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Anti-inflammation study of cellulose-chitosan biocomposite-based Tetrapleura tetraptera (Taub) dried fruits aqueous extract

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Abstract

Nanotechnology is making exceptional progress in the medical field. The development of new systems for the delivery of plant ingredients brings benefits such as good bioavailability, solubility, sustained release, and enhanced potential. To the best of our knowledge, there is no report on nanocomposites from *Tetrapleura tetraptera* despite its wide range of ethnopharmacological uses including management of the inflammatory diseases. This study aimed to prepare chitosan and cellulose-based nanocomposite with anti-inflammatory potential. The biocomposite showed an encapsulation efficiency of 69.4%. Their formation was confirmed by a peak at 290 nm in UV-Vis. Infrared spectrophotometry proves the formation of a mixed polysaccharide and polyphenols system. No signs of toxicity and no deaths were found, therefore the lethal dose 50 (LD_{50}) is higher than 2000 mg/kg. The biocomposite has an anti-inflammatory activity *in vitro* with a maximum inhibition of 99.5% and an anti-inflammatory activity *in vivo* with a maximum inhibition of 98.7% for a dose of 200 mg/kg. The synthesized biocomposite from the aqueous extract of the fruits of *Tetrapleura tetraptera* showed an anti-inflammatory property with satisfactory safety.

1. Introduction

The most important theory in disease prevention and care is inflammation. The inflammatory response can be defined as a complex orchestration of events involving an innate system of cellular and humoral responses following injury [1]. The inflammation process is involved in the threat related to depression, asthma, Alzheimer's disease, sinusitis, heart disease, stroke, diabetes, obesity, migraine and headaches, osteoarthritis, inflammatory bowel disease, fibromyalgia, periodontal disease, irritable bowel syndrome, cancer, systemic lupus erythematosus, multiple sclerosis, and rheumatoid arthritis [1, 2]. The treatment of inflammation is as old as humanity. In fact, from ancient Egypt to Hippocrates, the management of inflammation was done with plant extracts [3–5]. After a turn in modern medicine with the development of modern drugs against inflammatory diseases, the development of anti-inflammatory nutraceuticals is continuously growing with different forms of diets. All these highlight the interest in plant-based preparations for the management of inflammatory diseases.

In developing countries, it is established that the population uses plant-based preparations to manage medical issues. Among the rich Cameroonian Flora, *Tetrapleura tetraptera* (Taub) holds an important place. *Tetrapleura tetraptera* (family Fabaceae) is a regular tree distributed throughout tropical Africa, particularly in West, Central, and East African rain forests [6–11]. The plant is named *Tetrapleura tetraptera* (Schum et Thonn) Taubert (Fabaceae-Mimosoideae). The synonyms are *Tetrapleura thonningii* Benth, *Adenanthera tetraptera* Schum et T honn; Common names are Aidan tree (English), and Quatre cotés (French). The plants are vernacularly called Ora ora (Awka), Ighimiakia (Bini), Imiminje (Etsako), Dawo (Hausa), Uyayak (Ibiobio), Oshosho (Igbo), Prekese (Twi, Ghana), Aridan/Aidan (Yoruba), Sassas (Bassa in Littoral region of Cameroon), Djetk or Essissa (Ewondo, central region of Cameroon).[6] The four-winged fruit (see Fig. 1) with a fragrant, typically pungent aromatic scent, is used in folkloric medicine for the treatment of various ailments and as spice in the preparation of varieties of soups by Efiks and

Ibibios of the Niger Delta region of Nigeria, in North-West, South-West and West region of Cameroun in yellow sausage or Nkui and Littoral region of Cameroon for Mbongo sausage [6–11]. *Tetrapleura tetraptera* has been used as medicine for various ailments including inflammatory diseases.[10] Fruit decoctions and infusions are traditionally used for the management and control of wounds, diabetes mellitus, asthma, hypertension, convulsions, leprosy, mental illness, inflammation, arthritis and rheumatoid pain, control of schistosomiasis, as a general tonic and as a flavouring / spice [6, 12]. The plant is one of the potential hepatotoxic plants used in Nigeria, it enhances steroidogenesis, spermatogenesis, and improved testicular histological integrity and boosts sexual competence in male rats [8, 10]. The aqueous fruit extract possesses analgesic and anticonvulsant properties [12]. The fruit has protective potential against stroke through modulation of redox and electrolyte imbalances, and attenuation of neurotransmitter dysregulation and other neurochemical dysfunctions and is a smooth muscle relaxant [13, 14]. *Tetrapleura tetraptera* was reported in a review to contain various biologically active compounds such as hentriacontane, an anti-inflammatory agent and the flavanone-naringenin a strong antioxidant [6]. Despite this wonderful potential of *Tetrapleura tetraptera*, it is still poorly exploited in medicine; this may be due to the lack of delivery strategy.

In general, the transport of the pharmaceutically active agent at the right location, at the right concentration for the right period, to the right place remains a challenge for researchers. To achieve this goal, 'drug *delivery systems*" have been introduced, based on the conjugation of the active agent with a suitable carrier. Drug delivery systems offer many advantages in therapy, including preventing frequent, expensive, and unpleasant dosing, increasing the therapeutic window, and thus reducing toxicity and the risk of iatrogenic effects [15, 16]. This need has driven nanotechnology to medical applications. The US National Nanotechnology Initiative defines nanotechnology as the practice of R&D at the atomic, molecular, or macromolecular levels in the sub100 nm range to create structures, devices, and systems that have novel functional properties [17].

Cellulose and chitin are the most abundant biopolymers on Earth. Both are so-called structural polysaccharides, as they build hierarchical structures in plants and animals [18]. Work on the successful combination of the two biopolymers increased in the 1990s, mainly by combining them in the form of films [19]. The combination of the two polymers was envisaged because they are similar in their linear molecular structure. However, they have different properties that can be usefully combined. Cellulose is the most common natural biopolymer on earth, constituted by the assembly of glucopyranose units joined by β (1,4) bond type. It is characterized, by its high degree of polymerization that vary from plant to plant. Cellulose offers hydrophilic, structuring and mechanical properties, and chitosan has an electron donor function and antimicrobial properties due to the amino group present [18]. Cellulose nanocrystals are an assembly of polymeric chains of glucose. Nanoparticle preparation considers many parameters including the nature of the acid, the temperature, and the source of cellulose [18].

Chitosan and chitosan derivatives-based systems have been considered encouraging materials for the development of drug delivery systems because of their exceptional physicochemical characteristics. Being a mucoadhesive polymer, chitosan enhances the residence time of the system and consequently

the bioavailability of the drug [15, 16]. This material has been used in the form of nanocapsules vesicular systems. Drugs are confined in cavities consisting of an inner liquid core surrounded by a polymeric membrane, for the delivery of indomethacin in anti-inflammatory and nociceptive activities in a chemical model of acute inflammation, theophylline for the anti-inflammatory and antiallergic effect or more generally as effective transdermal delivery of various hydrophobic drugs [16, 20–22]. To the best of our knowledge, we did not find a report of chitosan-nanocapsules loaded with *Tetrapleura tetraptera* aqueous extract for its anti-inflammatory effect. This paper aims to assess the *in vitro* and *in vivo* anti-inflammatory effect of cellulose chitosan biocomposite cross-linked with *Tetrapleura tetraptera* polyphenols.

2. Materials and Methods

2.1 Plant material

The plant material consists of fresh and ripe fruits of *Tetrapleura tetraptera* harvested in the central region of Cameroon and identified at the National Herbarium of Cameroon under the reference number 1727/SRFK (Fig. 1). After harvesting, the fruits were washed, cut into slices, dried and then freeze-dried. After that, the dried material was ground using a blender to obtain a powder. The decoction was made by soaking 100 g of powder in 1 L of distilled water for 30 min and heated to 80 ºC. The mixture obtained was filtered using a Wattman N°1 filter paper. The filtrate was used for biocomposite preparation.

2.2 Animal material

The study was carried out on young adult Wistar rats (*Rattus norvegicus*) raised in the animal facility of the Faculty of Medicine and Pharmaceutical Sciences of Douala. The selected rats were 8 to 12 weeks old and weighed between 120 and 180 grams. Rats that became ill or died, as well as nonpregnant rats, during acclimatization were excluded. These rats were divided into batches for acclimatization before the test. The ethical clearance Nr. 3158 CEI-Udo/06/2022/T was obtained to the Institutional Research Ethics Committee for Human Health of the University of Douala (CEI-UDo).

2.3 Reagents and chemicals

Medium molecular weight chitosan (viscosity 200–500 cP, 0.5% acetic acid at 20°C), glacial acetic acid (AR grade), and sulfuric acid were purchased from Friedemann Schmidt. Glutaraldehyde (25%) was obtained from Thermo Fisher Scientific. Cellulose microcrystalline was purchased from Alpha Aesar.

2.4 Phytochemical screening

The phytochemical screening was performed using the well-documented protocol as follows. Briefly, ferric chloride ($FeCl_3$) was used for tannin detection, cyanidin reaction with cyanidin (hydrochloric alcohol) for flavonoids detection, Dragendorff reaction for alkaloids detection, absolute alcohol for the detection of mucilages, the Fehling liquor test for the detection of reducing compounds and the foam index test for the detection of saponins [2].

2.5 Synthesis of biocomposite of the aqueous extract of *Tetrapleura tetraptera* made from cellulose and chitosan

2.5.1 Preparation of cellulose nanocrystals

It was carried out following the method used by Hassan et al., 2014 [4]. Cellulose was hydrolyzed under stirring with 65% sulfuric acid at 45 ° C for 45 min. Hydrolysis was stopped by diluting the reaction mixture with 10 times the volume of cold distilled water (~ 4°C). The nanocrystals were collected by centrifugation for 10 min at 6500 rpm for 30 minutes and washed with distilled water until the pH of the supernatant reaches 6.5.

2.5.2 Preparation of the biocomposite

The preparation of the composite was carried out by the ionic gelation method as described by Calvo and coworkers [5]. Briefly, 50 g of chitosan was dissolved in 500 mL of 1% (w/v) acetic acid under magnetic stirring, and the pH was adjusted to 5 using sulfuric acid. Cellulose nanocrystals (2.5%) were added to the chitosan solution (S1). A solution of sodium tripolyphosphate (STPP) was prepared concomitantly by mixing 2400 mg of STPP powder in 600 mL of distilled water (S2). Biocomposite synthesis was made by dropwise addition of the STPP solution (S2) solution in the chitosan/nano cellulose solution containing the extract (S3). The mixing was carried out with magnetic stirring at 500 rpm at room temperature. The suspension of composite was subjected to ultrasound for 5 min at 20% amplitude, then they were collected after centrifugation at 6500 rpm for 30 minutes and finally washed with distilled water.

2.6 Encapsulation efficiency: dosage of total polyphenols

The strategy here was to assess the amount of polyphenol in the initial solution compared to that of the supernatant after encapsulation. Briefly, the determination of total phenols was carried out by the Folin-Ciocalteu method. 1 mL of the sample (S3 and supernatant) was added to 5 mL of a Folin-Ciocalteu (10% in water) solution and incubated for 30 minutes. 4 mL of a sodium bicarbonate solution (0.7 M) was subsequently added to the above mixture, then stirred vigorously and incubated for 2 hours at room temperature in dark. The absorbance of the final solution was measured by UV-Vis spectrophotometry at the wavelength of 765 nm. A calibration curve was done with a range prepared from gallic acid in the same way as the previous samples at concentrations of 0.3; 0.6; 0.9 and 1.2 µg/mL. Total polyphenol concentration was calculated from the regression equation of this calibration curve and expressed in milligram equivalents of gallic acid per gram of extracted weight (mg EQ/g E) [6]. The encapsulation efficiency is shown in Eq. (1):

EEP = [(TPT of S3-TPT of supernatant)/(TPT of S3)] x100 (1)

2.7 Ultraviolet-visible characterization (UV-Vis)

UV-Vis spectroscopy was monitored using an aliquot of 2 mL of biocomposite suspension on a UV-Visible GENESYS 10S UV-VIS Spectrophotometer (Thermo). Measurements were made between 200 and

800 nm.

2.8 Fourier Transformed Infrared spectroscopy (FTIR)

FTIR was performed using a Bruker Tensor 37 with attenuated total reflection, ATR unit by scanning between 600–4000 cm^{-1}.

2.9 Powder X-ray diffraction (PXRD)

The PXRD diffraction measurements of chitosan, cellulose and chitosan-cellulose *Tetrapleura tetraptera* nanocomposites were carried out using a Bruker D2 Phaser powder diffractometer (Cu K-Alpha1 [Å] 1.54060, KAlpha2 [Å] 1.54443, K-Beta [Å] 1.39225) by preparing a thin film on a low-background silicon sample holder.

2.10 Scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDX) investigations

Scanning electron microscopy (SEM) was performed with a Jeol JSM − 6510LV QSEM Advanced electron microscope with a LaB_6 cathode at 20 kV equipped with a Bruker Xflash 410 (Bruker AXS, Karlsruhe, Germany) silicon drift detector for energy-dispersive X-ray spectrometric (EDX) analysis. The samples were sputtered with gold by using a JEOL JFC-1200 Fine Coater.

2.11 Acute toxicity

The acute toxicity test was carried out according to the guidelines of the OECD 425 protocol with a test limit at 2000 mg/kg body weight (BW) [3]. Two batches of three female rats were used, the first batch received 2000 mg/kg BW of biocomposite of the aqueous extract of *Tetrapleura tetraptera* (NCt) and the other batch received the distilled water. The rats were weighed and fasted, but with free access to water 12 hours before the administration of the NCt. Doses were administered once per gavage. After that, the rats were individually observed for 14 days with particular attention to the first 30 min after administration: then 4, 8 and every 24 hours. The observations were based on changes in skin, hair, mucous membranes, eyes, and behavior and observation of various manifestations such as tremors, convulsions, salivation, diarrhea, lethargy, sleep, and coma. The rats were weighed every other day and the growth rate (% X) was calculated using Eq. (3):

%X=((mass on day x-initial mass))/(initial mass) x100 (3)

On day 14, rats were sacrificed under anesthesia using an ether solution in a closed jar. The liver, kidneys, lungs, heart, and spleen weighed and the combined organ mass was calculated by Eq. (4):

Organ mass = [(organ mass) / (rat mass on the day of sacrifice)] x100 (4)

2.12 Anti-inflammatory activities

2.12.1 *In vitro* anti-inflammatory

The inhibition of the heat-induced denaturation of bovine serum albumin was carried out with slight modifications [23]. Thus, the reaction medium consisted of 0.200 µL BSA, 2800 µL phosphate-buffered saline (pH 7.4) and 200 µL of biocomposite solutions at various concentrations (200, 100, 50, 25 µg/mL). Distilled water was used as negative control and diclofenac was used as positive control and tested under the same conditions as nanocomposite. The mixture was subsequently incubated at 37°C for 15 min and then heated at 70°C for 5 min [7]. After the mixing, the absorbance was measured at 660 nm. The percentage inhibition is calculated by Eq. (2):

$\%INH_{bsa}$=(Abs control-Abs samples)/(Abs control) x100 (2)

Abs: absorbance; $\%\ INH_{bsa}$: percentage inhibition.

2.12.2 *In vivo* anti-inflammatory activity

The carrageenan induces hind paw oedema was done as previously described in the literature [8]. Briefly, the female rats were weighed and divided into 6 batches of 4 animals and fasted for 12 hours before the experiment. The initial diameter of the left hind paw (D0) was measured using an electric calliper for each animal. Each batch of rats received, respectively, distilled water (10 ml/kg BW), diclofenac anti-inflammatory reference at a standard dose (100 mg/kg BW), biocomposite solutions made of the aqueous extract of *Tetrapleura tetraptera* at doses of 200, 400, and 800 mg/kg BW. 30 minutes after oral administration of the various substances, each rat received an injection of 100 µL of 1% carrageenan under the plantar pad of the left hind paw. For each rat, the diameter of the hindpaw was recorded at t = 30 min, 1 h, 2 h, 3 h, 4 h, 5 h, and 6 h after induction of oedema. The variation of the oedema (X) was assessed at each instant with the formula 5 below:

$X = D_{t+1} - D_t$ (5)

D_{t+1}: diameter of the oedema at time t + 1; Dt: diameter of the oedema at the time t

Anti-inflammatory activity was evaluated by calculating the percentage inhibition (% INH) of edema according to the formula 6 below:

$\%INH_{car}$=[(X control-X treated)/(X control)]x 100 (6)

2.13 Statistical analysis

Microsoft Excel software was used to record crude data. The results were compared by two-way ANOVA using a t-test (student test) followed by the Tukey's multiple comparisons at the 95% threshold using GraphPad Prism version 8.0.1 for Windows software.

3. Results and discussion

3.1 Synthesis and characterization of cellulose and chitosan biocomposite in the aqueous extract of Tetrapleura tetraptera fruits

The mixture of cellulose, chitosan and plant polyphenols can produce materials with enhanced biomedical properties. From 100 g of dried fruits of *Tetrapleura tetraptera*, 8.7 g of crude extract were obtained, which yielded 8.7% extractable. The phytochemical screening was performed and the main results are presented in Table 1. From that table it was found that the extract contained saponins, reducing compounds, tannins, flavonoids, mucilages, leucoanthocyanins, anthraquinone, coumarin, quinone derivatives, sterol, and polyterpenes. However, alkaloids, anthocyanins, free anthracenes, as well as O-heterosides and C-heterosides were not detected using standard screening protocols. The encapsulation efficiency 69% was obtained from 20 mL of chitosan solution (1% m/v)/cellulose nanocrystals containing (2.5% m/v) aqueous extract of *Tetrapleura tetraptera* (0.1% m/v) and 10 mL of (0.4% m/v) STPP. The material encapsulation efficiency was found lower than encapsulation obtained using only chitosan and *Strychnos phaetricha* liana bark in water (73%) [24].

Table 1
Phytochemical screening of the crude aqueous extract of *Tetrapleura tetraptera*

compounds	Results
Alkaloids	-
Flavonoid	+
Gallic tanin	+
Catechic tanin	+
Anthocyanin	-
Leuco-anthocyanins	+
Quinone Derivative	+
Sterol et polyterpenes	+
Mucilage	+
Reducer compounds	+
Anthraquinone	+
Coumarins	+
Free Anthracene	-
O et C-heterosids	-
Saponins	+
Present (+); Absent (-)	

3.2 UV-Vis of the biocomposite

The UV-Vis spectrum of the biocomposite showed a peak at 296 nm, and a shoulder. Absorption signals located at wavelengths < 250 nm are generally attributed to organic metabolites and/or polymers or noise (Fig. 2). Materials obtained by a mixture of chitosan and cellulose have presented transitions between 205–245 nm and shoulders between 300–450 nm [25]. This is in good agreement with the present results.

3.2 FTIR Spectroscopy

Infrared spectroscopy gives without sample destruction information about functional groups present in extract, polymer or powder samples. It was used to study the chemical changes in the hybrid composite material. FTIR spectra of cellulose and chitosan and the biocomposite (Fig. 3) show vibrations around 894 and 1153 cm^{-1} related to the saccharide structure [26]. The band at 1313, 1385, and 1385 cm^{-1} is attributed to the vibration of C-H for cellulose, chitosan and the biocomposite respectively [26]. The vibration at 1558 cm^{-1} corresponding to the amino group of chitosan is shifted to 1545 cm^{-1} in the biocomposite.[26] Bands at 1650, 1660, and 1646 cm^{-1} for cellulose, chitosan and the biocomposite respectively can be attributed to the amide I vibration.[26] Vibration at about 2900 cm^{-1} can be attributed to CH_2 and C–H stretching and CH_3 asymmetric and symmetric stretching vibrations [27, 28]. The broad band from 3000 to 3600 cm^{-1} is due to the stretching vibrations of the OH groups of polyphenols in the biocomposite [26]. The FTIR spectrum of cellulose and chitosan revealed the following specific maxima peaks at 3287, 3346 and 3287, 3360 cm^{-1} attributed to O–H stretching from hydroxyl groups and stretching vibrations of N–H bonds from amino groups [27]. Both peaks disappears in the biocomposite due to interactions between O-H and N-H or polyphenols secondary metabolites. Moreover the two bands at 3621, and 3694, cm^{-1} inside biocomposite can be assigned to the OH stretching of inner surface hydroxyl due to the effects of interlayer modification [29]. The FTIR of cellulose, chitosan, and the plant-derived biocomposite are widely similar. Some shifts are observed because a new polysaccharide is formed. Infrared spectroscopy analysis shows the presence of polyphenols within the biocomposite that can act as crosslinkers [30].

3.3 PXRD

A study by powder X-ray diffraction of the extract with cellulose-chitosan material proves the presence of both polymers and shows that the mixture is composed of amorphous and crystalline regions [25]. As shown in the diffractogram (Fig. 4), cellulose exhibited peaks around 2θ = 14.9°, 16.4°, 20.4°, 22.5° and 34.3 °, respectively represent the crystallographic planes (1–10), (110), (102), (200) and (004) of a typical cellulose I structure. In addition, a small peak appeared at around 2θ = 12.5°, which corresponded to the (1–10) crystallographic plane of cellulose II. Two characteristic broad diffraction peaks are evident in pure chitosan; these peaks are typical fingerprints of semicrystalline chitosan. These diffraction peaks, found at 2θ = 9.2°, and 2θ = 20.1° respectively represent the diffractions from the (020) and (110) planes

of the chitosan crystalline lattice.[31] Nanoparticles sizes were calculated using the strongest signals to yield: 5 nm in cellulose for the (200) crystallographic planes situated at $2\theta = 22.5°$; 52 nm in chitosan for the 020 crystallographic planes situated at $2\theta = 9.2°$: 53 nm in the biocomposite using the 200 planes situated at $2\theta = 22.6°$. The four crystallographic plane patterns appear slightly shifted and agree well with the mixed composition of both polysaccharides [32]. Previous reports on nanocomposites describe broad peaks that can be shifted together with decrease of crystallinity. Only the highest peak is used in the size calculation leading to similar grain size for chitosan and the biocomposite [33]. The cellulose-chitosan biocomposite size of 53 nm is inside the range of other chitosan hybrid materials (46–58 nm) obtained using *Eucalyptus* oils [34]. Maxima of PXRD peaks were found to be varied with increasing content of chitosan in blended films or by material grinding using a planetary ball mill [25, 35].

3.4 SEM and EDX

SEM shows aggregates with linear, spherical, cuboid and smooth forms (Fig. 5). The material is found composed of carbon and oxygen with energy dispersive X-ray spectroscopy. The surface morphology shows an aggregated material composed of C and O. Previous studies show that pure chitosan matrix exhibited a relatively smooth surface [31].

3.5 Acute toxicity

The results from the toxicity testing showed no death, no clinical signs of toxicity as well as no difference between the relative weights of the vital organs in comparison with the normal rats (Fig. 6). No sign of toxicity was observed using the acute toxicity texting. Animal growth was not affected by biocomposite treatment and organ weight wasn't significatively different compared to the control group that received water. This is consistent with the fact that the *Tetrapleura tetraptera* is an edible plant [36, 37]. Additionally, nanocomposites are well established as nontoxic agents [38–40].

3.6 Anti-inflammatory activity

3.6.1 *in vitro* anti-inflammatory effect of NCt on heat-induced protein denaturation

The results from the BSA stability (Fig. 7) revealed that the NCt biocomposite has a better inhibitory effect than diclofenac with an IC_{50} of 9.4 and 122.0 µg/mL respectively. Protein denaturation is one of the causes of complications of inflammatory diseases: therefore, *in vitro* anti-inflammatory activity was evaluated using the protein denaturation inhibition method [41,42]. The crude extracts of *Tetrapleura tetraptera* have shown inhibition of BSA denaturation lower than diclofenac [43]. The biocomposite in this report exhibits significantly higher BSA activity compared to diclofenac.

3.6.2 *in vivo* Anti-inflammatory effect of NCt biocomposite on carrageenan-induced paw oedema in rats

The Fig. 8 shows the effect of NCt on carrageenan-induced paw oedema in rats. NCt successfully inhibits the appearance of oedema in a time-dependent manner. There were no statistically significant differences between the effect of the NCt, that of the extract and that of diclofenac used here as a reference. A non significative anti-inflammatory effect on carrageenan-induced paw oedema in rats of the diclofenac is obtained between 0.5 and 6 hours of treatment ($P > 0.3346$). Moreover, NCt in this report, at all concentrations, 200–800 µg/mL, and each time (except at 30 minutes) has a similar activity than that of the crude extract at 800 µg/mL and to diclofenac 100 µg/mL (positive control). The biocomposite with cellulose and chitosan has a high effect when administered at low concentrations compared to the crude extract. Similar observations have been reported previously by using ibuprofen-loaded nanoparticles based on sodium alginate and chitosan [44]. Carrageenan induces oedema under experimental conditions, the volume of which is maximal after 1 hour. When injected into the foot paw, it causes local inflammation which causes the release of several chemical mediators. Carrageenan-induced oedema is a triphasic response that involves the release of various mediators including histamine and serotonin in the first phase (1 hour). The second phase is mediated by the release of kinins (2 hours) and the third phase is attributed to prostaglandins and cyclooxygenase products, which last 3–5 hours [45]. Amaral et al. reported that the anti-inflammatory effect of bullfrog oil nanocapsules was more effective in carrageenan induce paw oedema compared to bullfrog oil alone [46].

4. Conclusion

This study highlights the anti-inflammatory activity of the *Tetrapleura tetraptera* biocomposite made of cellulose and chitosan. The composite size 53 nm consist of smooth, spherical, and cuboids grains with carbon and oxygen as elementary material. The composite combine the polysaccharides cellulose and chitosan crosslinked together and with *Tetrapleura tetraptera* polyphenols. The biocomposite exhibits a high inhibitory effect on BSA-induced denaturation *in vitro* and a similar anti-inflammatory effect of on carrageenan-induced paw oedema in rats compared to diclofenac as positive control. New polysaccharides based *Tetrapleura tetraptera* cellulose and chitosan may find usability in the management of inflammation diseases.

Declarations

Ethical approval

The ethical clearance Nr. 3158 CEI-Udo/06/2022/T was obtained to the Institutional Research Ethics Committee for Human Health of the University of Douala (CEI-UDo).

Competing interests

The authors declare that they have no conflict of interest

Author contributions

Conceptualization: EMF; Synthesis: NAC; Instrumental Analysis: EMF, NAC, THYB, FNAM; Animal models and bioassay: NAC, MEN, CNMJ, NMP, NYBMTM, SSP, NKCR, KJ, SEB; validation: NAA, EMF, HFJB; investigation: HFJB, NAC; resources: HFJB; writing - original draft: HFJB, NAC, EMF; writing - review & amp; editing: EMF, CJ; visualization: HFJB, NAC, EMF; supervision: EMF.

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Figures



Figure 1

Fresh fruit of *Tetrapleura tetraptera*

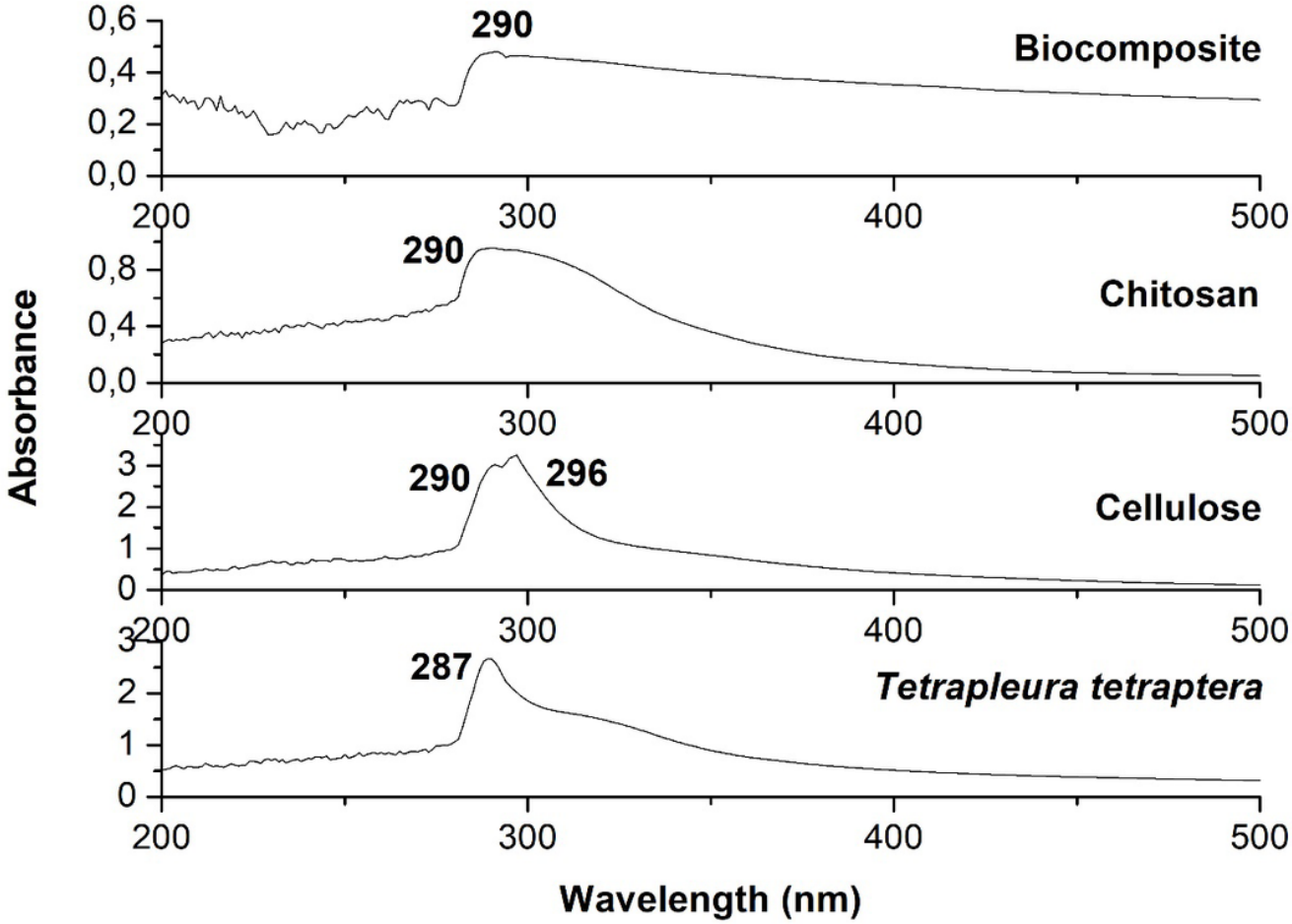


Figure 2

Spectrophotometry profile of cellulose, chitosan, *Tetrapleura tetraptera* extract and the biocomposite

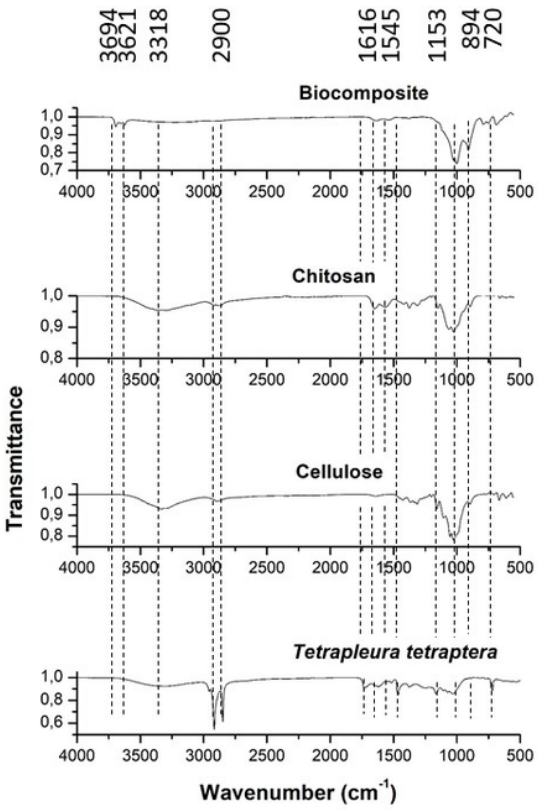


Figure 3

FTIR spectra of biocomposite, chitosan, cellulose, and *Tetrapleura tetraptera*.

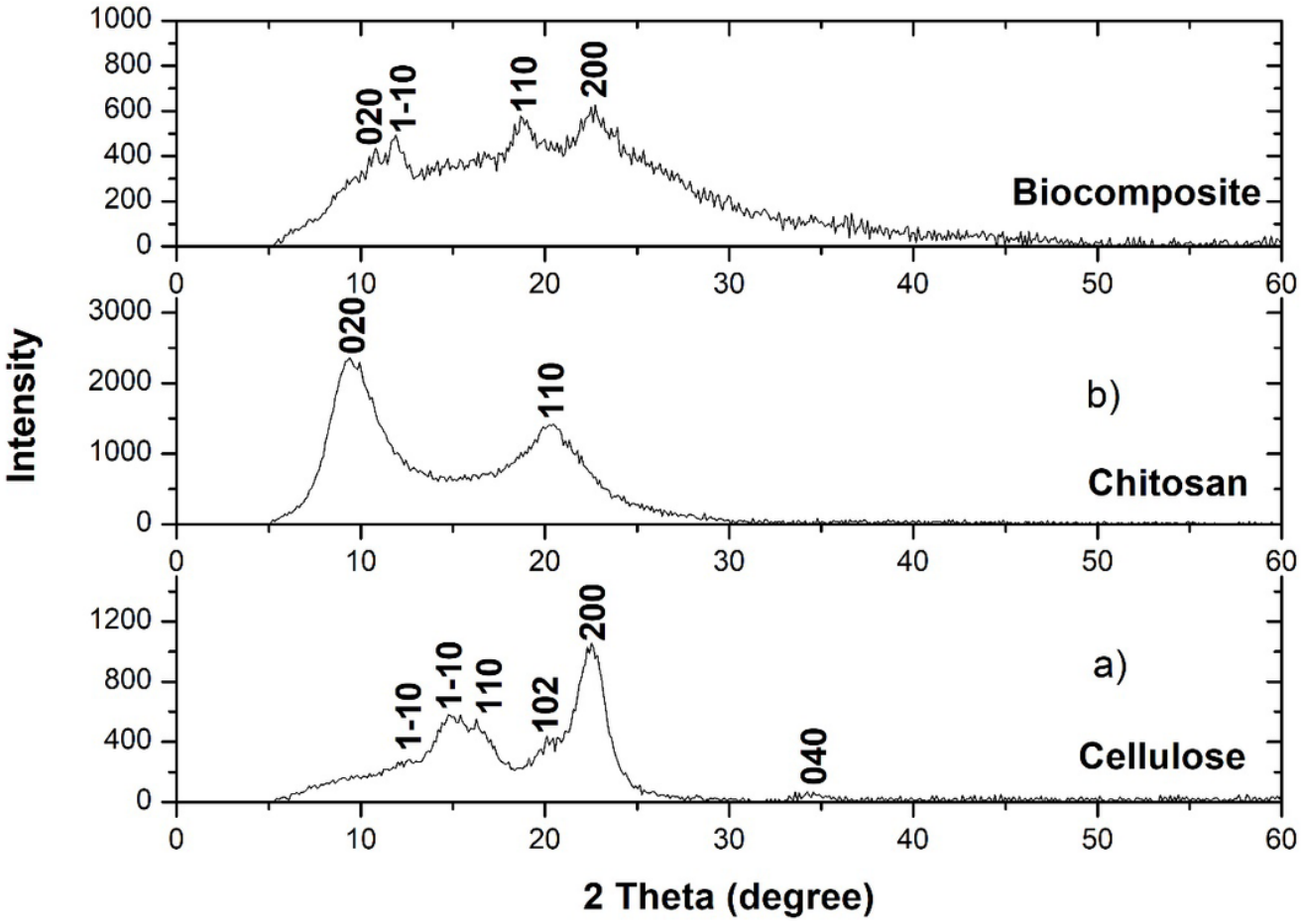


Figure 4

Powder X-ray diffractograms of biocomposite, chitosan, and cellulose

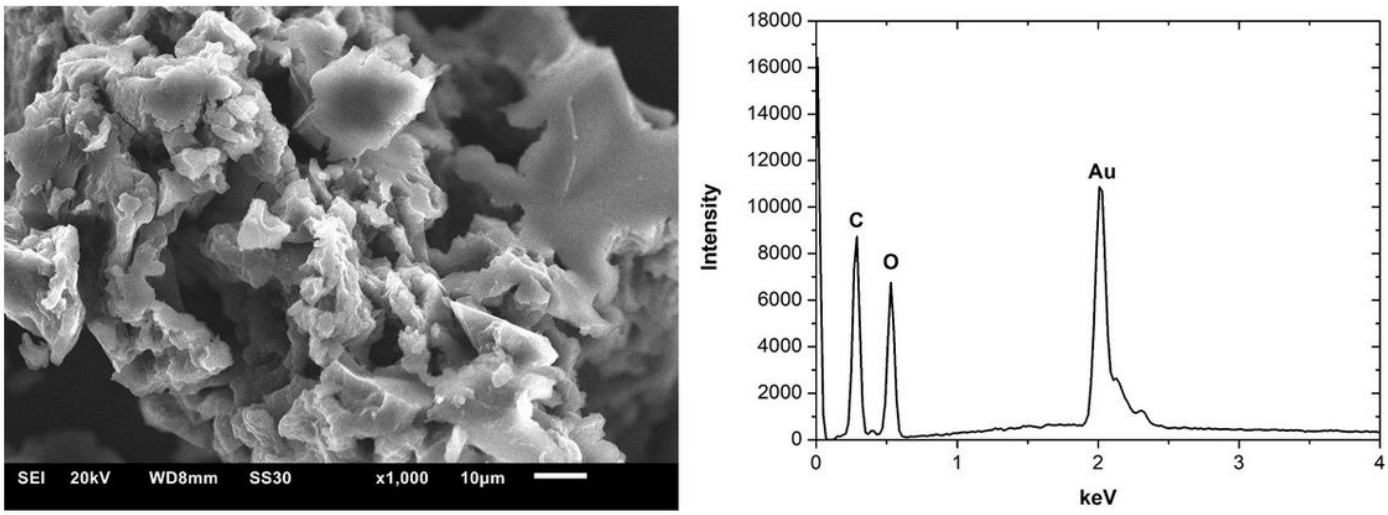


Figure 5

Scanning electron microscopy and EDX spectrum of the nanocapsule.

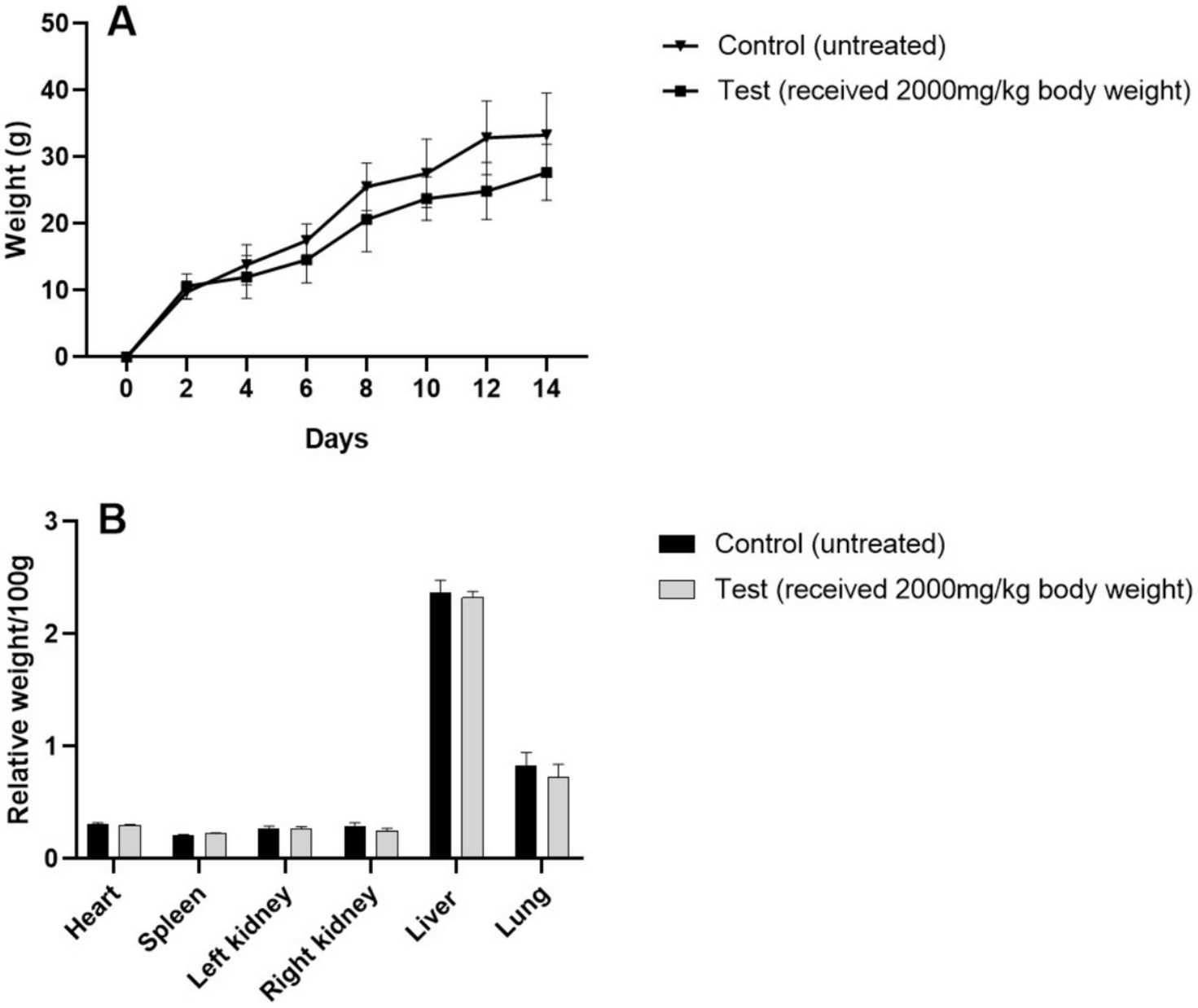


Figure 6

Body weight (A) and relative organ weight (B) of treated and untreated rats

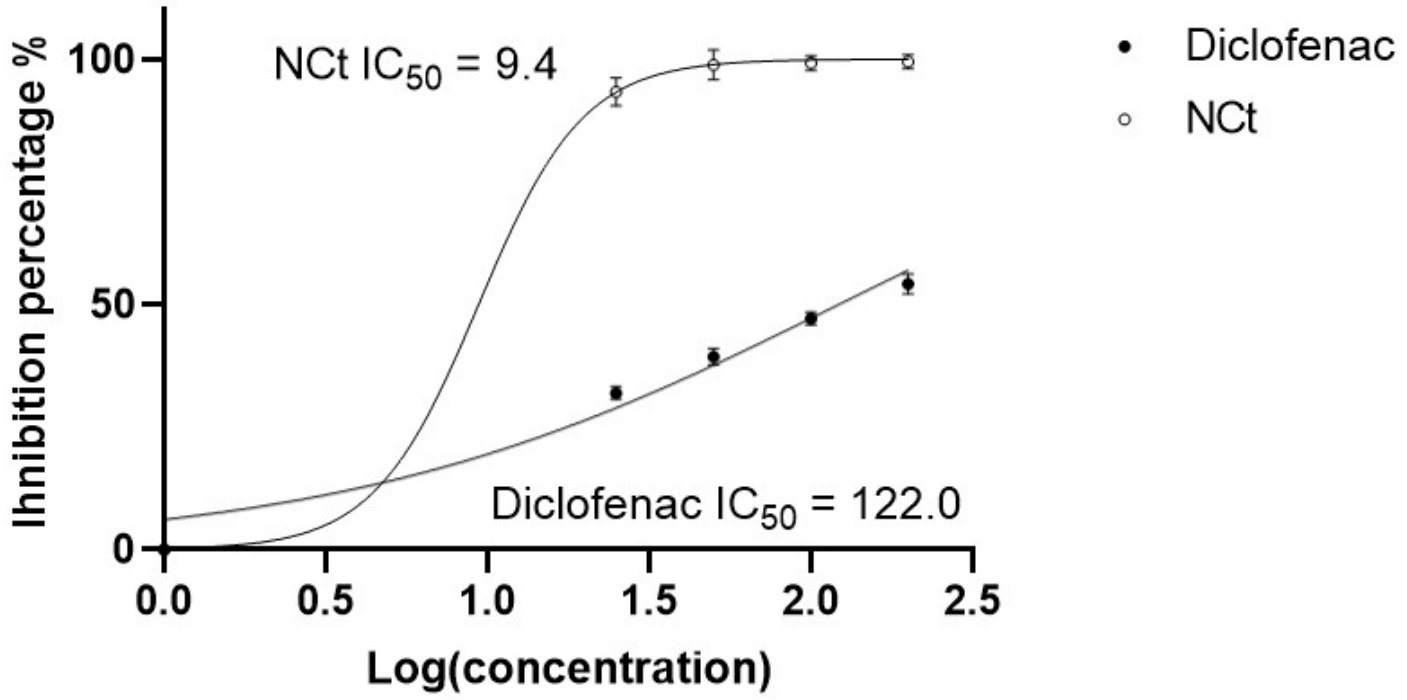


Figure 7

Inhibition of heat-induced denaturation of NCt and Dichlofenac

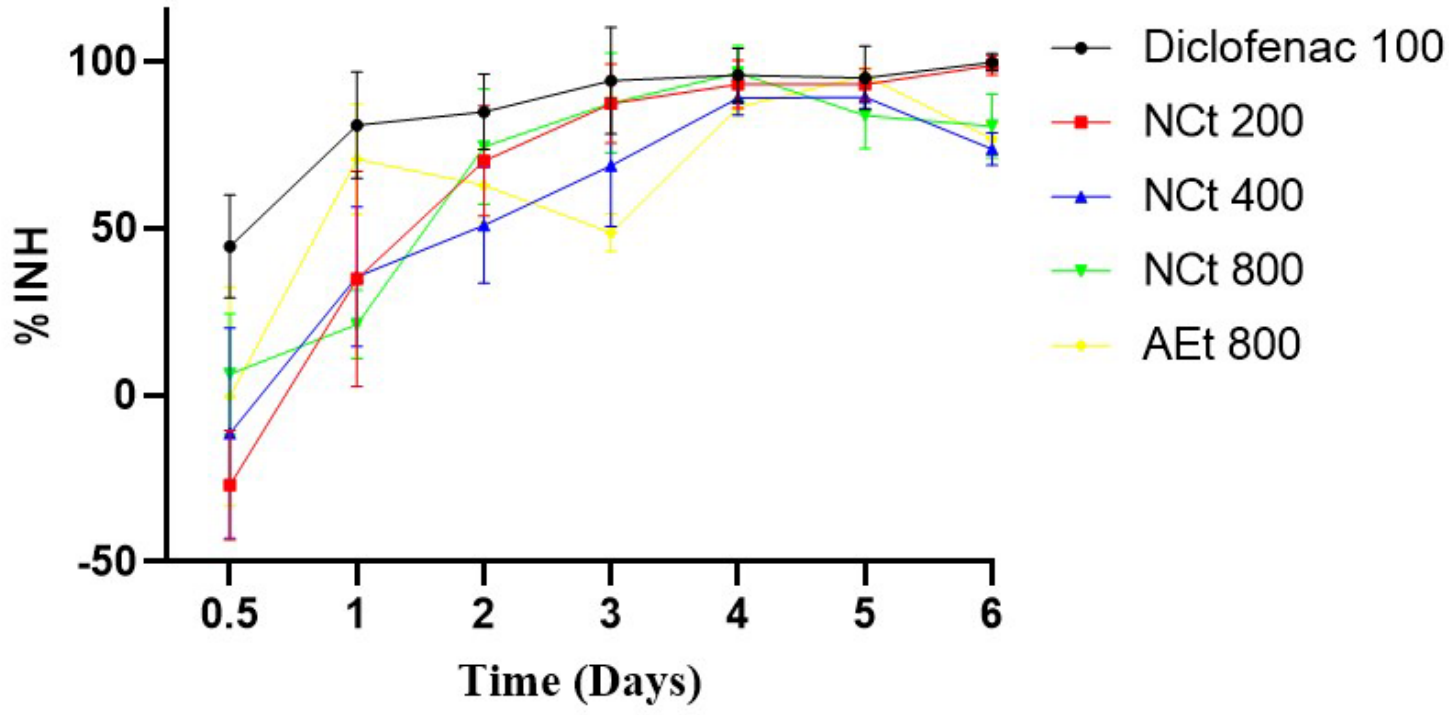


Figure 8

Effect of NCt, extract and Diclofenac on carrageenin-induced hind paw edema.

Diclo-100= diclofenact at 100mg/kg bw; NCt 200 = biocomposite of *Tetrapleura tetraptera* at 200mg/kg bw; NCt 400= biocomposite of *Tetrapleura tetraptera* at 400mg/kg bw; NCt 800= biocomposite of *Tetrapleura tetraptera* at 800mg/kg bw; AEt= Aqueous extract of *Tetrapleura tetraptera* at 800mg/kg bw