ANTINOCICEPTIVE AND ANTI-INFLAMMATORY STUDIES ON TRADESCANTIA ZEBRINA

NAVID BAGHALPOUR1, SEYED ABDULMAJID AYATOLLAHI1,2, NIMA NADERI1, TAHEREH HOSSEINABADI1,2, YASAMAN TAHERI1,2, JAVAD MAHROO-BAKHITIYARI1, ZABTA KHAN SHINWARI1,2, ALI TALHA KHALIL1,6 AND JAVAD SHARIFI-RAD1,2,3

1Phytochemistry Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
2Department of Pharmacognosy and Biotechnology, School of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran
3Department of Pharmacology and Toxicology, School of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran
4National Council for Tibb, Islamabad, Pakistan
5Pakistan Academy of Sciences, Islamabad, Pakistan
6Department of Biotechnology, Qarshi University Lahore Pakistan
*Corresponding author’s email: shinwari2008@gmail.com; javad.sharifirad@gmail.com

Abstract

Herein, we report phytochemical investigation and In vivo anti-inflammatory and antinociceptive potential of Tradescantia zebrina L. extracts. T. zebrina belongs to the family Commelinaceae and has important therapeutic uses like conjunctivitis, hemorrhoid, and superficial wound dressings in folkloric medicine. In phytochemical screening, detection tests for saponins, tannins, terpenes, flavonoids, and alkaloids were performed. Folin-Ciocalteau based procedure was for total phenolic content quantification. Three fractions, including ethyl acetate, n-hexane and chloroform, fractions, were prepared from the ethanolic extract. A total of 124 Wistar rats were treated with the ethanolic extract (100 mg/kg and 300 mg/kg), fractions (100 mg/kg and 300 mg/kg), and normal saline for negative control and Diclofenac (25 mg/kg) for positive control group by oral gavage route. Antinociceptive and anti-inflammatory properties were established using the formalin and carrageenan tests, respectively. Also, the acute toxicity of the ethanolic extraction was analyzed on ten rats. The ethyl acetate and chloroform fractions decreased the licking of the foot significantly in the formalin test (p<0.001). Treatment with the ethanolic extract decreased the paw edema caused by carrageenan at the doses of 100 and 300 mg/kg relative to control group significantly (p<0.001). Similar results were obtained at doses of 30 and 100 mg/kg. The dose of 100 mg/kg of the ethyl acetate fraction showed a major effect in the carrageenan paw edema test. There was no mortality in the acute toxicity test which indicates its non-toxic effect at this stage. The result of the total phenolic content assessment showed that 76.88% phenolic content as μg gallic acid equivalents/mg of the extract. To conclude, antinociceptive and anti-inflammatory effects were revealed by different fractions T. zebrina and can be studied further for potential isolation of bioactive compounds.

Key words: Tradescantia zebrina, Commelinaceae, Anti-inflammatory, Antinociceptive.

Introduction

Pain represents a significant healthcare problem linked to potential or actual tissue damage; however, it is defined as an unpleasant sensation that motivates the human being to find ways of pain relief (Goldman & Bennett, 2002). There is a paradigm shift towards the multi-target inhibition of inflammation through natural products and their derivatives (Zeb et al., 2016; Jan et al., 2020) In recent years, non-steroidal anti-inflammatory drugs and opioids have been the main medications for pain relief, yet there have been many reports representing major side effects from these drugs including gastrointestinal upset, renal function abnormalities, and risks of dependency (Calixto, 2005). Therefore, the scientist has been searching for a safer way for pain relief.

Inflammation is a condition manifested in different ways. Inflammation can compromise the immune system, cause infections, and delay the diagnosis and treatment of the disease. There is an obvious correlation between inflammation and pain due to the release of chemical mediators in the process of inflammation; this can increase the impulse on the nociceptors along sensory afferent fibers (Medzhitov, 2008). Nature can be a rich source for analgesic and anti-inflammatory effects. Natural compounds with their wide pool of bioactive agents can be used for the management of many diseases (Sahraie-Rad et al., 2015; Sharifi-Rad et al., 2016; Bagheri et al., 2016; Sharifi-Rad et al., 2017; Sharifi-Rad et al., 2018b). The effectiveness of natural compound has been tested in animal and human clinical trials (Sharifi-Rad et al., 2018a; Salehi et al., 2019). Herbs have been used for their anti-inflammatory and analgesic effects since ancient times (Sesterhenn et al., 2007; Ayatollahi et al., 2019). There are more than 60 flowering species and 37 genera in the family Commelinaceae (Faden, 1998; Edeoga & Ogbebor, 1999). One of the genera, called Tradescantia, comprises of 70 discovered species (Burns et al., 2011). Locally known as “Wandering Jew,” the plant Tradescantia is a perennial herbaceous plant possessing trailing stems with fleshy and oval leaves. The leaves are red or purple with broad, silvery stripes while appear purple on the lower side (Faden, 2008). T. zebrina is found in various parts of the world; however, it is native to East Mexico. It grows well in a variety of soils, in forests and open woodlands, in subtropical and warm temperate regions (Ribeiro et al., 2014).

Recently, significant analgesic as well as anti-inflammatory effects were revealed by the leaves extract of Tradescantia fluminensis using writing test, formalin test, and egg albumin induced edema test, performed on Wistar Albino rats and mice of both sexes (Waweru et al., 2017). 15-lipoxygenase from T. zebrina revealed possible applications in the treatment of asthma (Alaba & Chichico-Hernandez, 2014). Significant analgesic properties of Tradescantia patilla are also reported signifying the therapeutic potential of the members of Commelinaceae (Huq, 2015).
The Commelinaceae family are also part of traditional and folk medicine (Qasim et al., 2010). In Central America, *T. zebrina* is used to improve kidney function. The cataplasms are also used as dressing for superficial wounds (Pöll, 1997). In East Cuba, the extract of *T. zebrina* is used as a treatment for conjunctivitis (Cano & Volpato, 2004). In the traditional manuscripts of medicine, *T. pallida* is mentioned for its rich nutrition and its effects on increasing blood flow, inflammation and the improvement of gastrointestinal diseases (DeFilipps et al., 2004; Li, 2006). In early Chinese Medical Literature, *T. zebrina* is brought up for its effect on renal diseases and the improvement of the renal activity. They mention that 200 grams of the *T. zebrina* with 15 pieces of Phoenix dactylifera commonly known as Date Palm and 12 pieces of Zingiber officinale, should be boiled in water and the extract should be taken on an empty stomach (Amaral et al., 2006). In Jamaica, *T. zebrina* is used for the treatment of tuberculosis, high blood pressure and cough. The leaves of the plant are applied for the management of swelling and hemorrhoids. In Mexico, a beverage prepared from lemon and the leaves of *T. zebrina* called “Marali” is taked as a cold tonic drink (Dash et al., 2017). Leaves are used as tea for blood cleansing and influenza treatment in Guyana (Amaral et al., 2006). Extending on the rich folkloric literature on *T. zebrina*, herein, an attempt is made to scientifically validate the therapeutic potential of *T. zebrina* through diverse *In vivo* and *In vitro* experiments.

**Materials and Methods**

**Plant collection, preparation, and identification:** Of the 4 kg areal parts of *T. zebrina* were collected from Mohammad-Shahr region in Alborz, Iran, in the flowering season (June 2017). They were dried under shade at room temperature. A control herbarium specimen was prepared and the identity was confirmed by a positive comparison of its characteristics with in-house plant reference material. This herbarium specimen, identified by Dr. Javad Sharifi Rad (SBMU-1160), is kept in the Phytochemistry Research Center at Shahid Beheshti University of Medical Sciences.

**Preparation of total extract and fractions of *T. zebrina***: After shade drying, plant material was grounded. Powdered material (500 g) was repeatedly extracted with 80% ethanol using the maceration method. At first, the powder was macerated for 72 hours, filtered and replaced by the solvent every 24 hours. Afterwards, solvent was evaporated using rotary evaporator. After complete concentration of the extraction, doses 100 mg/mL and 300 mg/mL were prepared for the following steps. Also, three fractions were prepared with Ethyl acetate, N-hexane and Chloroform solvents, using liquid-liquid extraction method (Bibi et al., 2016) as indicated in Fig. 1.

**Fig. 1. Summary of the Scheme used for the fractionation process.**
Phytochemical screening tests: Phytochemical analysis was accomplished to confirm the presence of Terpenoids, Alkaloids, Saponins, Flavonoids and Tannins was carried out by the methods described previously (Evans, 2009; Jesionek et al., 2015). Table 1. Describe the details about the stationary phase and the solvents.

Antioxidant activity screening test using HTLC: The screening for the antioxidant activity of the crude extract and fractions was performed using a 0.2% methanolic solution of DPPH. DPPH solution was sprayed on the chromatography strip. The anti-oxidant activity is rapidly shown as yellow marks on a purple background on the strip (Cieśla et al., 2012; Jesionek et al., 2014).

In this assay, the mobile phase was chloroform-ethyl acetate-formic acid (4:3:2:1), and the stationary phase was silica gel HPTLC plate size 5 x 10 cm coated with fluorescent indicator F254.

Preparation of ethanolic extract and the fractions doses: A heterogeneous suspension of the concentrated ethanolic extract and the fractions of T. zebrina were made with distilled water and tween 80. Different doses (100 mg and 300 mg) of the ethanolic and 30 mg and 100 mg doses from the fractions were prepared. Diclofenac, with a dose of 25 mg/kg, was injected by the Intraperitoneal (IP) route as standard material for positive control. Oral gavage of NaCl solution was done for the negative control group. The extraction with the dose of 1 mL/kg was administrated by oral gavage route.

Study groups: A total number of 124 Wistar rats weighing 100–120 g were collected from the Pharmacy School of Shahid Beheshti University of Medical Sciences animal house, Tehran, Iran. Sixty-two rats were tested by the formalin test and 62 other rats were tested for the paw edema. The Rats were held at 37% humidity at 25°C and light/dark cycle of 12:12 h. Rats were kept under no restrictions on water and feed. The research scheme was approved from the biomedical and pharmacological ethics committee, the school of pharmacy, Tehran, Iran (Zimmermann, 1983).

Formalin test: Before the test, rats were kept in individual cages for 1 hr to adapt to their environment. Half an hour before running the test, the rats were treated with normal saline (negative control group), the ethanolic extract at doses of 100 mg/kg, and 300 mg/kg, the fractions at doses of 100 mg/kg, and 30 mg/kg by oral gavage route. For the positive control group, diclofenac 25 mg/kg was injected by the IP route. 40 µL Formalin (5%, in 0.9% normal saline) was used to induce paw edema in sub-planar tissue of paws. Behavior was rated for 60 min after the injection. Pain score was given originally described by Dubuisson & Dennis (1977) as “0 = normal weight-bearing on the injected paw; 1 = limping during locomotion or resting the paw lightly on the floor; 2 = elevation of the injected paw so that at most the nail touches the floor; and 3 = licking, biting or grooming the injected paw”. The score was recorded every five minutes (Dubuisson & Dennis, 1977).

Carrageenan-induced paw edema test: 0.1 ml carrageenan (1% w/v) was used to induce paw edema in sub-plantar tissues of the left hind paw of each rat. At first, the rats were treated with the extraction for the test groups and normal saline for a negative control group by oral gavage route and diclofenac 25 mg/kg injection for the positive control group. After 30 minutes, the carrageenan was injected. Thickness of paws was determined prior and after of the injection of carrageenan for three hours (Winter et al., 1962). For paw thickness measurement, the paw was marked at the level of the lateral malleolus and immersed in a glass open-top cylinder filled with mercury placed on a digital balance. When the paw is immersed, the liquid applies a force to attempt its expulsion, which is the weight of the volume of the displaced mercury. The digital balance measures this force. After that, the volume can be calculated by the density of mercury using the following equation:

\[ V = \frac{m}{\rho} \]

In this equation, V is the volume of the displaced liquid, \( \rho \) in the density of the liquid (for mercury \( \rho = 13.534 \text{ g/cm}^3 \)), and m is the weight of the displaced liquid (Fereidoni et al., 2000).

The anti-inflammatory potential was obtained after the measurement of the paw edema before and after the injection of carrageenan and treating with extract of T. zebrina.

Total phenolic content assay: For this assay, Folin & Ciocalteu's phenol reagent was used. The reaction is measured by the absorption at 765 nm and compared with a standard curve generated by gallic acid as a standard phenolic solution. In this matter, 1 mL of standard gallic acid (10 - 100 µg/mL) was introduced to 4.5 mL of dH2O and Folin Ciocalteu's reagent (0.5 mL) followed by incubation at room temperature for 10 minutes. Afterwards sodium carbonate solution 4 mL of (75 mg/mL) was introduced to the reaction mixture and kept at room temperature, without light exposure, for thirty minutes. After that, the absorption was measured at 765 nm for each gallic acid solution, and the calibration curve was drawn based on the absorption. The same process was done for the plant extract (concentration of 400 µg/mL) instead of gallic acid. TPC of the extract was calculated using the calibration curve, and the following equation (Nickavar & Esbatì, 2012; Velioglu et al., 1998).

\[ TPC = \frac{C \cdot V}{M} \]

In this equation, C, V, and M were the concentration of gallic acid established from the calibration curve (mg/mL), the volume of the extract (mL), and the weight of the extract (mg), respectively.

Statistical analysis: Results are analysed as mean ± standard deviation (SD). For analyzing the behavioral changes as a result of pain from the Formalin test, the area under the curve (AUC) of the pain score–time graph was assessed, and data were analyzed by one-way analysis of variance (ANOVA) followed by Bonferroni’s post-test, considering p<0.05 as significant. For the carrageenan test, the data were also analyzed by one-way analysis of variance (ANOVA) followed by Tukey’s post-test and also considering p<0.05 as significant. The statistical analysis was performed by Graphpad Prism® 8.0.
Results

Various tests were carried out on n-hexane, ethyl acetate and chloroform fractions, and ethanolic extracts for phytochemical evaluations are summarized in which is presented in the Tables 2-6, respectively.

Phytochemical screening: The results of phytochemical screening of the ethanolic extract and the fractions are according to Table 2.

Table 2. Phytochemical screening results.

<table>
<thead>
<tr>
<th>Photochemical component</th>
<th>Status</th>
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<tr>
<td>Terpenoids</td>
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<td>Flavanoids</td>
<td>++++</td>
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<tr>
<td>Alkaloids</td>
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<td>Tannins</td>
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<td>Saponins</td>
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Compounds were identified by HPTLC method. The results of terpenoids, flavonoids, and alkaloids identification, and antioxidant screening test are presented in Tables 2-6, respectively.

HPTLC method for anti-oxidant assessment: HPLC plates were recorded before and after derivatization, at visible and UV spectrum, and fluorescence light. The yellow spots on the purple background (visible) confirm the presence of compounds with antioxidant activity.

Effects of T. zebrina on behavioral changes of animal models in the formalin test: Administration of T. zebrina extracts induced significant lessening of pain score relative to control group in the formalin test. Treatment with ethanolic extract at 300 mg/kg revealed highest reduction of pain score which indicates a dose-dependent effect from the extract. The results are illustrated in Fig. 2.

Behavioral studies in the formalin model showed decreased pain scores in the animal models treated with T. zebrina extracts relative to control.

The area under the curve calculated from figure 2 shows a significant effect of the ethanolic extract of T. zebrina. The pain score of the group treated with 25 mg/kg of Diclofenac and the plant extract at the dose of 300 mg/kg was the lower compared to other treatments (Fig. 3).

In the post-test, n-hexane, chloroform, and ethyl acetate fractions showed a significant reduction in pain score as compared to the control group. Ethyl acetate and chloroform fractions decreased the pain score remarkably. Furthermore, these two fractions showed more effect at the dose of 100 mg/kg. This can demonstrate a dose-dependent impact on pain score. Figure 4 displays a comparison between positive control, negative control, and test groups.

Fig 2. Effects of T. zebrina on behavioral changes of animal models in the formalin test in comparison with the positive and negative control groups.

Fig. 3. The area under the curve of pain score from figure 1. Results are expressed as mean ± SD. Significant difference compared with negative control group (saline) p <0.0001****
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Table 3. Terpenoid compounds identified in \textit{T. zebrina} detected by HPTLC. Anisaldehyde-sulfuric acid reagent was used for identification. Terpenoids appeared as purple-blue lines after spraying the plate with the reagent under the visible light. The majority of terpenoids were detected in fraction 1 (n-hexane) and fraction 2 (chloroform).

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<thead>
<tr>
<th>F</th>
<th>UV</th>
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<td>Before derivatization</td>
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Table 4. Flavonoid compounds identified in \textit{T. zebrina} detected by HPTLC. Natural product reagent was sprayed on the HPTLC plate. Sharp blue lines in fluorescent light and light yellow spots at the visible spectrum reveal the presence of flavonoids. A great part of flavonoids was found in fraction 2 (chloroform) and fraction 3 (ethyl acetate).

<table>
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Table 5. Alkaloid compounds identified in *T. zebrina* detected by HPTLC. Dragendorff reagent was applied for identification. Alkaloids appeared as yellow, orange, and brown lines in the visible spectrum. Most of the flavonoids were detected in ethyl acetate fraction 3.

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Table 6. Anti-oxidant screening test using HPTLC method.

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<td>Before derivatization</td>
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<td><img src="image8" alt="Image" /></td>
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<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
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<tr>
<td>After derivatization</td>
<td><img src="image10" alt="Image" /></td>
<td><img src="image11" alt="Image" /></td>
<td><img src="image12" alt="Image" /></td>
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<td>1</td>
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Fig. 4. Effects of *T. zebrina* chloroform, n-hexane and ethyl acetate fractions on behavioral changes of animal models compared to positive and negative control groups. Results are expressed as mean ± SD. Significant difference compared with negative control group (saline) p <0.0001****. Significant difference with positive control group diclofenac) p <0.05$, p <0.01$$, p <0.0001$$$$

Fig. 5. Effects of *T. zebrina* ethanolic extract on carrageenan-induced paw edema. Results are represented as mean ± SD. Significant difference compared with negative control group (saline) p <0.0001****. Significant difference with positive control group (diclofenac) p <0.05$, p <0.01$$, p <0.0001$$$$

Fig. 6. Effects of *T. zebrina* of ethyl acetate, n-hexane and chloroform fractions and standard drug Diclofenac on carrageenan-induced paw edema. Results are presented as mean ± SD. Significant difference compared with negative control group (saline) p <0.0001****. Significant difference with positive control group (diclofenac) p <0.01++.

Fractions show a significant impact on pain score as compared to the control groups. Bar charts show noteworthy effects on pain scores from ethyl acetate and chloroform (Fig. 4).

The effects of *T. zebrina* on behavioral changes of animal models in the paw edema test: The anti-inflammatory potential of the test samples at a dose of 100 and 300 mg/kg, and 30 and 100 mg/kg for the ethanolic extract and the fractions, respectively, against the acute paw edema induced by carrageenan is depicted in figure 5. The ethanolic extract and the fractions induced a remarkable and dose-dependent response.

Figure 5 reveals the results of paw volume after administration of ethyl acetate, n-hexane, and chloroform fractions and Diclofenac in carrageenan-induced edema. A dose-dependent reduction of the paw volume was observed.

As shown in figure 6, the fractions have a substantial and dose-dependent impact on paw edema reduction in the carrageenan-induced rat paw edema test.

**Total phenolic content (TPC) of *T. zebrina*:** TPC was deterred using gallic acid calibration curve. The results appear in Table 7.

TPC of the ethanolic extract was 76.88 ± 0.14 µg gallic acid/g extract using the regression equation calculated from the calibration curve.

**Table 7. Total phenolic content of *T. zebrina*.**

<table>
<thead>
<tr>
<th>Extract</th>
<th>Total phenolic content (µg Gallic acid/g extract)</th>
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<tr>
<td>Ethanol extract</td>
<td>76.88 ± 0.14</td>
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**Discussion**

Medicinal flora is a vital resource for seeking bioactive compounds (Hussain et al., 2010; Ayaz et al., 2019; Khan et al., 2020). These medicinal plants are used in different preparations and are playing a vital role in fulfilling the health vacuum as nearly 80% of the global population relies on alternative and herbal medicines (Khalil et al., 2014). Bioactive natural compounds represent a rich resource for the expansion of antinociceptive and anti-inflammatory agents. Ethnomedicinal scriptures reveal the antinociceptive and anti-inflammatory potential of *Tradescantia zebrina*. Previously, it was explained in studies that flavonoids of this plant could have anti-inflammatory and antinociceptive potentials (Waweru et al., 2017). Among different species like *T. spathacea*, *T. pallida*, *T. zebrina* revealed the highest amount of flavonoids which are considered to impart antinociceptive and anti-inflammatory potential (Mohamed, 2018).

Furthermore, another group in 1993 reported 42 genera of this family had a great amount of glycosylated form of the phenolic compounds apigenin, luteolin, and flavonols named 6-hydroxy-luteolin and tricin (Martinez & Martinez, 1993). As stated by the studies, it is clear that tremendous amounts of the flavonoids are soluble in ethyl acetate and chloroform fraction (Routray et al., 2013; Chikezie et al., 2015). Hence we initiated the current work to appraise the anti-inflammatory and antinociceptive effects of the plant *T.*
zebrina. Our approach led to the extraction and fractionation of this plant, which was investigated for its antinociceptive and anti-inflammatory activities in animal models.

The formalin test as a model of nociception discriminates pain in two phases, which can be separated by time: the first phase (0-5 minutes) generates peripherally through the direct stimulation of nociceptive neurons; this phase is called the neurogenic phase. The second phase (20-25 minutes) is taken place through the activation of central neurons, especially the neurons of the dorsal horns in the spinal cord. This phase is thought to be an inflammation-induced pain due to the activity of cytokines (like prostaglandins, serotonin, histamine, and bradykinin). These facts can be used to explain the antinociceptive mechanism. Our results indicates that the extracts and fractions of T. zebrina created antinociception against both the inflammatory and neurogenic phases of the formalin test. Based on our results from the carrageenan-induced paw edema test, the ethanolic extract, chloroform, n-hexane, and ethyl acetate fractions have anti-inflammatory activity.

The herbal plant extracts possess diverse phytochemicals like polyphenols which are a key source of antioxidant agents (Shinwari & Qasair, 2011; Walter et al., 2011). Antioxidant activity is revealed because of the redox potential of phytochemicals which plays a role in free radical scavenging. There are many polyphenols in plants including flavonoids, flavonols, tannins, stilbenes, phenolic acids, etc. while the main among them are flavonoids that indicate extraordinary antioxidant potential. Previous studies indicate various benefits of medicinal plants for human health like anticancer, anti-inflammatory, antinociceptive, antibacterial, antiviral and anti-allergic effects (Shinwari et al., 2020; Sharif-Rad et al., 2018). Our results indicates the presence of terpenoids, phenols, flavonoids and alkaloids that possesses the potential to manifest the antioxidant activity, anti-inflammatory potential and antinociceptive activities of T. zebrina.

Conclusion

This study demonstrated that ethanolic extract, n-hexane, ethyl acetate and chloroform fractions of T. zebrina displayed activity against nociceptive responses triggered in animal models by chemical (formalin) stimuli. Also, it showed anti-inflammatory activity against carrageenan-induced paw edema. Both of these effects were more significant in ethyl acetate and chloroform fractions. The flavonoids in these fractions are the main compound responsible for their effectiveness. The mechanism of action for antinociceptive and anti-inflammatory activity is not completely clear, and it requires further investigation.

References


