Original Research Article

ANTINOCICEPTIVE AND ANTI-INFLAMMATORY PROPERTIES OF *Triumfetta cordifolia* A. RICH. (TILIACEAE) ROOT METHANOL/DICHLOROMETHANE EXTRACT

Abstract

Objective: *Triumfetta cordifolia* is a terrestrial plant of the Tiliaceae family used traditionally for the treatment of many diseases. Different parts are traditionally used for the treatment of many diseases that cause pain and inflammation. This study aims to evaluate the antinociceptive and anti-inflammatory activities of the methanol/dichloromethane extract of *T. cordifolia* roots in animal's model.

Methodology: Phytochemical screening and the study of toxicity methanol/dichloromethane extract of roots of *Triumfetta cordifolia* was performed. Antinociceptive activity was evaluated using acetic acid and formalin tests. Anti-inflammatory activity was evaluated *in vitro* using bovine serum albumin (BSA) denaturation method and *in vivo* on carrageenan-induced oedema. The extract was given orally at the doses of 50, 100 and 150 mg/kg of body weight.

Results: The phytochemical analysis revealed the presence of alkaloids, saponins, flavonoïds, polyphenols, reducing sugars, tannins, coumarins and steroids. No signs of toxicity were observed after 14 days experiment. Regarding antinociceptive activity, *Triumfetta cordifolia* (200 mg/kg) has decreased significantly ($P < 0.0001$) the number of abdominal contortions and also significantly inhibited the formalin-induced neurogenic pain at the dose of 50 mg/kg body weight. In anti-inflammatory tests, the extract inhibited oedema ($P< 0,001$) at 50mg/kg at the fourth hour and also protected the serum albumin bovine denaturation ($P <0.05$) at a concentration of 50 µg/ml.

Conclusion: The results obtained indicate that the methanol/dichloromethane extract is without acute toxicity and has peripheral and central analgesic properties, as well as anti-inflammatory properties in vivo and vitro.

Keywords: *Triumfetta cordifolia*, methanol/dichloromethane extract, analgesic, anti-inflammatory, phytochemical screening, toxicity.

1. INTRODUCTION

Pain is defined as an unpleasant sensory and emotional experience associated with or resembling that of actual or potential tissue damage [1]. Pain can play a protective role, as is the case of acute inflammation that results in the perception of pain, leading to the avoidance of harmful stimuli and the encouragement of tissue healing [2]. Inflammation is defined as a process of immune defense of the body in response to a stimulus with endogenous or exogenous origin, which can be transmitted through infection, trauma, surgery, burns or necrotic tissue [3]. Painful and inflammatory conditions are a major health, social, and economic problem. According to International Association for the Study of Pain (IASP) criteria, the prevalence of chronic pain ranges from 15 to 35% in the global population. Approximately 1.71 billion peoples worldwide live with osteoarticular disorders such as low back pain, osteoarthritis, trauma, and rheumatoid arthritis [4]. Pain and inflammation are ailments that can cause significant physical / moral suffering, responsible for the major alteration in the quality of life of patients. Therefore, regardless of the origin, painful and inflammatory conditions must be managed. In conventional medicine, the World Health Organization (WHO) recommends the use of analgesics (non-opioids weak opioids, and strong opioids) and anti-inflammatory drugs as a treatment. However, their use exposes the patient to many risks and side effects. The risks encountered are gastric ulcers, kidney failure, withdrawal syndrome, and addiction [5].

The use of plants in herbal medicine dates back to antiquity and has grown with the evolution of civilization in all regions of the world. Plants have always held an important place in medicine. They are considered an essential source of raw material necessary for the discovery of molecules useful in the manufacture of new drugs [6]. *Triumfetta cordifolia* is a species of the Tiliaceae family whose decocted leaves and macerated bark are used to treat lumbago and muscle pain [7]. The plant is consumed in western Cameroon as a traditional meal named "Nkui" [8]. HPLC analyses of methanol extract of *T. cordifolia* leaves collected in Bayelsa State, Nigeria, showed that kaempferol was the major flavonoid compound, followed by quercitrin, (+) catechin, luteolin, quercetin, apigenin, rutin and myricetin [9]. Moreover the plant extract possess a notable anti-inflammatory effectiveness against formalin-induced paw edema [9]. This research was carried out to increase the literature on the anti-inflammation properties by confirming their traditional use in the treatment of pain. The analgesic

and anti-inflammatory properties of the methanol/dichloromethane extract of the roots of *T. cordifolia* is described.

2. MATERIALS ANS METHODS

2.1. Chemicals used.

Paracetamol 500 mg (Ubigen Laboratory), acetic acid 1%, formaldehyde 1%, Tramadol 50 mg (Tramadol^{R} 50 mg Cooper Laboratory), BSA (bovine serum albumin) 4%, PBS buffer at pH 6.6, carrageenan 1%, diclofenac 10 mg/kg (Ubigen Laboratory).

2.2. Plant material

The roots of *Triumfetta cordifolia* (Fig. 1) were collected in October 2021 in the commune of Bonaléa, canton of Abo south; village of Penda Mboko in Moungo Department, Littoral region, Cameroon. The identification was made at the National Herbarium of Cameroon, in comparison with the material of Breteler N^{o} 515 of the specimens of the Herbarium Collection N^{o} 10792 SRF Cam.

A **B**

Fig. 1. Photography of Aerial part (A) and roots (B) of *Triumfetta cordifolia*

2.3. Preparation of the extract

After harvest, the fresh roots (2200g) of *T. cordifolia* were cleaned and grounded to obtain the dough. The dough obtained was macerated three times in the mixtures of methanol /dichloromethane (70 / 30) for 72 hours at room temperature. After filtration, the filtrate was concentrated to obtain 59.9 g (yield: 2.72%) of crude extract.

2.4. Animals

Wistar albino rats aged 50 -70 days, weight 95 - 115 g were used for the acute toxicity test; rats aged of 60 - 90 days ,120-180 g for anti-inflammatory activity and *Swiss* mice (age 70 - 100 days, 19 and 32 g) for analgesic tests. The animals were raised in the animal facility of the Faculty of Medicine and Pharmaceutical Sciences of Douala in plastic cages, under normal conditions of temperature (23-30 °C) and alternation 12 h of light /dark cycle. They had free access to water and food. All experiments were carried out according to the protocol approved by the Institutional Ethics Committee for Health Science of the University of Douala (protocol approval number 3165 CEI-Udo/06/2022/T).

2.5. Evaluation of acute toxicity assessment

The acute toxicity assessment of the extract was conducted using the protocol for the limit test proposed by the Organization for Economic Co-operation and Development (OECD) guidelines number 425 (2008) [10]. The study was carried out over 14 days in 9 females Wistar rats. After five-day acclimatization animals were fasted for 12 hours with free access to water prior to the study and distributed as follows: control batch consisting of 3 females receiving distilled water (10 mL/kg); two experimental batches of three females each receiving a single dose of extract dosed at 2000 mg/kg and 5000 mg/kg by oral route. Animals were observed for mortality and clinical signs of toxicity such as respiration, fecal consistence, aspects of the coat, diarrhea, vomiting, tremors, tachycardia, unrest, convulsions/coma/death during 3 hours after administration of extract. After those first hours of observation, the rat were further monitored daily for their body weight as well as the mortality over a period of 14 days. At the end of experiment, the rats were sacrificed under anesthesia with ether solution and internal organs such as liver, kidneys, lung, heart and spleen were removed, cleaned, weighed, and macroscopically observed.

2.6. Phytochemical screening

Phytochemical screening was carried out on the methanol/dichloromethane extract of *Triumfetta cordifolia* using standard procedures described by [11-13].

2.7. Evaluation of analgesic activity

2.7.1. Acetic acid test

The method used was described by Koster et al. (1959) [13]. Abdominal contractions were induced by intraperitoneal injection of acetic acid (1%, 10 ml/kg) in mice. Thirty minutes before acetic acid injection, mice were pretreated with the methanol / dichloromethane extract of *T. Cordifolia* (50, 100 and 200 mg/kg), distilled water (10 mL/kg) and paracetamol (150 mg/kg). The number of abdominal contortions per mouse was recorded for 30 minutes and this directly after the injection of acetic acid [14]. The analgesic effect was evaluated using the following formula 1 [15].

(Average of the contortions of the control group - Average of the contortions of the treated group) × 100/ Average of the contortions of the control. (1)

2.7.2. Formalin test

The formalin test method was described by Dubuisson and Dennis (1977) [16]. Formalin (1%, 20 µL) was injected into the subplantar of the right hind paw of the animals to induce pain. The paw licking time was observed for 0-5 min (early phase) and 15-30 min (late phase) after formalin administration [17]. Mice were treated with the methanol / dichloromethane extract of *T. cordifolia* (50, 100, 200 mg/kg), distilled water (10 ml/kg) or tramadol (10mg/kg); Thirty minutes before formalin injection. The percentage of inhibition (PI) of paw licking time was evaluated using equation 2:

$$(T - T')/T \times 100 \quad (2)$$

Where T represents the mean value of the time licking of the vehicle control group for each phase and T' represents the means value of the time licking of the assays group for each phase [16].

2.8. Evaluation of anti-inflammatory activity

2.8.1. *In vitro*: Bovine Serum Albumin denaturation test

The method used will be that of inhibition of heat induced Bovine Serum Albumin denaturation [18]. The reaction medium consisted of 0.2 ml BSA (0.4%, w/v) prepared in phosphate-buffered-saline buffer (PBS), 2.8 ml of PBS and 2 ml of our extract solution at the of concentrations 50, 100 and 200 µg/mL). As a controls, we used distilled water and diclofenac (reference drug). Subsequently, the mixture was incubated at 37°C for 15 minutes and then heated to 70°C for 5 minutes in water bath. After cooling the mixture, the absorbance is measured at 660nm and each experiment is repeated three times. The percentage of inhibition is calculated using formula 3:

Percentage of inhibition (%) = (absorbance of the test - absorbance of the negative control) x 100 / absorbance of the negative control. (3)

2.8.2. *In vivo*: carrageenan-induced rat paw edema experiment

The rats were fasted for 12 hours before the experiment. They were randomly divided into 5 groups of 5 rats each: Batch 1: negative control received a vehicle, distilled water at 10 ml/kg body weight. Batch 2: positive control received a reference drug, diclofenac at 10 mg/kg of body weight. Groups 3, 4 and 5 received extract at increasing doses of 50,100 and 200 mg/kg thirty minutes after oral gavage. Edema was induced in rats by injection into the right plantar aponeurosis of the rat of 0.1 ml of carrageenan 1%. Plethysmometric measurements were recorded before and after injection at 1/2h, 1h, 2h, 3h, 4h, 5h and 6h [19]. The percentages of inhibition (PI) were calculated using formula 4:

$$PI\ (\%) = ((Vt - Vo) - (Vt - Vo) \times 100 / (Vt - Vo) \quad (4)$$

Vt = average paw volume in each group at a time t

Vo = mean volume in each group before injection

2.9. Statistical analysis of results

Data are expressed as an average ± SEM. The calculation of averages and percentages of inhibition were done using the Microsoft Excel 2016 spreadsheet. comparisons between experimental and control groups were performed by one-way analysis of variance (ANOVA) followed by Turkey's post test and two-way analysis of variance followed by Turkey's post test using GraphPad Prism® software version 8.0.1. Differences are considered significant at the $p < 0.05$.

3. RESULTS

3.1. Phytochemical study

Phytochemical analysis of *Triumfetta cordifolia* extract revealed the presence of: alkaloids, saponins; flavonoids, sterols, terpenes, tannins, reducing sugars and coumarins (Table 1).

Table 1: Phytochemical screening of Triumfetta cordifolia methanol/dichloromethane root extract of *Triumfetta cordifolia*

Metabolites	**Methanol/dichloromethane roots extract of *Triumfetta cordifolia***
Alkaloïds	+
Saponins	+
Flavonoids	+
Sterols	+
Tannins	+
Reducing sugars	+
Coumarins	+
Terpenes	+
Anthraquinones	-

Anthocyanins	-

(+) Present (-) absent

3.2. Acute toxicity

3.2.1. Mortality and lethal dose 50

Triumfetta cordifolia root extract administered at doses of 1000 mg/kg and 2000 mg/kg body weight did not produce any mortality during the 14 days monitoring after extract administration. No signs of major toxicity or mortality were observed at any dose level after 14 days of acute toxicity testing. The lethal dose 50 (LD_{50}) has been estimated above 5000 mg/kg of body weight.

3.2.2. Behavioral changes and adverse reactions

During the first 3 hours after administration of *Triumfetta cordifolia* root extract at doses 2000 mg/kg and 5000 mg/kg; no signs of toxicity were revealed during these hours. During 14 days of observation, there was not major variation signs of toxicity in this work and no deaths were recorded (Table 2).

Table 2: Clinical signs of rats in acute toxicity of *Triumfetta cordifolia* root extract

Clinical Signs	Groups		
	Distilled water	Extract 2000mg/kg	Extract 5000mg/kg
Fecal consisting	N	N	N
Aspects of the coat	N	N	N
Diarrhea	A	A	A
Vomiting	A	A	A
Respiration	N	N	N
Tremors	N	N	N
Tachycardia	N	N	N
unrest	N	N	N

Convulsions	A	A	A
Death	A	A	A

Absence (A)/ Normal (N)

3.2.3. Effects of *Triumfetta cordifolia* root extract on body weight

We noticed a normal increase in the masses of all rats without significant differences between the control batch and the test batches (Fig. 2).

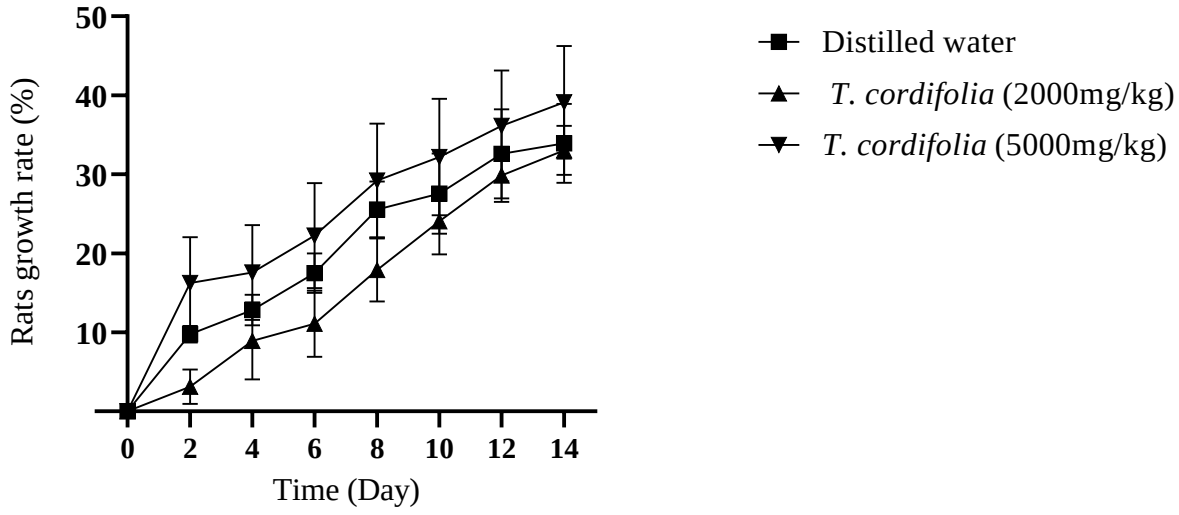

Fig. 2. Evolution of variation in rat body weight as a function of time

Each points represents the growth rate weight ± ESM, *T. c = Triumfetta cordifolia* at doses of 2000 and 5000 mg/kg.

3.2.4. Effects of *Triumfetta cordifolia* root extract on organ weights

There was no significant difference in organ weights of *Triumfetta cordifolia* root extract treated rats as compared to the control rats at the end of the acute toxicity study (Fig. 3).

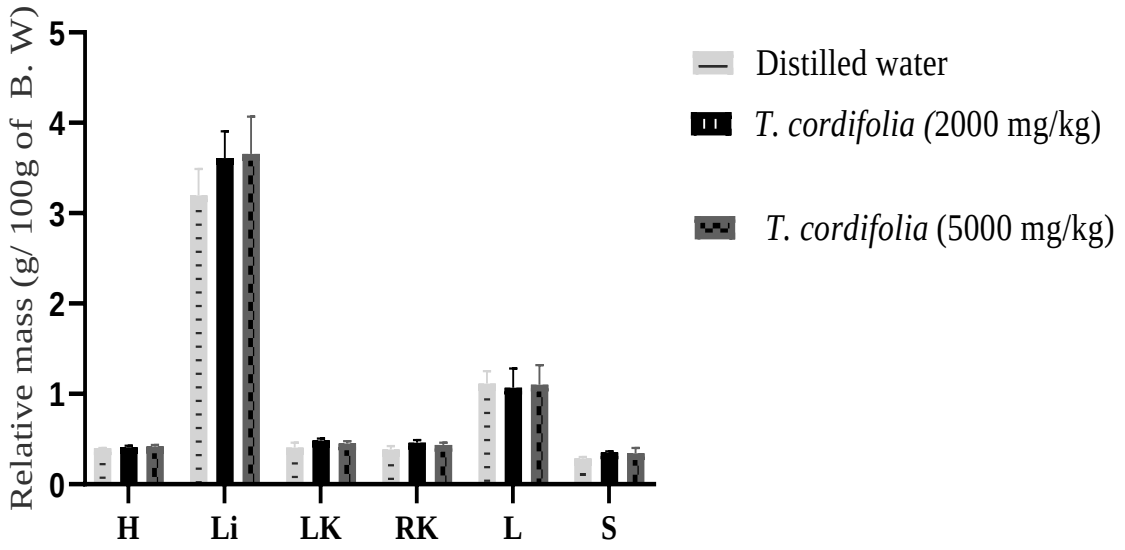

Fig. 3. Effect of *T. cordifolia* extract on relative organs mass

Each bar represents the average of relative organs weight; n=5; H= Heart, l=liver, LK=left kidney, RK=right kidney, L=lung, S=spleen.

4. Analgesic effect of the roots extract of *Triumfetta cordifolia*

The antinociceptive activity of the methanol/dichloromethane extract was evaluated in several experimental models: the acetic acid-induced writhing and the formalin test.

4.1. Effect of the extract on acetic acid-induced pain

The manifestation due to an intraperitoneal injection of acetic acid is characterized by abdominal writhings as shown in Fig. 4. We noticed that the plant extract significatively decrease ($P < 0.0001$) the number of abdominal writhings induced by intraperitoneal injection of acetic acid in rats. The highest inhibition was 79.45% at the dose of 200 mg/kg of body weight. Paracetamol used as a reference drug, has decreased the number of writhings (67.12%) at 50 mg/kg.

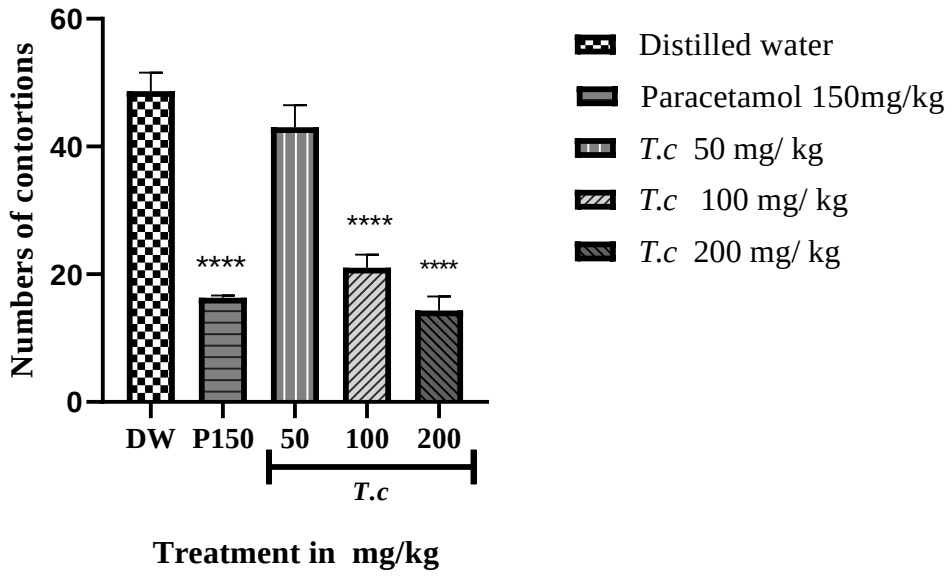

Figure 4: Effect of extract *Triumfetta cordifolia* on Number of abdominal contortions induced by acetic acid injection according to treatments

Each bars represents the average of abdominal contortions; n=5; ****P<0.0001 significant difference from negative control; NC = negative control, P150 = paracetamol at 150 mg/kg, *T. c = Triumfetta cordifolia* at doses of 50, 100 and 200 mg/kg.

4.2. Effect of the extract on formalin-induced pain

Subplantar injection of formalin induces biphasic pain in mice characterized by paw licking. Figure 5 shows us the licking time of the paw in the different phases of observation. It follows from this figure that in the first phase (0-5min) the licking time decreases from 46.92±3.99 seconds in animals of the negative control to 41.72±4.82s, 43.69±6.90s, and 30.92±3.76s respectively in animals treated with the extract at the respective doses of 50, 100, and 200 mg/kg. The respective percentages of inhibition obtained are: 11.07%, 6.86%, 34.10% ($p<0.0001$) compared to the negative control. During the second phase, the paw licking time decreased from 97.77±11.30s to 52.35±11.51s, 77.01±7.953s, and 93.77±11.83s for animals treated with the extract at 50, 100 and 200 mg/kg. Furthermore, tramadol used as reference substance inhibited pain at 35.44% ($P< 0.001$) in first phase (0-5min) and 53.04% ($P< 0.0001$) in second phase II (15-30 min).

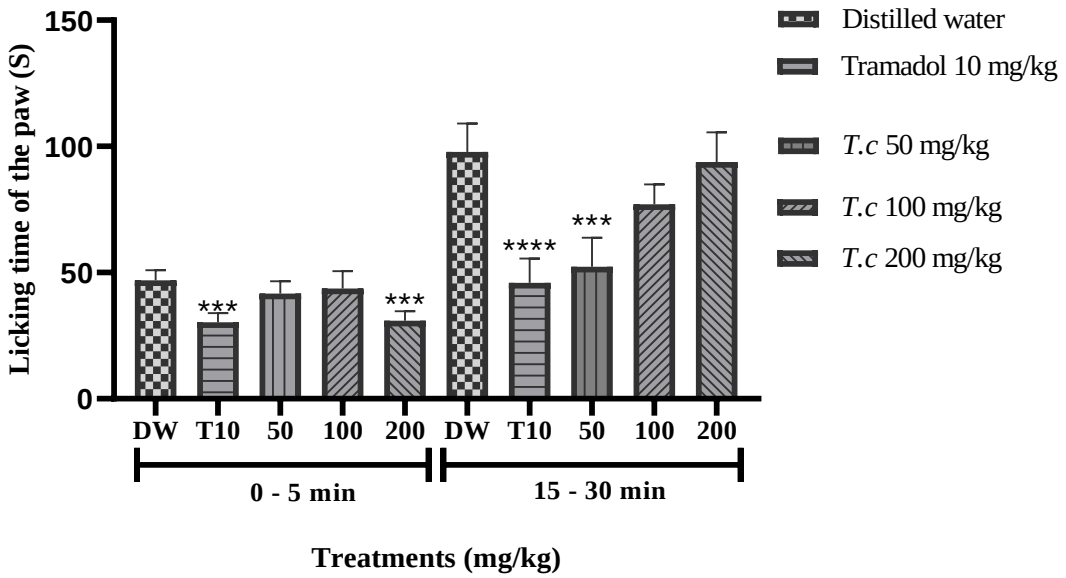

Figure 5: Effect of *T. cordifolia* on paw licking time induced by formalin injection according to treatments

Each bar represents the average of licking time ± SEM, n=4, ****P<0.0001 and *** P <0.001: significant difference from the negative control; NC = negative control, PC = positive control (tramadol 10 mg/kg), *T. c = Triumfetta cordifolia* at doses of 50, 100 and 200 mg/kg.

4.3. Anti-inflammatory effect of the root extract of *Triumfetta cordifolia*

4.3.1. Effect of *Triumfetta cordifolia* extract on BSA denaturation

The result of the evaluation of the *in vitro* anti-inflammatory effect of the bark of *Triumfetta cordifolia* is shown in Fig. 6. It is observed that the maximum inhibition of 99.66% is obtained at the concentration of 50µg/ml. Statistical analysis shows a significant difference (P< 0.05) between different concentrations of extract or diclofenac compared to the negative control.

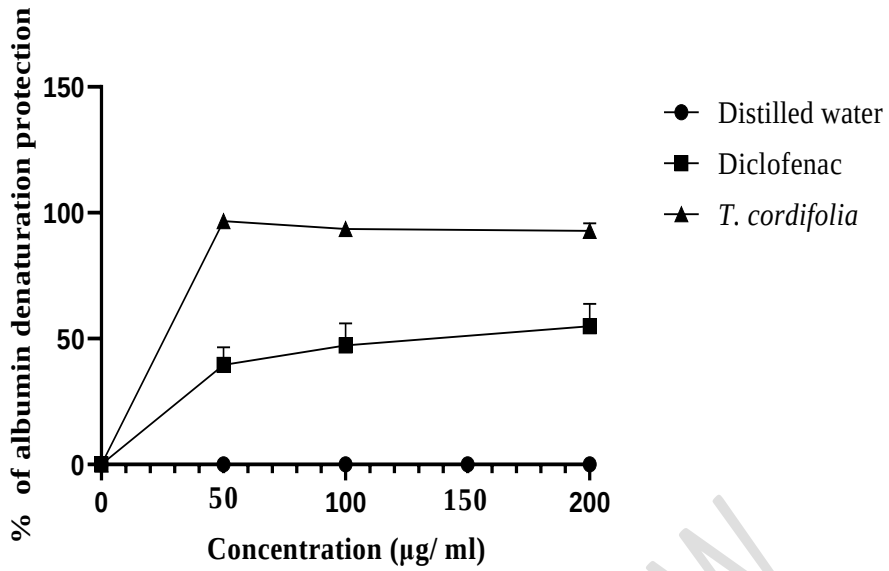

Fig. 6. Variation of the percentage of inhibition of methanol/ dichloromethane extract due to concentration. n=3

4.3.2. Effect of *Triumfetta cordifolia* extract on carrageenan-induced paw oedema

The anti-inflammatory effect of *Triumfetta cordifolia* is evaluated on carrageenan-induced plantar edema induced by carrageenan injection; indeed, in the control group, the edema induced in rats by carrageenan was maintained throughout the experiment. Treatment with the extract significantly ($P< 0.001$) reduced edema at all times. The maximum inhibition was observed at a sixth hour with the percentage of 71.38% (50 mg/kg); extract at the dose of 100 mg/kg has its maximum inhibition at the 30th minute (55.73%) and the 1st hour (55.12%) respectively while 200 mg/kg inhibited at the 3rd hour for 56%. These percentages are obtained compared to the negative control. Similarly, the reference molecule (diclofenac) showed significant ($P<0.0001$) inhibition every hour with the maximum inhibition of 88.58% at the 4th hour (Table 3).

Table 3: Variation of paw edema on carrageenan test

Treatments	Dose	Variation in the volume of paw oedema (ml)

	(mg/kg)	1/2h	1h	2h	3h	4h	5h	6h
Distilled water	10	0.28± 0.02	0.30± 0.04	0.91± 0.02	1.13 ± 0.02	1.64± 0.39	1.36 ± 0.05	1.47 ± 0.05
Diclofenac	10	0.17 ± 0.04 (41.59%)	0.12 ± 0.07 (62.48%)	0.19 ± 0.07 (78.29 %)	0.20 ± 0.07 (82.00 %)	0.18 ± 0.06 (88.58 %)	0.64 ± 0.05 (52.94 %)	0.73± 0.03 (49.82 %)
T. cordifolia	50	0.21 ± 0.05 (23.01%)	0.21 ± 0.04 (29.85 %)	0.55 ± 0.02 (39.28%)	0.46 ± 0.03 (59.11 %)	0.47 ± 0.02 (71.38 %)	0.81 ± 0.09 (40.07%)	0.77 ± 0.11 (47.61%)
T. cordifolia	100	0.12 ± 0.03 (55.75%)	0.13 ± 0.02 (55.13%)	0.46 ± 0.06 (48.9%)	0.53 ± 0.03 (52.44%)	0.79 ± 0.10 (51.59%)	0.93 ± 0.04 (31.61%)	0.95 ± 0.06 (35.20%)
T. cordifolia	200	0.17± 0.01 (39.82%)	0.20 ± 0.04 (32.79%)	0.58 ± 0.08 (35.44%)	0.49 ± 0.03 (56.00 %)	0.98 ± 0.03 (40.03%)	1.07 ± 0.03 (20.95 %)	1.06 ±0.07 (28. 03%)

5. DISCUSSION

For the acute toxicity study of the methanol/dichloromethane extract of *T. cordifolia*, all animals treated at a single dose of 2000mg/kg or 5000mg/kg showed no signs of toxicity. The roots extract of *T.cordifolia* did not cause any death. Suggesting that the median 50 lethal dose (LD_{50}) of the extract is higher than the limit doses administered (5000 mg/kg). This is consistent with the results obtained obtained by Wansi et al. (2016) which showed that the LD_{50} of *T. Pentandra* is greater than 5000 mg/kg [20].

Phytochemical screening of the methanol/dichloromethane extract of *T. cordifolia* revealed the presence of polyphenols, flavonoids, alkaloids, coumarins, saponins, reducing sugars, steroids and tri-

terpenes while anthocyanins and anthocyanosides are absent. A similar result has been obtained by Sandjo *et al*. (2010) [21]. The phytochemicals present in the roots of the plant may be related to their pharmacological properties [22-24].

Regarding the analgesic study effects of *Triumfetta cordifolia* root extract in mice, intraperitoneal injection of acetic acid causes pain in animals characterized by abdominal contortions and abdominal stretching [25]. Indeed, acetic acid indirectly generates inflammatory pain related to stimulation of peripheral nociceptive neurons by endogenous mediators such as serotonin, histamine, bradykinin, and prostaglandins that activate the nociceptors responsible for the transmission of pain impulses by cell trauma [26,27]. Pretreatment with the extract significantly reduced ($p<0.0001$) contortions and maximum inhibition was achieved at a dose of 200mg/kg, for an inhibition percentage of 79. 45%. The results obtained indicate the peripheral analgesic activity of the extract and suggest that it would have an inhibitory action on mediator's release or a direct inhibitory action on nerve endings involved in peripheral pain.

The acetic acid test is useful to screen substances with analgesic properties; the intraplantar injection of formalin was chosen as an additional test to elucidate whether the extract acts on peripheral or central nervous systems. The pain induced by intraplantar injection of formalin in mice is a biphasic pain characterized by licking the paw. Phase I or early phase (neurogenic pain) occurs during the first 5 minutes after injection and phase II or late (inflammatory pain) begins at the 10^{th} minute with a maximum between the 20^{th} and 30^{th} minutes [28]. During the first phase, nociception triggered by activation of primary afferent sensory neurons (fibers C) and during the second phase, nociception triggered by the release of inflammatory cytokines [29,30]. Administration of the methanol/dichloromethane extract of *T. cordifolia* showed significant inhibition in both phases. The highest percentages of inhibition of our extract were 34.10% (($p<0.001$; 200 mg/kg) at the first phase and 46.45% ($p<0.001$) 50 mg/kg) at the second. The extract could act as a central analgesic either by preventing the increase in impulse generated at the peripheral ends of the C fibers or by inhibiting the release of chemical mediators [31]. Tramadol significantly inhibited ($p<0.0001$; $p<0.001$) respectively both phases of formalin-induced pain.

The action of the extract on visceral pain and on the late phase at 50mg/kg of formalin-induced pain suggests that our extract may have anti-inflammatory activity. To prove this hypothesis, we performed an anti-inflammatory assay *in vitro* using bovin serum albunim denaturation protection power and in *vivo* by the carrageenan-induced paw edema test. Protein denaturation is one of the causes of inflammation. The possible mechanism of denaturation consists of alteration of electrostatic, hydrogen, hydrophobic, and disulphid bonds that maintain the three-dimensional structure of proteins [32]. The results of the anti-inflammatory activity *in vitro* showed a highest percentage of protection of 96.66 % at 50 mg/kg which would justify that the plant could be endowed with anti-inflammatory property.

Carrageenan-induced paw edema is an *in vivo* model commonly used in the evaluation of the anti-inflammatory properties of various compounds [33]. This is a triphasic response that involves the release of several inflammatory mediators. The first phase lasts about an hour after the injection of carrageenan is characterized by the release of histamine and serotonin, the second phase is related to the release of kinins and, the third phase is attributed to prostaglandins and leukotrienes, which last 3 to 5 hours [34]. These chemicals increase vascular permeability during their production, thus promoting the accumulation of fluid in the tissues that explains the edema [35]. In this study, pretreatment with the methanol/dichloromethane extract of *T. cordifolia* resulted in an effective inhibition of the volume of edema compared to the negative control. Maximum inhibition (71.38%) is achieved at a dose of 50 mg/kg 4 h after carrageenan injection. These results suggest that our extract may exert inhibitory activity on the release of pro-inflammatory mediators, including histamine, serotonin, bradykinin, prostaglandins, and leukotrienes. Diclofenac (10 mg/kg) used as the reference molecule also showed a maximum inhibition of 88.58% in the 4th hour also. Non-steroidal anti-inflammatory drugs such as diclofenac are cyclooxygenase inhibitors, which lead to inhibition of prostaglandin, prostacyclin, and thromboxane. Diclofenac can also affect the release and absorption of arachidonic acid and may also inhibit lipoxygenases [36].

Anti-inflammatory and analgesic activities can be justified by the result of phytochemical screening. Indeed, the result of phytochemical screening of the methanol/ dichloromethane extract of *T. cordifolia* reveals the presence of secondary metabolites such as alkaloids, flavonoids, triterpenes, and

steroids. These results are consistent with those obtained by Sandjo et al. (2010) [21]. A study by Ngolsou et al. (2022) reveals that the proportion of total flavonoids in *T. cordifolia* represents 14% of the total amount of phenolic compounds [37]. A flavonoid: trans-tiliroside had been isolated from the leaves of *T. cordifolia* [21,38]. Sandjo et al. (2010) identified steroids: stigmasterol, β-sitosterol and triumfettosterol Id; they also isolated nine triterpenoids, including lupeol, betulin [21]. The presence of compounds such as steroids, triterpenes and flavonoid alkaloids could give the plant analgesic and anti-inflammatory properties. The presence of flavonoids in the studied extract could generate the anti-inflammatory activity by inhibiting the production of the very active pro-inflammatory molecules: prostaglandins. This effect would be due to the reduction in arachidonic acid metabolism by inhibiting lipoxygenase, cyclooxygenase, and phospholipase A2 [38,39]. The results are in line with those obtained by Imomotimi et al. (2023) [9] in Nigeria, who isolated flavonoid compounds from the methanolic extract of *T. cordifolia* and demonstrated analgesic and anti-inflammatory activity in an animal model. These findings confirm that different parts of the *T. cordifolia* plant may be responsible of the anti-inflammatory properties.

6. Conclusion

The results obtained indicate that the root extract of *T. cordifolia* in methanol/dichloromethane is without acute toxicity and possesses peripheral and central analgesic properties as well as anti-inflammatory properties *in vivo* and *in vitro*. These effects could be due to the presence of phytochemicals observed in the plant. These experimental results support the traditional use of this plant in the treatment of inflammatory and painful diseases.

CONSENT

It is not applicable

ETHICAL APPROVAL

All experiments were carried out according to the protocol approved by the Institutional Ethics Committee for Health Science of the University of Douala (protocol approval number 3165 CEI-Udo/06/2022/T)

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