ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25jul1897

The Potential Analgesic Activity of Methanolic Leaf Extract of *Carmona retusa* (Vahl) Masam (Boraginaceae)

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Publication Date: 2025/08/14

Abstract: ***Carmona retusa*** **(Tsaang Gubat), a traditional medicinal plant in the Philippines, has been reported in preliminary studies to exhibit analgesic properties. However, limited scientific validation exists for its use in acute pain management. The study demonstrated that the methanolic leaf extract of Carmona retusa produces a dose-dependent analgesic effect in mice using the acetic acid-induced writhing test. The highest dose (5000 mg/kg) showed the strongest response, with a mean writhing count of 0.2 ± 0.4 comparable to the positive control (15.6 ± 8.55) and significantly lower than the negative control (41.4 ± 6.92). The middle dose (3750 mg/kg) showed strong analgesic activity (3 ± 3.79), while the lowest dose (2500 mg/kg) produced a more variable effect (21.2 ± 15.78). Statistical analysis confirmed significant differences between groups, indicating that analgesic activity increases with dose. These findings scientifically validate the plant's traditional use for pain relief, especially at higher concentrations of the extract.**

Keywords: *Pain, Analgesic, Carmona Retusa, Boraginaceae, Mefenamic Acid, Acetic-Acid, Mice, Writhing.*

How to Cite: Jehan P. Macawadib; Norjanah M. Tabua; Norlaicah G. Solaiman; Irene B. Tolibas; Junnin Gay L. Garay (2025) The Potential Analgesic Activity of Methanolic Leaf Extract of *Carmona retusa* (Vahl) Masam (Boraginaceae). *International Journal of Innovative Science and Research Technology*, 10(7), 3567-3570. https://doi.org/10.38124/ijisrt/25jul1897

I. INTRODUCTION

Pain management remains a pressing concern in healthcare. While synthetic analgesics such as non-steroidal anti-inflammatory drugs (NSAIDs) and opioids are widely used, they are often linked to adverse effects, including dependency and gastrointestinal issues (Wu et al. 2022; Puppala et al. 2020). These risks have raised the interest in plant-based alternatives that may provide relief with fewer side effects (Arena et al. 2021).

Carmona retusa (Vahl) Masam, a plant traditionally used in Southeast Asia for pain relief, has gained attention as a potential option. Its methanolic leaf extract is rich in bioactive compounds, including alkaloids, flavonoids, saponins, and terpenoids (Chandrappa et al. 2012). Notably, alkaloids have been recognized for their analgesic properties (Rauwald et al. 2018; Rajkumar et al. 2018).

Despite its traditional use and potential, the analgesic activity of *C. retusa* remained understudied. This research aimed to investigate the analgesic effects of its methanolic leaf extract using in vivo mice models. The findings would provide to the growing body of knowledge on natural analgesics and might inform the development of novel pain management strategies.

II. MATERIALS AND METHODS

A. Collection and Extraction of C. retusa

Leaves of *Carmona retusa* were collected from the National Steel Corporation (NSC), Iligan City (8.2077° N, 124.2107° E) and authenticated at MSU-IIT, Iligan City. The leaves were washed, rinsed with distilled water, and air-dried at 20–25°C. Dried leaves were pulverized, sieved (0.25 mm, no. 60 mesh), and stored in airtight containers at 10–15°C (Ogunshe et al. 2008). For extraction, the powder was soaked in 80% methanol (1 g:10 mL) for 3–7 days at room temperature with a 12-hour interval of maceration. The extract was filtered (Whatman no. 1) and concentrated by evaporation at 65°C, then stored in amber glass containers (Ingle et al. 2017; Azwanida 2015; Ogunshe et al. 2008; Pandey & Tripathi 2014; Doughari 2012; Sasidharan et al. 2011).

B. Phytochemical Screening

The methanolic leaf extract of *C. retusa* was screened for secondary metabolites. The presence of these bioactive compounds was performed through specific tests, including Mayer's test for alkaloids (Auwal et al. 2014; Tiwari et al. 2011; Dhanani et al. 2017), lead acetate test for flavonoids (Silva et al. 2017; Singh & Kumar 2017; Tiwari et al. 2011), foam test for saponins (Tiwari et al. 2012), and gelatin test for tannins (Raaman 2006).

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C. Acute Oral Toxicity Study

An acute oral toxicity test was performed following OECD Guideline 425 using the Up-and-Down Procedure (UDP) (Ashagrie et al. 2023; OECD 2022), which reduced animal use while estimating the LD50. Young, non-pregnant female Swiss albino mice (8–10 weeks old) were randomly selected and housed under standard conditions with free access to food and water. After a 7-day acclimatization, mice received single oral doses of 2000 mg/kg and 5000 mg/kg of *C. retusa* methanolic leaf extract. Mice were fasted before dosing and observed closely for signs of toxicity for 14 days (OECD 2022). Surviving mice were examined via necropsy to detect abnormalities in the liver, GI tract, and kidneys. The outcomes of the acute oral toxicity study were taken into consideration when choosing three different doses of *C. retusa* leaf extract.

D. Animal Grouping and Dosing

A preliminary toxicity assessment was conducted using increasing doses of the methanolic leaf extract of *C. retusa* to determine the optimal dosage for analgesic testing. Mice were divided into five groups, each consisting of five mice. Group I served as the negative control and received 10 mL/kg of PNSS with 2% Tween 80, group II as the positive control and was given 150 mg/kg of mefenamic acid with PNSS and 2% Tween 80, while groups III, IV, and V received 2500 mg/kg, 3750 mg/kg, and 5000 mg/kg of the *C. retusa* leaf extract with PNSS and 2% Tween 80, respectively. All treatments were administered orally using oral gavage (Ayanaw et al. 2023). The selected doses for analgesic evaluation were based on the toxicity findings, ensuring effectiveness without inducing harm.

E. Acetic Acid Writhing Test

Analgesic activity was evaluated by acetic-acid–induced writhing tests in mice. Each mouse was administered an intraperitoneal injection of 0.6% acetic acid at a dose of 10 mL/kg in each mouse, one hour after giving *C. retusa* methanolic extract, mefenamic acid, or saline. The number of writhing movements (abdominal contractions) was then recorded for 30 minutes only. The analgesic effect was calculated as a percentage reduction in writhing responses using the formula below (Yimer 2020).

% Analgesic Activity= (Mean writhing count of control group - Mean writhing count for treated group / Mean writhing count of control group) x 100

F. Statistical Treatment

The data that were gathered from the analgesic activity tests were recorded as the number of writhes per group. Descriptive statistics such as mean and standard deviation were computed using Microsoft Excel. Inferential analysis was done using one-way ANOVA to determine if there were statistically significant differences among the treatment groups. Tukey's post hoc test was performed to compare means pairwise and identify significant group differences. Significance was set at $p<0.05$.

III. RESULTS AND DISCUSSION

A. Phytochemical Screening

Phytochemical confirmatory tests revealed that the methanolic leaf extract of Carmona retusa contains alkaloids, flavonoids, tannins, and saponins. These compounds are known to exhibit analgesic properties, supporting the traditional use of the plant in pain management.

B. Acute Oral Toxicity

The extract was found to be safe at 5000 mg/kg with no observed mortality or signs of toxicity in mice within the 14-day observation period. This indicates that the methanolic extract has a wide safety margin for oral administration.

C. Analgesic Activity

The acetic acid-induced writhing test demonstrated that the extract produced a dose-dependent analgesic effect. At 5000 mg/kg, the writhing count was 0.2 ± 0.4, significantly lower than the negative control (41.4 ± 6.92) and comparable to the positive control (mefenamic acid, 15.6 ± 8.55). ANOVA results confirmed significant differences between groups ($F=14.1858$, $p<0.01$). Post hoc analysis indicated that high and middle doses had comparable efficacy to the positive control, validating the plant's traditional analgesic use. ANOVA showed significant differences among groups ($F=14.1858$, $p<0.01$).

Table 1. Mean Number of Writhes in Treatment Groups

Treatment Group	Dose (mg/kg)	Writhes (Mean ± SD)
Negative Control	Saline 10 ml/kg	41.4 ± 6.92
Positive Control	Mefenamic Acid 150 mg/kg	15.6 ± 8.55
Low Dose	2500	21.2 ± 15.78
Middle Dose	3750	3 ± 3.79
High Dose	5000	0.2 ± 0.4

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IV. CONCLUSION

The methanolic leaf extract of *Carmona retusa* exhibited significant analgesic activity in a dose-dependent manner. The high dose (5000 mg/kg) showed comparable effect to mefenamic acid, validating the ethnomedicinal use of the plant in treating pain. The extract was also found to be non-toxic in acute oral toxicity evaluation. Further studies on mechanism of action and isolation of active constituents are recommended.

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