

## Analgesic Effect of Ethanolic Extract of *Bougainvillea X Buttiana* (Var. Rose) Holttum & Standl

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### Abstract

**Background:** *Bougainvillea sp* is a medicinal plant traditionally used in Mexico to alleviate several diseases including painful disorders. The present study was carried out to evaluate the analgesic activity of ethanolic extracts of *Bougainvillea x buttiana* (var. Rose) Holttum & Standl.

**Methods:** *Bougainvillea x buttiana* bracts were extracted with ethanol. The extract was submitted to phytochemical qualitative analysis. The extract was orally administered at dose of 0.04, 0.4, 4 and 40 mg/kg. For analgesic evaluation were included methods for a peripheral mechanism pain such as the acetic-acid induced writhing method, and a central mechanism of pain as tail flick and formalin tests were used.

**Results:** The results obtained from the phytochemical qualitative analysis showed the presence of alkaloids, carbohydrates, fatty acids, lipids, saponins and tannins. Our results also clearly indicate that the oral administration of the *Bougainvillea x buttiana* extract caused a significant ( $p < 0.05$ ) dose-dependent reduction in the number of abdominal writhes. The extract also produced a remarkable increase in tail immersion latency time and paw licking time.

**Conclusion:** The results obtained demonstrated that *Bougainvillea x buttiana* extract showed analgesic potential in all models of nociception implying that both peripheral and central pathways of analgesia are involved. This might be due to the presence of various classes of phytochemicals in the plant extract.

**Keywords:** Analgesic; Phytochemical; Ethanol Extract; Tail Immersion Test; Writhing Test; Paw Licking

### Abbreviations

BxbREE: Ethanolic Extract of *Bougainvillea x buttiana*

### Introduction

According to the International Association for the Study of Pain (IASP), pain is defined as an unpleasant sensory or emotional experience associated with actual or potential tissue damage. This definition emphasizes pain as a complex multidimensional sensory-perceptual phenomenon that represents a unique subjective individual experience [1]. Various studies have shown that the pharmacologic management of pain requires the use of analgesic drugs. These drugs are divided into two groups, opioid and non-opioid analgesics [2]. In the literature, different studies have shown the serious side effects such as: physical dependency, tolerance, addiction, gastrointestinal disorders [2,3]. The search for potent analgesic agents with minimal side effects remains the goal of many scientific studies [4]. Several natural products have been used in traditional medicine and the research of these products is guided by ethnopharmacological knowledge and that brings a substantial contribution to the innovation of drugs through new chemical structures or by the mechanisms of ac-

tion [5]. *Bougainvillea* is a genus of the Nyctaginaceae family, has been widely used as a Mexican traditional medicine to treat respiratory infections, gastric ulcer, and stomach pain [6]. From the scientific point of view, in the last years several medicinal properties in extracts from *Bougainvillea x buttiana* (var. orange) have been reported including antinociceptive, anti-inflammatory and immunomodulation activities [6,7]. Extracts of *Bougainvillea x buttiana* of different colors also have antioxidant activity and differences in phenol content [8]. These differences observed for the ethanol extracts of *B. x buttiana* from different colors suggest a synergistic effect of the bioactive components, played an important role in the observed activities [8]. In this context, the objective of the present work was to investigate the antinociceptive activity of the ethanol extract of *B. x buttiana* (var. Rose-Cuernavaca).

### Materials and Methods Chemicals and solvents

Ethanol, acetonitrile HPLC grade and standards (gallic acid), and carrageenan, were purchased from Sigma-Aldrich Chemical Co., (St. Louis, MO, USA). Standards such as Aspirin (Bayer Mexico, TOL, MEX). Dexamethasone (Generic, GDA, MEX).

### Extraction of plant material

The flowers and bracts of *Bougainvillea x buttiana* were manually harvested in Cuernavaca, Morelos, Mexico, during summer 2015 and identified. A voucher specimen *Bougainvillea x buttiana* Holttum & Standl, was registered with the foil: 33870 for later reference and deposited at the Herbarium HUMO, CIByC (UAEM). The plant collection 110 g of flowers and bracts have been dehydrated at 25°C and grinded into powder were submerged in the ratio of 50/50 (w/v) for 3 days by continuous shaking. The extract was filtrated by using Whatman filter paper No 1, and the solvent was removed using a rotary evaporator at 50°C. The crude ethanolic extract of *Bougainvillea x buttiana* (var. Rose) was named as (BxbREE) and stored at -20°C for screening antinociceptive activity.

### Phytochemical Screening

The phytochemical screening methods to detecting the compounds in ethanolic extract of *Bougainvillea x buttiana* were performed as per standard methods [9,10]. To detect the presence or absence of different constituents including: Dragendorff's test for detecting alkaloids, Borntrager's test for anthraquinone, Fehling's test for carbohydrates, saponification for lipids, Foam test for saponins and Ferric chloride test for tannins.

### Animals

The animals were purchased from Bioterio del Instituto Nacional de Salud Pu blica (Cuernavaca-Morelos, Me xico) BALB/c. female mice (20 - 25 g) were acclimatized to the laboratory for 1 week prior to the experiment. All the experimental animals had free access to water. The animals used for the experiments was made in accordance to the protocol approved by the Committee (CCUAL-FM-UAEM N. 002/2016).

### Analgesic Activity

#### Mouse writhing assay

The animals were divided into 7 groups of 5 mice each. Writhing activity in mice was evaluated according the method described by Koster, *et al.* 1959 [11]. The different amounts of BxbREE were orally (v.o.) administered. One hour later, 100 µL of 0.6% acetic acid was administered intraperitoneally. The number of writhes/mice was counted during a period of 20 minutes, starting 10 minutes after the injection of acid acetic. Aspirin® and Dexamethasone 4 mg/kg and saline solution were used as standard and control, respectively, and were also intraperitoneally administered one hour before the acetic acid administration.

#### Tail Immersion Test

The tail immersion test was performed as described by Parimaladevi, *et al.* 2003 [12]. Experimental animals were randomly selected and separated into 7 groups of 5 mice each. The lower 4 cm portion of the tail was immersed in a 1 liter beaker of water maintained at

51 ± 1 °C. For all groups of control and experimental the reaction time (seconds) was chosen as the time when the animals completely withdrew their tails from hot water, a cut-off time of 10 seconds was allowed to avoid tissue damage. For control groups the animals were treated with saline solution. BxbREE extracts at doses of 0.04, 0.4, 4 and 40 mg/kg were orally administered at 1 hour before the assay. The standards Aspirin® and Dexamethasone were intraperitoneally administered with 4 mg/kg one hour before assay. The time of tail immersion was recorded at intervals of 0, 30, 60 and 120 minutes after administration.

### Formalin Test

The formalin test was performed by using a method described by Hunskaar, *et al.* 1985 [13]. In brief: control groups were treated with saline solution. Groups of 5 mice were treated orally at 1 hour before the assay with different amounts of BxbREE extract. Groups of 5 mice were intraperitoneally treated with standards Aspirin® and/or dexamethasone (4 mg/kg). After 60 minutes, treatment for all tested drugs 20 µL of 1.0% formalin solution was given into the right hind paw of mice. After the different treatments, the animals were placed in separated boxes and licking/biting and flinching were recorded in two phases. The early phase (0 - 5 minutes) and the late phase (15 - 30 minutes) after formalin injection were evaluate.

### Statistical Analyzes

The statistical significance of differences between the groups was obtained by the analysis of variance (ANOVA) test complemented by GraphPad Prism 6;  $p < 0.05$  was considered significant.

## Results and Discussion Phytochemical Screening

Qualitative analysis of ethanolic extract of BxbREE was performed for alkaloids, anthraquinones, carbohydrates, fatty acids, lipids, saponins and tannins. Table 1 shows the presence of the alkaloids, carbohydrates, fatty acids and tannins. The anthraquinones and saponins were absence in this sample.

Phytochemical Test	Results
Alkaloids	+++
Anthraquinone	-
Carbohydrates	+++
Fatty acids	++
Saponins	-
Tannins	+

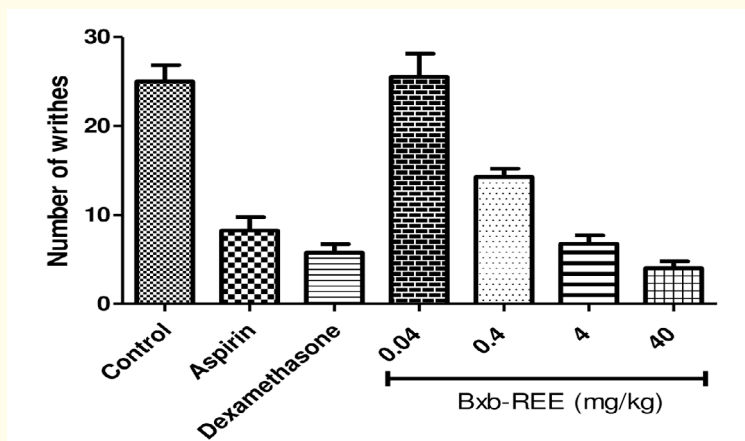
**Table 1:** Qualitative analysis of ethanolic extract of BxbREE.

Absent (-), present (+), moderate (++), and abundant (+++)

### Analgesic Activity

The analgesic drugs act on peripheral or central nervous system to selectively relieve pain without significantly altering consciousness [14]. Centrally acting analgesics act by raising the threshold for pain and, also altering the physiological response to pain. On the other hand, peripherally acting analgesics act by inhibiting the generation of impulses at chemoreceptor site of pain [2]. Three murine pain models, acetic acid-induced writhing, tail immersion test and formalin test, were employed in our study for screening of analgesic activity of *Bougainvillea* extract, all methods are useful. Administration of different amounts of ethanol extract of *Bougainvillea x buttiana* prior to acetic acid administration caused a dose-dependent reduction in the number of writhes compared to control (Figure 1). The greatest inhibition was observed at doses of 4 and 40 mg/kg. For these doses the number of writhes were similar those results observed

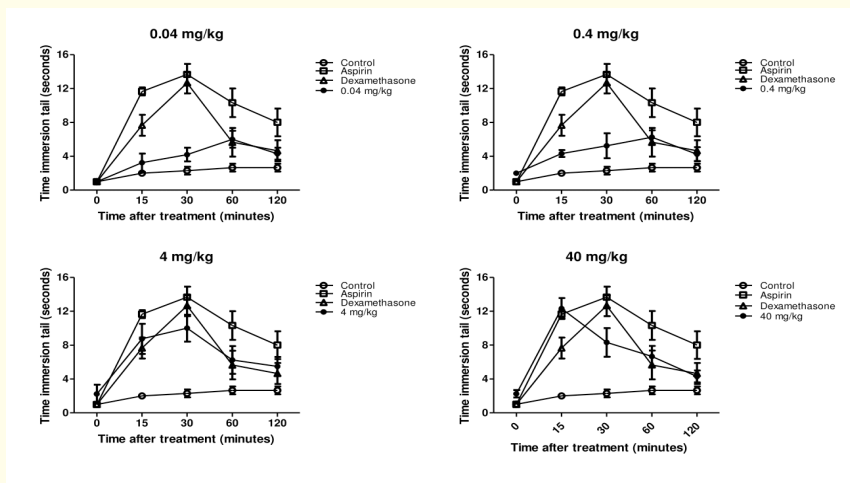
in standard groups (Figure 1). The results of this study demonstrate that *Bougainvillea x buttiana* ethanolic extract possesses analgesic activity. Various studies have shown that the acetic acid-induced abdominal constriction is described to involve peripheral mechanisms. Like this, in peritoneal fluids the presence of acetic acid which can act indirectly by inducing the release of endogenous mediators such as prostaglandins, cyclooxygenase and lipooxygenase. The increase in prostaglandin levels within the peritoneal cavity then enhances inflammatory pain by increasing capillary permeability [15]. These products are capable to stimulate the nociceptive neurons sensitive to non-steroidal anti-inflammatory drugs [16]. The marked reduction of writhes number caused by the BxbREE suggests that its effect may be peripherally-mediated via the inhibition of synthesis of the inflammatory mediators.



**Figure 1:** Effect of BxbREE extract on acetic acid-induced mouse writhing. Groups of mice were orally administered with various amounts of BxbREE extract, other groups were orally administered with 4 mg/kg of Aspirin® and/or dexamethasone 60 minutes before of acetic acid administration, were used as described in Materials and Methods. Each bar corresponds to the results (mean  $\pm$  SD)  $n = 5$ .

### Tail Immersion Test

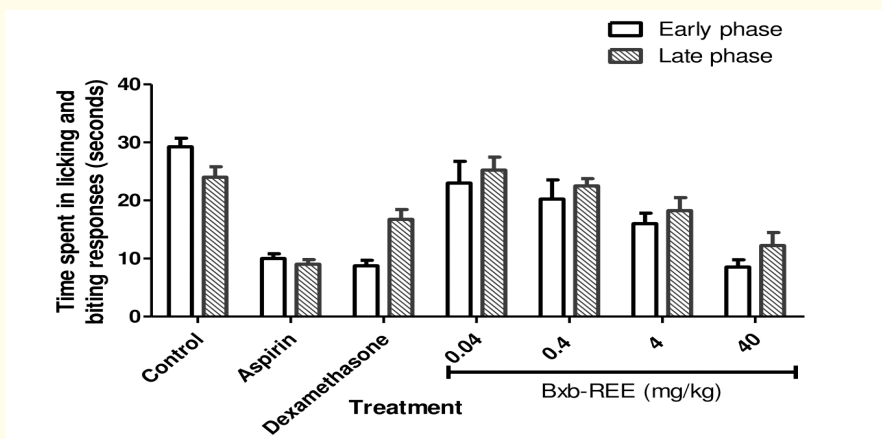
The tail immersion test is used for evaluating the centrally analgesic compounds [17]. This test consists of a thermal stimulus and increase in the reaction time is used for evaluating central antinociceptive activity [18]. The effects of the tested extract on tail immersion response in mice are presented in Figure 2. The antinociceptive extract caused a significant ( $p < 0.05$ ), in a dose-dependent increase in the mean reaction time on tail immersion (Figure 2). The antinociceptive activity of Aspirin® and dexamethasone started at 15 minutes; however, the highest activity was observed at 30 minutes. Different dose of BxbREE extract displayed a significant activity in the tail immersion when compared with results obtained in control group ( $p < 0.05$ ). In groups of mice treated with 0.04 and 0.4 mg/kg the maximum increase in tail immersion time was observed at 60 minutes after the treatment. For groups of mice treated with 4 and 40 mg/kg of BxbREE the maximum response was at 30 and 15 minutes, respectively (Figure 2). In 15<sup>th</sup> minute after treatment with 40 mg/kg of extract the tail immersion time was similar with those results obtained for groups treated with Aspirin®. The results obtained in this study suggested that the central antinociceptive property of the BxbREE extract was probably due to their anti-inflammatory activities.



**Figure 2:** Effect of BxbREE extract on time immersion tail. Groups of mice were orally administered with various amounts of BxbREE extract, other groups were orally administered with 4 mg/kg of Aspirin® and/or dexamethasone 60 minutes before of tail immersion, were used as described in Materials and Methods. Each bar corresponds to the results (mean ± SD) n = 5.

**Formalin Test**

Figure 3 shows the effect of BxbREE extract on formalin induced pain. There was significant dose-dependent reduction of time spent in licking and biting responses at both phases by the extract. At a 40 mg/kg of the extract used produced similar reductions in both phases when compared with those results obtained for Aspirin® group (Figure 3). The BxbREE extract inhibited both phases of formalin induced pain in mice. Formalin test is one of the most important analgesic models to correlate with clinical pain. The injection of formalin into plantar aponeurosis is able for induces biphasic nociceptive behavior in animals which seem to involve two distinctly different stimuli. In the early phase, formalin test pain occurs due to the direct stimulation of the sensory nerve fibers with the release of substance P and bradykinin. On the other hand, the late phase, is characterized by the releasing of compounds such as serotonin, histamine, bradykinin, and prostaglandins [19]. In this study show that the BxbREE was able cause significant inhibition of both phases in formalin test and suggests that it possesses central and peripheral analgesic activity.



**Figure 3:** Effect of BxbREE extract on time spent in licking and biting responses. Groups of mice were orally administered with various amounts of BxbREE extract, other groups were orally administered with 4 mg/kg of Aspirin® and/or dexamethasone 60 minutes before of formalin administration, were used as described in Materials and Methods. Each bar corresponds to the results (mean ± SD) n = 5.

## Conclusion

The results obtained in this study indicate that the ethanolic extract of *Bougainvillea x buttiana* possesses potential analgesic activity which is mediated via central and peripheral mechanisms and confirmed its medicine use in the management of pain and inflammatory disorders.

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## Conflict of Interest

The authors declare no conflicts of interest.

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