000 plant species have been studied, many ofwhich contain valuable therapeutic agents, and the applications of

novel compounds from plants forpharmaceutical purposes have been gradually increasing in recent years[2].

In contemporary medication, even though now we have the supply to enhance the synthesis of medicines inthe lab, plants are still contributor in health care. However, medicinal plants acquire a great concentration towards them, due to their prolonged use in folk drugs as good as their prophylactic residences, especially in developing countries[3].

Plants have played an important role in human health care since ancient times. In an adaptationagainst attacking pathogens and environmental stress, plants produce several substances that exertbiological activities. These small organic molecules come from secondary metabolism and have severalbiological activities. Among the diverse functions, anti-inflammatory actions are highlighted[4].

Chronic inflammatory diseases have been recognized as the most significant cause of death in the world today, with more than 50% of all deaths being attributable to inflammation-related diseases such as ischemic heart disease, stroke, cancer, diabetes mellitus, chronic kidney disease, non-alcoholic fatty liver disease (NAFLD) and autoimmune and neurodegenerative conditions [5].

Non-steroidal anti-inflammatory drugs are commonly prescribed for the treatment of pain and inflammatory conditions such as rheumatoid arthritis, osteoporosis, andAlzheimer'sdisease. However, because many NSAIDs are associated with side effects such as gastrointestinal bleeding and suppressed function of the immune system, attention has shifted to alternative pharmacotherapies[6]. Many of these natural compounds also work by inhibiting the inflammatory pathways in a similar manner as NSAID[7].

In Cameroon,ethnobotanical survey shows that medicinal plantsare used traditionally for many diseases such as infectious diseases, inflammatory diseases and many others. For most of these plants their bioactivity is still unknown hence the need for continuous studies

Inflammation is the body's immune defense process in response to aggression of exogenous origin(Burns, infection, allergy, trauma) or endogenous (Cancer cells or autoimmune Pathologies).The purpose of which is to eliminate the pathogen, repair tissue damage and promote a return to homeostasisand tissue healing[8].Clinical signs of these processesinclude Heat, redness, swelling, and pains. In addition, an alternation of the function of the affected organ may occur. Acute Inflammation is a beneficial phenomenon for the body but if left unchecked, persistent inflammation becomes unfavorable and may be an etiological factor in various chronic diseases such as rheumatoid arthritis,atherosclerosis, cancer, cardiovascular and neurodegenerative diseases.

Non-steroid anti-inflammatory drugs have been commonly prescribed for the treatment of pain and inflammatory condition such asmuscle pain, dysmenorrhea, arthritic conditions, pyrexia, gout, migraines,and rheumatoid arthritis,osteoporosis and Alzheimer'sdisease. [9]. However,NSAIDs

areassociated with side effects such as gastro-intestinal bleeding and suppressed function of the immune system. Attention has shifted to alternative pharmacotherapies. The use of plants in the treatment of inflammatory diseaseshas been widely practiced as manyshows lesser adverse effects compare to the NSAID[9].Plants and their derivatives have been a source of medicines for ages and have been propose for management of inflammatory disease. *Ficusexasperata*. is a herbal plants of the Moraceae family comprising of more than an 800 species and are rich in polyphenols, flavonoids and tannins which are responsible for their antioxidant and anti-inflammatory effects. This plant from ethnobotanic survey and long usage for local treatment of pains in the community presents mostly anecdotal evidence of treatment. There is a need for evidence based studies to establish proof of therapeutic activity, safety and environmentally friendly nature of use. The study is intended to provide information to categorize uses of Ficus exasperate into class 2 of traditional medicine[10]. The objective of this study was to evaluate the anti-inflammatory properties and acute toxicity of leaves extracts of *Ficusexasperata*

## METHODOLOGY

### Plant material- Harvesting and Identification.

Freshly collected leaves of *Ficusexasperata*were harvested at the locality of OBALA in the Center Region of Cameroon, 45km North of Yaoundé, the capital of Cameroon.The plant sample were identified by a Botanist at the National Herbarium by comparing with the voucher specimen: Botanical collection No **697** registered at the national Herbarium **as No 14506/SRF Cam.** The leaves were shade-dried. The dried leaves were pulverized into powder form using a clean mechanical grinder.

### PREPARATION OF THE DIFFERENT EXTRACTS.

Different extraction methods Maceration, decoction and infusion were used to prepare the plant extracts as a standard methods used in the extraction of phytochemicals and for the test selection of best yield [5]..

The abstract is generally comprehensive but can be improved by including:

1. A brief mention of the study's methodology (e.g., "using heat-induced protein denaturation assays").
2. A clearer statement about the significance of the results relative to NSAIDs.
3. More explicit mention of the implications of the findings for therapeutic development.

Consider removing redundant information about the general role of NSAIDs, which is already well-known.

The manuscript is scientifically sound, and the methodologies and results are appropriately detailed. The statistical analysis is adequate, but further clarification on certain points, such as the rationale for extraction methods, would enhance the robustness of the study.

The references are sufficient and cite foundational and recent studies. However, the manuscript would benefit from including additional recent studies focusing on plant-based anti-inflammatory agents or comparative analyses with NSAIDs.

The language is generally suitable for scholarly communication, but minor grammatical and stylistic improvements are needed. Attention to sentence structure and word choice will enhance clarity and readability.

1. Include a clearer explanation of why specific extraction methods were chosen.
2. Improve figure captions to make them more informative.
3. Provide additional context on how these findings could contribute to clinical applications or the development therapeutics.
4. Consider adding a comparison of cost-effectiveness between plant-based and synthetic anti-inflammatory treatments.



**Preparation of Aqueous extract**

In this process, 100g of the coarsely powdered crude plant was put in a stoppered container with 1000ml of distilled water and allowed to stand at room temperature for a period of 48 hours with frequent agitation until the soluble matter was dissolved. The mixture was then strained, the marc (the damp solid material) was pressed, and the combined liquids were clarified by filtration using Whatman paper (No 2) and the supernatant was collected. This filtrate was evaporated and the extract collected. The percentage yield was calculated as follows

$$PercentageYield = \frac{Massofextractobtained}{Massofpowderinitiallyuse} \times 100$$

**Preparation of Hydroethanolic extract**

In this process, 100g of the coarsely crude powder was put in a stopper container containing 500ml of ethanol and 500ml of water and allow to stand at room temperature for a period of 48h with frequent agitation until soluble matter has dissolved, the mixture was then strained. The marc (the damped solid material) was pressed and the combined liquid were clarified by filtration using Whatman paper(No 2) the supernatant was collected. This filtrate was evaporated and the extract collected. The percentage yield was then calculated as follows.

$$Percentage\ Yield\ = \frac{Mass\ of\ ectrac\ obtained}{Mass\ of\ powder\ initially\ use}\ x\ 100$$

**Preparation of infusion extract**

In this process, water was heated using the water bath. The warm water was then poured in the container containing 100g of the plants. The mixture was strained, the marc (the damped solid material) was pressed and filtered using Whatman paper(No 2) and then collection of the supernatant. This filtrate was evaporated and the extract collected. The percentage yield was then calculated as follows

$$Percentage\ Yield\ = \frac{Mass\ of\ extract\ obtained}{Mass\ of\ powder\ initially\ use}\ x\ 100$$

**Preparation of extract of Decoction**

In this process 100g of the powdered plant was dissolve in 1000ml of water. The mixture was placed in a water bath to boil at 98$^{o}$C for 30 min. The mixture was then allowed to cool.

The mixture was then strained, and pressed and the combined liquid was clarified by filtration using Whatman paper (n$^{o}$ 2) and the supernatant collected. This filtrate was evaporated and the extract collected. The percentage yield was then calculated as follows

$$Percentage\ Yield\ = \frac{Mass\ of\ extract\ obtained}{Mass\ of\ powder\ initially\ use}\ x\ 100$$

***INVITRO* EVALUATION OF ANTI-INFLAMMATORY ACTIVITY**

**Anti-inflammatory effect of extracts on protein denaturation by the Heat induced protein denaturation assay.**

**Principle**

Protein denaturation is a biochemical reaction that occurs during a chronic inflammatory response that can lead to loss of tissue function. Previous research has indicated that protein denaturation is the main cause of inflammatory and arthritic disorder which thus proceed to the generation of autoantigens, thus leading to rheumatic disorder. Denaturation possibly involves variation of hydrogen, electrostatic disulfide and hydrophobic bonds [11]. Many anti-inflammatory drugs are dose deterrence heat induced protein denaturation [12]. Therefore, substance that can prevent protein denaturation would be considered valuable for the development of anti-arthritic drugs.

**Procedure**

Ovalbumin was extracted from chicken egg white according to the method described by Dharmadeva et al with little modification [15]. A fresh egg was broken and the yolk was separated from the white, the egg white was collected in a beaker placed in an ice bath and mixed with 50ml of phosphate buffer (0.1M, pH=6.6) then homogenized with magnetic stirring for 5 min, then the homogenate obtained was centrifuged at 3000 rpm (4°C.) for 5 min, the supernatant obtained was filtered through a strip of gauze to remove the precipitant. Finally, the filtrate obtained was divided into aliquots with a volume of 2 to 2.5 ml then stored at -20°C.

Inhibition of protein denaturation with chicken egg albumin was achieved as follows:5000 µl of reaction mixture containing 200 µl of fresh egg albumin, 2800 µl of phosphate-buffered saline solution of pH 6.4 and 2000µl of extract solutions of different concentrations were prepared (50-1000 µg/ml). Additionally, a similar volume of distilled water was taken as a control. The reaction mixture was placed at 37 ± 2°C in an incubator for 15 min followed by heating at 70°C for 5 min. After cooling, absorbance was taken at 660 nm using vehicle blank. Similarly, indomethacin and aspirin served as standard controls and absorbance was then measured. Percentage inhibition of protein denaturation was calculated using the following formula.

$$Percentageinhibition = \frac{Absorbanceofcontro - Absorbanceoftest}{Absorbanceofcontrol} x\ 100$$

This assay was used for the determination of inhibition of protein denaturation using Bovine serum albumin. Reaction mixture (0.5 mL) of various concentrations consisting of 0.45 mL bovine serum albumin (5% aqueous solution) and 0.05 mL of the different plant extract of *F. exasperata* along with aspirin and indomethacin were prepared. pH was calibrated at 6.3 using 1N HCl. After preparation mixtures were incubated at 37°C for 20 min subsequently heating at 57°C for 30 min. After cooling the samples, 2.5 mL phosphate buffer saline (pH 6.3) was added to each test tube. Moreover, 0.05 mL distilled water was used in place of plant extract/fractions in control test tube whilst product control did not contain bovine serum albumin. In due course, absorbance was measured spectrophotometrically at 660 nm and percentage inhibition of protein denaturation was calculated as follows.

$$Percentage\ inhibition = \frac{Abs\ control - Abs\ Sample}{Abs\ Control} x\ 100$$

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**Statistical Analysis**

Raw data on weight, alimentation and biochemical parameters were collected and entered in Microsoft Excel 365. The GraphPadInstat version 5.1 software was used for comparison between the groups which were analyzed using one-way analysis of variance, the ANOVA test followed by Turkey's Kramer post hoc test. The results were expressed in terms of mean ± standard deviation. *p*-values ≤0.05 were considered as statistically significant

## RESULTS

### ANTI-INFLAMMATORY ACTIVITY

**Effects of extracts on protein egg albumin denaturation.**

Figure 1 shows the kinetics of percentage protection of plant extracts and reference drugs from heat-induced egg protein denaturation. It was observed that the protection against denaturation increased with the concentration of the extracts.

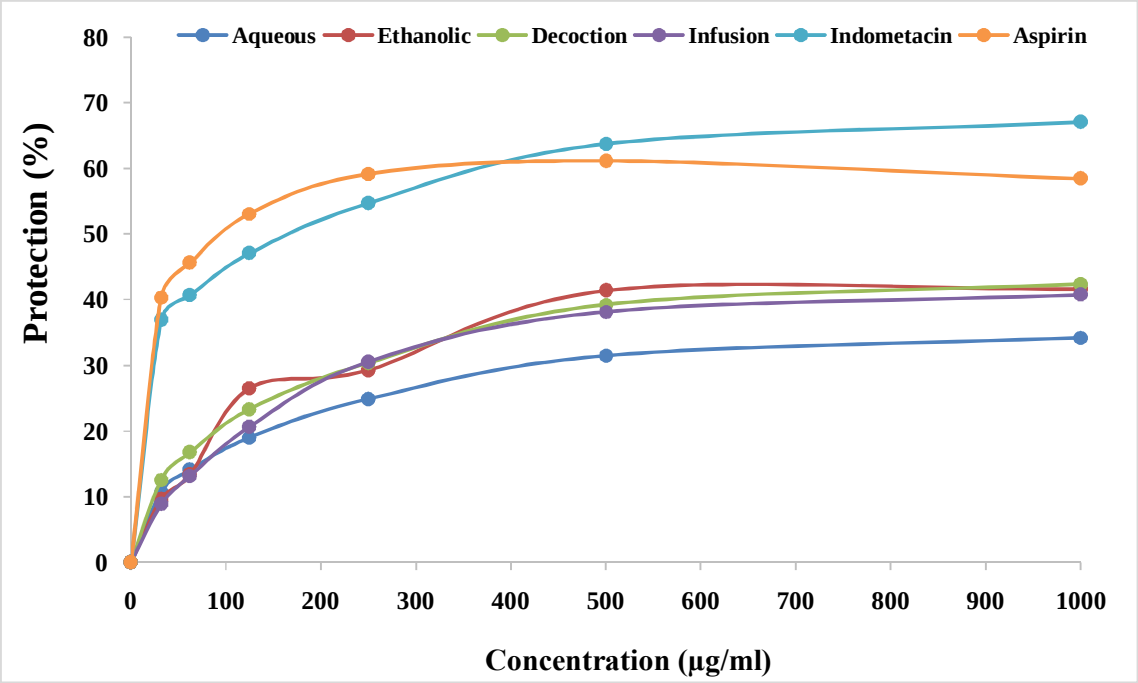


**Figure 1**: Anti-inflammatory protection effects of plants extract using the egg albumin protein denaturation method

The table 1shows the protective capacity of the plant extracts against protein denaturation, it can be seen that indomethacin had the greatest activity, followed by aspirin, the ethanolic extract of the plant, decoction, infusion and aqueous extract, showed the weakest protection.

**Table1.** : Anti-inflammatory effect of plant extract on from denaturation of protein of Ovalbumin method

| Conc (µg/ml) | 32,125 | 62,25 | 125 | 250 | 500 | 1000 | IC50 |
|---|---|---|---|---|---|---|---|
| **Aq** | 10,79 ± 2,26 | 14,10 ± 1,39 | 19,03 ± 0,88 | 24,86 ± 1,22 | 31,46 ± 1,34 | 34,22 ± 0,71 | 1382,71 |
| **Eth** | 9,66 ± 2,33 | 13,36 ± 0,38 | 26,51 ± 12,15 | 29,26 ± 1,70 | 41,40 ± 4,01 | 41,61 ± 0,96 | 1011,72 |
| **Dec** | 12,62 ± 1,42 | 16,78 ± 1,02 | 23,31 ± 1,89 | 30,36 ± 1,68 | 39,26 ± 1,10 | 42,34 ± 1,07 | 1029,41 |
| **Inf** | 8,89 ± 0,91 | 13,15 ± 0,72 | 20,64 ± 2,01 | 30,55 ± 2,63 | 38,16 ± 1,25 | 40,77 ± 1,60 | 1057,83 |
| **Ind** | 36,94 ± 1,83 | 40,68 ±1,50 | 47,07 ± 0,67 | 54,69 ± 0,95 | 63,67 ± 0,68 | 67,06 ± 3,87 | 408,77 |
| **Asp** | 40,36 ± 1,35 | 45,63 ± 2,15 | 53,10 ± 0,53 | 59,12 ± 1,91 | 61,12 ± 1,16 | 58,45 ± 1,30 | 423,84 |

All values expressed as mean ± standard error of the mean, n=3; Statistical analysis is done by one-way One-Way Analysis of Variance. Ag-Aqueous extract, Eth-Ethanolic extract; Dec=Decoction extract; Inf=Infusion extract; Ind=Indomethacin; Asp=Aspirin.

Based on the results from albumin denaturation test, the aqueous extracts and the decoction of the leaves of the plant showed an inhibition of more than 10% at the dose of 32.125 µg/ml while the ethanolic extract and the infusion showed a weaker inhibition respectively of 9 and 8%, Indomethacin 36% and Aspirin 40%. At a concentration of 125µg/ml the Ethanolic extract presents a maximum of 26% inhibition followed by decoction23% then infusion 20% and 9% aqueous in comparison to indomethacin and aspirin which had 47% and 53% respectively. At the dose of 500 µg/ml the Hydroethanolic extract showed inhibition of 41%,followed by decoction 39%, then infusion 38 and aqueous 31 compared to indomethacin and Aspirin which showed an inhibition of 63% and 58% respectively.The highest activity was seen in indomethacin with the lowest IC50 value. Indomethacin showed a greater percentage inhibition than aspirin.

**Anti-inflammatory effects of extracts on protein bovine serum albumin denaturation method.**

The figure 2 shows the kinetics of percentage protection of plant extracts from heat-induced bovine serum albumin protein denaturation. It was observed that the protection against denaturation increased with the concentration of the extracts.

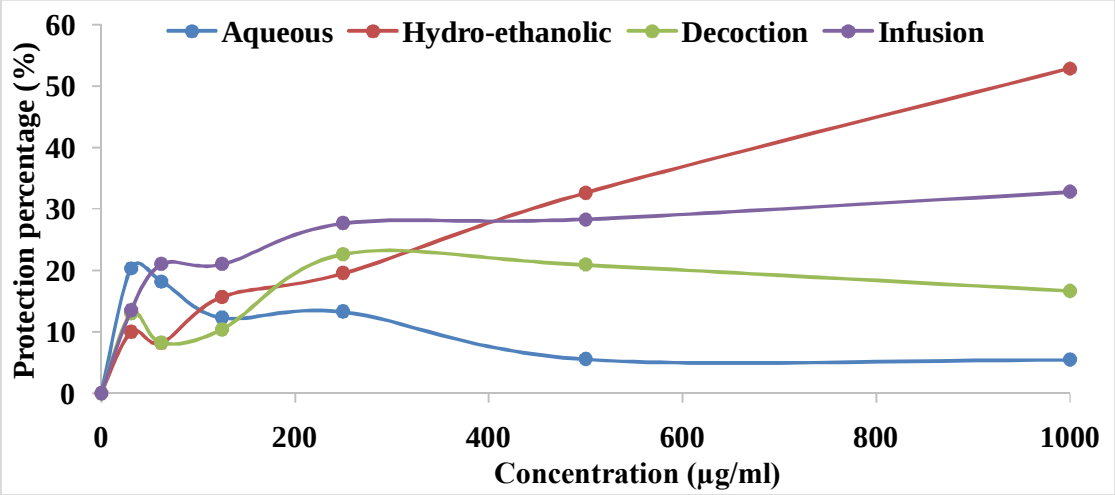


**Figure 2 :Anti-inflammatory p**rotection property of plants extract from albumin bovins proteins denaturation.method

The table 2 shows the protective capacity of the plant extracts against protein denaturation, it can be seen that ethanolic extract had the greatest activity, followed by infusion, decoction and aqueous extract which had the weakest protection.

**Table 2**: Anti-inflammatory effect of plant extract from denaturation of protein of Ovalbumin method

| Conc (µg/ml) | 0 | 31,125 | 62,5 | 125 | 250 | 500 | 1000 | IC50 |
|---|---|---|---|---|---|---|---|---|
| **Aq** | 0 | 20,37 ± 11,75 | 9,23 ± 4,10 | 12,28 ± 5,54 | 13,25 ± 4,26 | 5,53 ± 0,58 | 5,44 ± 0,73 | 4567,78 |
| **Eth** | 0 | 9,97 ± 6,73 | 8,25 ± 5,57 | 15,64 ± 4,59 | 19,54 ± 4,63 | 32,59 ± 3,42 | 52,85 ± 3,15 | 902,92 |
| **Dec** | 0 | 12,98 ± 7,01 | 8,20 ± 5,29 | 10,37 ± 4,08 | 22,60 ± 3,05 | 20,88 ± 2,58 | 16,62 ± 3,14 | 3436,62 |
| **Inf** | 0 | 13,51 ± 3,74 | 21,01 ± 4,58 | 21,01 ± 3,07 | 27,63 ± 3,26 | 28,30 ± 11,89 | 32,72 ± 7,38 | 1581,90 |

All values expressed as mean ± standard error of the mean, n=3; Statistical analysis is done by one-way One-Way Analysis of Variance.; Ag-Aqueous extract, Eth-Ethanolic extract; Dec=Decoction extract;Inf=Infusion extract

**DISCUSSION**

The leaves of *Ficusexasperata* was effective in inhibiting albumin denaturation. This process was evaluated across varying concentrations of the plant extracts, and the results showed a concentration-dependent inhibition of albumin denaturation. At lower concentrations (32.25 µg/ml), the leaf extracts demonstrated modest inhibition, with the infusion extract showing 8.89 %, hydroethanolic extract at 9.66 %, aqueous maceration at 10.29 %, and decoction at 12.6 %. These values were compared to the positive controls, indomethacin and aspirin, which showed 36.94 % and 40.36 % inhibition, respectively.At the highest concentration of 1000 µg/mL, the *Ficusexasperata* leaf extracts showed notable increases in their ability to inhibit albumin denaturation. Specifically, the aqueous maceration extract exhibited a 34.22 % inhibition, the infusion extract showed 40.77 %, the hydroethanolic extract demonstrated 41.61 %, and the decoction extract had the highest inhibition at 42.34 %. When compared to the standard anti-inflammatory drugs, indomethacin and aspirin, which exhibited inhibition rates of 67.06 % and 58.45 %, respectively, the plant extracts displayed relatively lower but still significant inhibitory effect The trend observed in this study aligns with the findings of Leelaprakash et al., where similar plant extracts were shown to possess anti-inflammatory properties through the inhibition of protein denaturation[8].

Indomethacin had a greater percentage inhibition than aspirin showing indomethacin is a more potent Anti-inflammatory agent than aspirin. This result is similar to that conducted by Crook *et al*[16]. This suggests that *Ficusexasperata* contains bioactive compounds capable of inhibiting the process of albumin denaturation, a key marker for inflammation. While the plant extracts demonstrated some level of efficacy, their effects were less potent compared to indomethacin, which is considered a strong anti-inflammatory agent. The inhibition of albumin denaturation by *Ficusexasperata* extracts indicates that the plant may possess compounds that stabilize proteins and prevent their structural changes induced by external factors, such as heat or chemicals, commonly associated with inflammation. In inflammatory diseases like arthritis, protein denaturation is a common occurrence, which contributes to the symptoms of swelling, pain, and tissue damage. Therefore, the ability of *Ficusexasperata* to inhibit this process highlights its potential for managing such conditions.

The variation in the inhibition rates across different extracts suggests that the method of extraction plays a role in the effectiveness of the plant's bioactive compounds. The decoction and hydroethanolic extracts demonstrated the highest inhibition, suggesting that these

extraction methods may be more efficient in extracting the active compounds responsible for the observed anti-inflammatory effects. In contrast, the aqueous maceration and infusion extracts exhibited lower inhibition, indicating that their extraction methods might not fully extract the most potent bioactive compounds.

Overall, while the inhibition observed for *Ficusexasperata* extracts is lower than that of indomethacin and aspirin, these results are promising. The plant shows potential as a natural anti-inflammatory agent, and further studies, including chemical analysis and bioactive compound identification, could help in understanding the specific compounds responsible for these effects. Additionally, the concentration-dependent inhibition and the promising results at higher concentrations suggest that the plant could be developed into a therapeutic agent for inflammation-related conditions.

*Ficusexasperata* has been shown in this study as a promising anti-inflammatory herbal plant, and with the history of long use in the community, and environmentally friendly a safety study concluded shall enable us formulate the product for use in category 2 traditional medicine. The homologation process will be necessary after formulation and testing. An alternative phyto anti-inflammatory is significant to serve the local community, considering the hepatotoxic nature of use and abuse of NSAID. On the Pharmacoeconomic point of view *Ficusexasperata* is readily available and used by many people in the community as a category 1 traditional medicine. Our studies confirm the use in category 1 improved traditional medicine.

**Conclusion**:The presence of secondary metabolites reported in earlier studies, in the studied extracts justifies the promising anti-inflammatory properties expressed by the rate of , which were confirmed by protecting against protein denaturation. More sensitive and specific methods are required to test for these secondary metabolites in serum.



**DISCLAIMER (ARTIFICIAL INTELLIGENCE)**
Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

**ETHICAL APPROVAL**
All experiments are in phase with the terms of the Institutional Ethical Review Board of Faculty of Medicine and Biomedical Sciences, University of Yaoundé I. Ethical clearance approval reference No 277/UY1/FMBS/VDRC/ESD of 09 May 2023.

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