Analgesic potential of dichloromethane leaf extracts of *Eucalyptus globulus* (Labill) and *Senna didymobotrya* (Fresenius) in mice models

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**IntRODUCTION**

Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage (1). Pain serves as an instant warning to the nervous system to commence a motor response thus minimizing physical damage (2). Insufficient pain relief is a problem globally (3). In pain management, nonsteroidal anti-inflammatory drugs (NSAIDs) are highly prescribed (4). However, these drugs have many side effects such as gastrointestinal complications namely peptic ulcers, bleeding, and mental dependence. Hence, their clinical uses have been limited in pain management (5). Selective

**Keywords:**

Eucalyptus globulus
Senna didymobotrya
Pain
Phytochemicals
Formalin test

**ABSTRACT**

**Introduction:** Pain is managed using conventional drugs like paracetamol, aspirin and diclofenac among others. Synthetic drugs have many side effects. This study aimed at evaluating the analgesic potential of the dichloromethane leaf extracts of *Eucalyptus globulus* and *Senna didymobotrya* in mice.

**Methods:** The dichloromethane leaf extracts of *E. globulus* and *S. didymobotrya* were subjected to quantitative phytochemical analysis using gas chromatography-mass spectrophotometry (GC-MS). *In vivo* analgesic evaluation comprised of nine groups of animals (Swiss albino mice): normal, positive, negative control and six experimental groups that received 25, 50, 100, 150, 200 and 250 mg/kg body weight of each plant extract intraperitoneally. Thirty minutes later, they were injected with 0.01 mL of 2.5% formalin. The animals in positive control group were administered diclofenac (15 mg/kg) and formalin, the normal control mice received 3% dimethyl sulfoxide (DMSO) in normal saline, while the negative group received DMSO in normal saline and formalin. All the doses were administered intraperitoneally. The duration of shaking and licking of the injected paw was scored and analyzed.

**Results:** The analysis revealed that *E. globulus* contained alpha-pinenes, endo-fenchol, alpha-eudesmol, myrcene, camphene, alpha-phellandrene, limonene, and camphor while *S. didymobotrya* possessed camphene, alpha-phellandrene, limonene, and camphor. In the late phase, *E. globulus* at the doses of 25, 50, 100, 150, 200 and 250 mg/kg reduced the paw licking time by 34.03%, 60.79%, 84.33 %, 90.65%, 94.49%, 98.52%, respectively while *S. didymobotrya* extract reduced the paw licking time by 26.48%, 32.96%, 87.04%, 91.27%, 93.40%, 90.97%, and 96.82%, respectively.

**Conclusion:** The results of this study validate and support the traditional uses of these plants as analgesics.

**Implication for health policy/practice/research/medical education:** The leaf extracts of *Eucalyptus globulus* and *Senna didymobotrya* possess analgesic activity in mice formalin model that might contribute to the development of alternative drugs for management of pain.

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cyclooxygenase-2 inhibitors have some benefits in preventing such side effects, while the risk of cardiovascular adverse effects demands important consideration (6). Narcotics are also used in pain management (7). Narcotics refer to opium, opium derivatives, and their fully synthetic or semi-synthetic substitutes, including leaf of cocoa and cocaine (8).

In Kenya, *Eucalyptus globulus* was introduced in 1902 for use as fuel wood for the Kenya-Uganda railway. Junevile leaves of *E. globulus* are opposite, cordate-ovate, sessile and covered with bluish-white bloom. Adult leaves are lanceolate, alternate, 1-2 inches broad and 6-12 inches long (9). *E. globulus* is used as antiseptic, deodorant, astringent, anthelmintic, expectorant, inhalant, diaphoretic, insect repellent, sedative, rubefacient, vermifuge and suppurative (10). Besides, *E. globulus* is used in the management of abscess, asthma, burns, sore throat, bronchitis, cancer, cough, diphtheria, diabetes, arthritis, malaria, dyspepsia, dysentery, miasma, boils, tuberculosis, spasms, wounds, worms and vaginitis (11-13).

*Senna didymobotrya* is a flowering plant that belongs to the legume family. The plant grows to a maximum height of about 30-90 cm. It is found across the country in several types of the habitat. The root extracts of *S. didymobotrya* are utilized in the management of intestinal worms and malaria (14).

Pain is managed using medicinal plants such as *Artemisia dracunculus* (15), *Acacia mellifera*, *Carissa spinarum* (Linn) (16), *Harrisonia abyssinica* (17), among others. *E. globulus* and *S. didymobotrya* are in traditional use in the management of pain by the people of Embu county. However, there are no scientific data to support this biological activity. Therefore, this study was designed to determine the chemical constituents and in vivo analgesic potential of dichloromethane leaf extracts of *E. globulus* and *S. didymobotrya* in mice models.

**Materials and Methods**

Based on the literature review on ethno-medicinal uses of these plants, leaves from each of these plants were harvested from Embu county, Kenya. The plant samples were put in Khaki bags and then transported to Kenyatta University. Further processing of the plant samples was carried out in the Department of Biochemistry, Microbiology, and Biotechnology. The leaf samples were identified and taxonomically assigned voucher specimen numbers by an acknowledged taxonomist. *E. globulus* and *S. didymobotrya* were assigned voucher specimen numbers “JKM001” and “JKM002” respectively. The samples were deposited at Kenyatta University Herbarium for future reference.

The plant leaf samples were air-dried at 25°C for two weeks and then ground into a homogenous fine powder. The powder of each sample was kept at room temperature in well-sealed and labeled airtight khaki papers until use in extraction. Five-hundred grams of each plant sample was weighed separately and then put into well-labeled conical flasks, separately. Two liters of dichloromethane (DCM) were put into each conical flask, corked and then let to stand for one day. The mixtures were then filtered separately using Whatman number 1 filter papers. To each of the remnants, 1 L of DCM was added and left to stand for 24 hours. This was followed by a second filtration. This procedure was repeated until the solvent appeared clear. The concentration of each extract was carried out using a rotary evaporator at 40°C. The concentrated extracts were then separately put in clean open beakers to permit the evaporation of the remaining solvents. The extracts obtained were stored at -4°C until use (18).

Gas chromatography-mass spectrophotometry (GC-MS) analysis

Agilent Gas Chromatograph 7890A/5975C Mass Spectrometer in full scan mode was used to analyze the samples with the following conditions: gas chromatography column “HP-5 MS low bleed capillary column” (0.25 μm, 30 m by 0.25 mm i.d) (J and W, Folsom, California, United States of America), flow rate (Helium) “constant flow mode, 1.25 mL/min”, injection split mode, oven temperature of 35°C for the initial 5 minutes and then raised by 10°C per minute to 28°C for 10.5 minutes and run time of 70 minutes.

For analysis, a protocol was used, which was reviewed by the Principal Scientist, and the Head of the Department of Behavioral and Chemical Ecology in the International Centre of Insect Physiology and Ecology (ICIPE), Prof. Baldwyn Torto. A mass of 1.2 mg of DCM leaf extract of *E. globulus* and 1.1 mg of *S. didymobotrya* were diluted by partitioning between methanol and hexane. This was followed by vortexes and centrifugation. Bypassing through anhydrous Na₂SO₄, the hexane layer was dried and analyzed using GC-MS. Authentic serial dilutions of 1,8-cineole 99% as standard (Gillingham, Dorset, England) (50, 150, 250 and 550 ng/μL) were prepared and analyzed using GC-MS. The peak areas were used for quantification purposes.

Experimental animals

Swissalbinomice of both sexes aged between 2-3 months and weighing approximately 20 g were used to assess analgesic activities of the two DCM leaf extracts. The approval for experimentation of animals was obtained from the National Commission for Science, Technology, and Innovation (Reference number NACOSTI/P/16/6765/14525). The animals were taken care of, and handled as per Kenyatta University ethical guidelines and procedures for handling experimentation animals. Mice were selected 24 hours before experimentation based on their normal response to sensorimotor testing. The sensorimotor test was done by holding the mice in a fully extended and inverted position.
Experimental design

A completely randomized experimental design was adopted in this study. Swiss albino mice were randomly allocated nine groups of 5 mice and treated as follows: group 1 (normal control) comprised mice that received intraperitoneal 3% DMSO. Group 2 (negative control) received 3% DMSO and then pain was induced after 30 minutes by injection of 0.01 mL of 2.5% formalin. Group 3 (positive control), received 0.1 mL of diclofenac sodium at a dose of 15 mg/kg body weight and after 30 minutes were administered with 0.01 mL of 2.5% formalin to induce pain.

Groups 4, 5, 6, 7, 8 and 9 (5 mice/group) received 25 mg/kg, 50 mg/kg, 100 mg/kg, 150 mg/kg, 200 mg/kg and 250 mg/kg DCM leaf extract in 3% DMSO respectively. After 30 minutes, pain was induced through injection of the left hind paw tissue with 0.01 mL of 2.5% formalin. Table 1 summarizes this experimental design.

The formalin-induced pain was carried out by a previously described method (21), where all the animals received 0.1 mL of treatments intraperitoneally and 30 minutes later with 0.01 mL injection of 2.5% formalin in the left hind paw to induce pain. The time taken on licking, shaking, biting or lifting of the injected paw was scored and recorded (22). The Swiss albino mice were placed inside a transparent plexiglass chamber with a mirror put at the side of the chamber to provide a clear observation of the pain response. Two phases of intensive pain were observed and recorded separately (the early phase of 1-5 minutes and late phase of 15-30 minutes). The percentage of pain inhibition was computed using the following formula.

\[
\text{Percentage pain inhibition} = \frac{C - T}{C} \times 100
\]

Where, \(C\) = Vehicle control group value for each phase

### Table 1. Grouping of the mice for evaluation of antinociceptive effects of the extracts

| Animal group | Treatment                      |
|--------------|--------------------------------|
| I            | DMSO only                      |
| II           | Formalin + DMSO                |
| III          | Formalin + 15 mg/kg diclofenac |
| IV           | Formalin + 25 mg/kg extract    |
| V            | Formalin + 50 mg/kg extract    |
| VI           | Formalin + 100 mg/kg extract   |
| VII          | Formalin + 150 mg/kg extract   |
| VIII         | Formalin + 200 mg/kg extract   |
| IX           | Formalin + 250 mg/kg extract   |

Statistical analysis

Data on pain was entered in a Microsoft Excel broadsheet and then transferred to Minitab statistical software (version 17.0) for statistical analysis. Descriptive statistics (mean±SEM) were computed. One-way analysis of variance was employed to analyze for statistical variation among various sets of treatment groups followed by Tukey's post hoc test for mean separations and comparisons. Analgesic activity of the two plant extracts was compared using unpaired \(t\) test. The level of significance was set at 99.5% (\(P \leq 0.005\)).

Results

GC-MS results of *E. globulus* (Labill) and *S. didymobotrya* (Fresenius)

The GC-MS results revealed that these plants were endowed with several bioactive agents that possessed analgesic activity (Table 2). Representative total ion chromatogram of the DCM leaf extracts of *E. globulus* and *S. didymobotrya* with their retention times are respectively presented in Figures 1 and 2.

The molecular and structural formula of compounds identities of DCM leaf extracts of *E. globulus* and *S. didymobotrya* are shown in Figure 3.

Analgesic effects of DCM leaf extracts of *E. globulus* and *S. didymobotrya* in mice

Two phases were used to assess the antinociceptive activities of DCM leaf extracts of *E. globulus* and *S. didymobotrya* on formalin-induced noiception in Swiss albino mice. They included the early phase which lasted between 1-5 minutes and a late phase that lasted between 15-30 minutes after injection of formalin. *E. globulus* leaf extracts possessed analgesic activity in mice. This was evident by a reduction in paw shaking, licking and lifting time (Table 3).

The Swiss albino mice that received *E. globulus* extract at the doses of 25, 50, 100, 150, 200 and 250 mg/kg body weight as well as diclofenac (15 mg/kg), decreased the paw licking time in the early phase by 8.29%, 31.87%, 19.84%, 31.71%, 29.76%, 30.57%, and 31.87%, respectively (Table 3). The analgesic activity of *E. globulus* DCM extract at the six dosages exhibited a significant difference in the early phase (\(P < 0.005\), Table 3). On the other hand, the analgesic effect of diclofenac was not statistically significant compared with *E. globulus* at dosages of 50, 150, 200 and 250 mg/kg body weight in the early phase (\(P > 0.005\), Table 3). The antinociceptive effect of *E. globulus* extract showed a dose-independent response in the early phase (Table 3).

The mice that were administered with DCM leaf extract of *E. globulus* at the doses of 25, 50, 100, 150, 200 and 250 mg/kg body weight including the diclofenac, reduced the paw licking time by 34.03%, 60.79%, 84.33 %, 90.65%,
94.49%, 98.52%, and 98.32%, respectively in the late phase (Table 3). The analgesic activity of the leaf extract at the six dosages revealed a significant difference in the late phase ($P < 0.005$; Table 3). In contrast, the analgesic activity of the diclofenac was comparable to that of DCM leaf extract of E. globulus (Labill) at a dose of 250 mg/kg body weight in the late phase ($P > 0.005$; Table 3). The analgesic effect of the leaf extract of E. globulus showed a dose-dependent response in the late phase (Table 3).

The analgesic effect of DCM extract of E. globulus at all the six dose levels was significantly effective in the late phase compared to the early phase ($P < 0.005$).

On the other hand, the mice that received DCM leaf extract of S. didymobotrya (Fresenius) had reduced analgesic activity compared to the control group ($P < 0.005$).

### Table 2. GC-MS results of E. globulus (Labill) and S. didymobotrya (Fresenius) phytocompounds associated with analgesic activity

| Compound name     | Chemical class | E. globulus retention time (min) | % Abundance | S. didymobotrya Retention time (min) | % Abundance |
|-------------------|----------------|----------------------------------|-------------|--------------------------------------|-------------|
| Endo-fenchol      | Terpenoids     | 13.25                            | 1.15        | -                                    | -           |
| α-Eudesmol        | Terpenoids     | 20.64                            | 14.81       | -                                    | -           |
| Myrcene           | Terpenoids     | 11.01                            | 0.75        | -                                    | -           |
| p-Cymene          | Terpenoids     | 11.64                            | 7.81        | -                                    | -           |
| Camphene          | Terpenoids     | 10.11                            | 2.01        | 10.11                                | 9.11        |
| α-Phellandrene    | Terpenoids     | 11.26                            | 12.73       | 11.26                                | 3.24        |
| Limonene          | Terpenoids     | 11.71                            | 10.44       | 11.73                                | 24.9        |
| β-Pinene          | Terpenoids     | 10.70                            | 10.68       | 10.72                                | 3.85        |
| Camphor           | Flavonoids     | 13.74                            | 4.03        | 13.74                                | 34.01       |
| α-Pinenes         | Terpenoids     | 9.78                             | 5.73        | 9.80                                 | 5.47        |

The representative total ion chromatogram of the DCM leaf extract of E. globulus with retention time is shown in Figure 1.
Analgesic effects of *Eucalyptus globulus* and *Senna didymobotrya*

Figure 2. Representative total ion chromatogram of the DCM leaf extract of *S. didymobotrya* with retention time.

Figure 3. The structural and molecular formula of compounds identified by gas chromatography-mass spectrometry analysis of the DCM leaf extract of *E. globulus* and *S. didymobotrya*.

formalin-induced pain which was evident by reduced paw time licking in the two phases (Table 4). The mice that received DCM extract of *S. didymobotrya* at the doses of 50, 100, 150, 200 and 250 mg/kg bw as well as diclofenac (the reference drug) reduced paw licking time in the early phase by 0.43%, 1.61%, 5.81%, 7.20%, 7.96%, and 38.17%, respectively (Table 4). However, the plant extract at 25 mg/kg body weight dose level did not show significant analgesic effect in the early phase as shown in Figure 3. In the early phase, the analgesic activity of *S. didymobotrya* at the six dose levels revealed significant differences ($P<0.005$; Table 4). The analgesic effect of diclofenac was significantly higher compared to that of the DCM extract of *S. didymobotrya* at all dose levels in the early phase ($P<0.005$; Table 4). The antinociceptive effect of *S. didymobotrya* demonstrated a dose-dependent response in the early phase (Table 4). The *S. didymobotrya* extract at the doses of 25, 50, 100, 150, 200 and 250 mg/kg bw including diclofenac, lowered the paw licking time by 26.48%, 32.96%, 87.04%, 91.27%, 93.40%, 90.97%, and 96.82%, respectively in the late phase (Table 4). The analgesic activity of the *S. didymobotrya* extract at the six dose levels was statistically significant in the late phase ($P<0.005$, Table 4). The analgesic effect of diclofenac was significantly
higher compared to those of the six of extracts dose levels in the late phase \((P<0.005, \text{ Table 4})\). The analgesic effect of \textit{S. didymobotrya} had a dose-dependent response in both phases, except the dose of 250 mg/kg bw dose in the late phase that was not as potent as 200 mg/kg bw \((\text{Table 4})\). In comparison, the analgesic effect of \textit{S. didymobotrya} DCM leaf extract in the late phase was significantly higher compared to the early phase at the six dose levels tested in mice \((P<0.005, \text{ Table 4})\).

Comparatively, the analgesic effect of \textit{E. globulus} DCM extract was significantly higher than that of \textit{S. didymobotrya} DCM extract at all the tested dose levels in the early phase \((P<0.005, \text{ Figure 4})\).

The analgesic effects of \textit{S. didymobotrya} (Fresenius) and \textit{E. globulus} DCM leaf extracts demonstrated no significant differences at the doses of 100, 150 and 200 mg/kg body weight in the late phase \((P>0.005, \text{ Figure 4})\). On the other hand, the analgesic effect of the DCM extract of \textit{E. globulus} was significantly higher compared to that of \textit{S. didymobotrya} in the late phase the doses of 25, 50 and 250 mg/kg body weight \((P<0.005, \text{ Figure 5})\).

**Discussion**

This study aimed at determining the analgesic activity of DCM leaf extracts of \textit{E. globulus} and \textit{S. didymobotrya} on formalin-induced nociception in mice. The \textit{E. globulus} and \textit{S. didymobotrya} leaf extracts showed significant analgesic effects by reducing pain in mice in the early and late phases. The demonstrated effects were both peripheral and central \((23)\). The central analgesic activity could have been due to inhibition of the nociceptive effects of noradrenaline, bradykinin, prostaglandins, adrenaline, adenosine, serotonin, and acetylcholine. On the other hand, the peripheral analgesic effect could be attributed to inhibition of the discharge of endogenous pain mediators like prostaglandin-2 (PGE\textsubscript{2}) and PGE\textsubscript{2α} in peritoneal fluids including lipoxygenase which triggers the nociceptive neurons \((24)\).

The significant antinociceptive effects of DCM leaf extracts of these plants could be attributed to the existence of analgesic components that acted by blocking the prostaglandin pathways \((25)\). The DCM leaf extracts mechanisms of action can be postulated to be similar to those of NSAIDs like diclofenac and ibuprofen. These

| Group | Treatment | Early Phase (1-5 min) | Late Phase (15-30 min) |
|-------|-----------|----------------------|------------------------|
| Normal control | DMSO only | 0.00±0.00* (0.00) | 0.00±0.00* (0.00) |
| Negative control | Formalin + DMSO | 123.00±1.53* (100.00) | 297.40±9.58* (100.00) |
| Positive control | Diclofenac 15 mg/kg bw + Formalin | 83.80±1.39* (31.87) | 5.00±0.89* (98.32) |
| DCM leaf extract of \textit{E. globulus} | 25 mg/kg bw + Formalin | 112.80±1.24* (8.29) | 196.20±1.05* (34.03) |
| | 50 mg/kg bw + Formalin | 83.80±1.62* (31.87) | 116.80±0.93* (60.79) |
| | 100 mg/kg bw + Formalin | 98.60±1.08* (19.84) | 46.60±0.93* (84.33) |
| | 150 mg/kg bw + Formalin | 84.00±0.55* (31.71) | 27.80±1.16* (90.65) |
| | 200 mg/kg bw + Formalin | 86.40±1.96* (29.76) | 16.40±1.03* (94.49) |
| | 250 mg/kg Bw + Formalin | 85.40±1.78* (30.57) | 4.40±0.75* (98.52) |

Values are expressed as mean ± SEM for 5 mice in each group. Values with the different superscript letters are statistically significant along the same column using one-way ANOVA accompanied by Tukey’s post hoc test \((P≤0.005)\). The values in brackets represent % pain inhibition.

| Animal Group | Treatments | Early Phase (1-5 min) | Late Phase (15-30 min) |
|--------------|------------|----------------------|------------------------|
| Normal control | DMSO only | 0.00±0.00* (0.00) | 0.00±0.00* (0.00) |
| Negative control | Formalin + DMSO | 186.00±0.01* (100.00) | 321.00±0.01* (100.00) |
| Positive control | Diclofenac 15 mg/kg bw + Formalin | 115.00±3.29* (38.17) | 10.00±0.49* (96.82) |
| DCM leaf extract of \textit{S. didymobotrya} | 25 mg/kg bw + Formalin | 189.60±0.93* (-1.94) | 236.00±1.70* (26.48) |
| | 50 mg/kg bw + Formalin | 185.20±1.69* (0.43) | 215.20±0.97* (32.96) |
| | 100 mg/kg bw + Formalin | 183.00±1.22* (1.61) | 41.60±1.8* (87.04) |
| | 150 mg/kg bw+ Formalin | 175.20±1.39* (5.81) | 28.20±0.97* (91.17) |
| | 200 mg/kg bw + Formalin | 172.00±0.93* (7.20) | 21.20±1.07* (93.40) |
| | 250 mg/kg Bw + Formalin | 171.20±1.77* (7.96) | 29.00±1.26* (90.97) |

Values are expressed as mean ± SEM for 5 mice in each group. Values with the different superscript letters are statistically significant along the same column using one-way ANOVA accompanied by Tukey’s post hoc test \((P≤0.005)\). The values in brackets represent % pain inhibition.
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Drugs block the synthesis of prostaglandins by truncating the cyclooxygenase-1 pathway (26). This inhibition lowers the peripheral nervous tissue sensitization leading to less nerve stimulation and pain reduction (27). The DCM leaf extracts of the two plants exhibited analgesic effects by decreasing paw licking time in early and late phases on formalin-induced pain in mice (28).

GC-MS results revealed the presence of bioactive phytochemicals that possessed analgesic activity. According to previous study, Aniba canelilla was found to have analgesic effects on the acetic acid-induced writhing, hot plate test and formalin-induced pain (29). Studies by (30), on antihypernociceptive activity of O. gratissimum essential oil, reported that myrcene at doses of 10, 20 and 40 mg/kg body weight showed significant antinociceptive activity against von Frey and hot plate tests models in mice. Similarly, a study by Shah et al (38), reported that myrcene had antinociceptive potential, by reducing latency time in tail-flick and hot plate test models in rodents.

Conclusion

In conclusion, the DCM leaf extracts of E. globulus and S. didymobotrya were found to contain bioactive compounds that were able to significantly reduce formalin-induced analgesia in mice. This study validates the traditional use of these plants in the management of pain.

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Authors’ contributions

JKM, CMK, JNN and MPN: Designed the project, collected the specimen and performed the experiments, analyzed and data. The first draft was prepared by JKM. All authors read the final version and confirmed it for the publication.

Conflict of interests

All the listed authors declare no conflicts of interest.
Ethical considerations

The approval for experimentation of animals was obtained from the National Commission for Science, Technology and Innovation (Reference Number: NACOSTI/P/16/6765/14525).

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