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Anti-Nociceptive and Anti-Inflammatory Potential of Stem Bark Fractions of *Dalbergia candenatensis* (Dennst.) Prain: Insights from Experimental Models into Underlying Mechanisms

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Abstract

Dalbergia candenatensis (Dennst.) Prain (family: Fabaceae), a mangrove species with traditional medicinal use, was evaluated for its phytochemical composition and the *in vivo* anti-nociceptive and anti-inflammatory effects of its stem bark fractions in Swiss albino mice. Acute toxicity was studied at doses up to 2000 mg/kg body weight (b.wt.) and animals were observed for 14 days. Anti-nociceptive activity was evaluated using acetic acid-induced writhing (chemically induced pain), formalin-induced paw licking (neurogenic and inflammatory pain), tail immersion, and hot plate methods (thermally induced pain). Anti-inflammatory activity was evaluated in carrageenan and formalin-induced paw edema models. Results showed that mice exhibited no mortality or noticeable behavioral alterations at doses up to 2000 mg/kg b.wt. during the 14 days observation period. In comparison to control, all fractions at 200 mg/kg b.wt. showed significant ($p < 0.001$) anti-nociceptive activity. The n-hexane fraction produced the highest inhibition (50.86%) in the acetic acid test. In the formalin test, the ethyl acetate fraction exhibited maximum inhibition (59.69% in the early phase and 48.00% in the late phase). In the tail immersion and hot plate methods, ethyl acetate and dichloromethane frac-

tions produced significant ($p < 0.001$) increases in reaction time compared with standard drugs. In the anti-inflammatory assays, the ethyl acetate fraction showed maximum inhibition of paw edema in both carrageenan (47.37%) and formalin (51.30%) models. The results suggest that stem bark fractions of *D. candenatensis* possess significant ($p < 0.001$) anti-nociceptive and anti-inflammatory activity, possibly mediated by both central and peripheral mechanisms.

Keywords

Dalbergia candenatensis, Phytochemical Composition, Indomethacin, Analgesic, Anti-Inflammatory, Swiss Albino Mice

1. Introduction

Oxidative stress, resulting from an imbalance between reactive oxygen species (ROS) production and the body's antioxidant defenses, plays a pivotal role in the pathogenesis of various chronic conditions, including pain, inflammation, neurodegenerative disorders, and cardiovascular diseases [1] [2]. ROS such as hydrogen peroxide (H_2O_2), superoxide anions (O_2^-), and hydroxyl radicals ($OH^{\cdot}$) can damage proteins, lipids, and DNA, thereby triggering pathological processes that contribute to both nociceptive and inflammatory responses [3]. While conventional synthetic analgesics and nonsteroidal anti-inflammatory drugs (NSAIDs) remain the primary therapeutic options, their long-term use is often limited by gastrointestinal, cardiovascular, and other systemic adverse effects [1] [4] [5]. This has driven increasing interest toward plant-derived agents with lower toxicity profiles that can offer comparable efficacy in pain and inflammation management [6].

Natural products have historically provided an invaluable source of pharmacologically active compounds, with more than half of all modern drugs originating directly or indirectly from plants [7] [8]. Herbal medicines, in particular, exert their effects through synergistic actions of multiple bioactive constituents, which may enhance therapeutic potential while minimizing adverse reactions [9]. Mangrove plants, in particular, have evolved remarkable biochemical and physiological adaptations to survive in extreme saline and tidal conditions. This ecological resilience is reflected in their rich and diverse array of secondary metabolites, many of which exhibit potent pharmacological activities, including antioxidant, antimicrobial, anti-inflammatory, and analgesic effects [10] [11]. Such unique chemical diversity positions mangrove-associated species as promising yet underexplored candidates for drug development [12].

Dalbergia candenatensis (Dennst.) Prain, a mangrove-associated species belonging to the Fabaceae family, is traditionally used in South and Southeast Asia for various ailments, including infections, respiratory disorders, and skin diseases [13]-[15]. The deep red heartwood is valued in Thailand as both a natural red dye and an antibacterial agent [15]. Ethnomedicinally, the heartwood has also been

recognized for its therapeutic benefits as a blood tonic and expectorant, and for its antibacterial and antifungal properties [16]. Phytochemical investigations have revealed a diverse profile of secondary metabolites, including flavonoids, isoflavonoids, neoflavonoids, quinones, triterpenes and glycosides, some of which exhibit antimicrobial, cytotoxic, and anti-inflammatory activities [15] [17]-[22]. Several bioactive molecules have been identified, notably a series of candenatenins (A-K) and flavonoid derivatives such as 4-hydroxy-3-methoxy-8,9-methylenedioxypterocarpan, 3,5-dihydroxy-7-methoxyflavanone, claussequinone, 5-hydroxybowdichione, formononetin, (R)-4-methoxydalbergione, and melilotocarpan A [15] [17]-[19]. Despite this rich ethnomedicinal background, the analgesic and anti-inflammatory properties of different solvent fractions of *D. candenatensis* stem bark remain largely unexplored [22]-[27]. The present study investigates these pharmacological activities using different solvent fractions of *D. candenatensis* stem bark in Swiss Albino mice. By integrating phytochemical screening with *in vivo* models, this work aims to provide scientific validation for its traditional uses and identify bioactive fractions that could serve as promising leads for the development of safer, plant-based analgesic and anti-inflammatory agents.

2. Materials and Methods

2.1. Plant Collection and Crude Extraction

Fresh mature stem barks of *Dalbergia candenatensis* were harvested from the Sundarbans, Bangladesh, in January 2023. A voucher specimen for this collection was deposited at the Bangladesh National Herbarium, Mirpur, Dhaka, and the plant was authenticated by Mr. Ahmed Saqee, Scientific Officer, with an accession number DACB 94873.

Extraction of Plant Materials

The freshly collected stem barks were thoroughly washed with water to remove adhering debris, excess moisture was blotted off, cut into small pieces, and the samples were air-dried followed by oven drying at 40˚C. The dried material was then finely ground to a 100-mesh powder using a heavy-duty blender and stored in airtight containers under refrigeration until further use.

A cold maceration of 1 kg powdered stem bark was performed in 3 liters of 99% ethanol at room temperature for 7 days with occasional stirring. The mixture was filtered through cotton and Whatman No. 1 paper, repeated three times with fresh ethanol. The combined filtrates were concentrated under reduced pressure at 40˚C using a rotary evaporator, yielding a crude ethanolic extract. This was fractionated by a modified Kupchan method [28] into n-hexane (HDCB), dichloromethane (DDCB), ethyl acetate (EDCB), and aqueous (ADCB) fractions, respectively, and stored at −20˚C until use.

2.2. Qualitative Phytochemical Screening

Various extracts of *D. candenatensis* stem bark was subjected to phytochemical

screening following established quantitative protocols [29]. The analysis focused on detecting the presence of key secondary metabolites, including alkaloids, phenolics, steroids, reducing sugars, saponins, tannins, and flavonoids [30].

2.3. Ethical Permission and Experimental Animals

All animal procedures adhered to the ARRIVE 2.0 guidelines for animal research reporting. The study protocol received approval from the Ethical Review Committee (ERC) of the Committee on Ethical Compliance in Research, Department of Pharmacy, Southeast University (SEU/Pharm/CECR/113/2025). Twenty-four Swiss Albino mice (20 - 25 g) were housed under standard laboratory conditions (22˚C ± 2˚C temperature, 55% ± 10% relative humidity, 12-hour light/dark cycle) with free access to standard pellet diet and water ad libitum. Animals were randomly allocated to experimental groups, and all behavioral tests were performed by an investigator blinded to treatment assignments [1]. Humane endpoints were established, with no unexpected mortality observed. All procedures conformed to the National Institute of Health (NIH) Guide for the Care and Use of Laboratory Animals.

2.4. Oral Acute Toxicity Test

The acute oral toxicity of *D. candenatensis* stem bark fractions was evaluated in mice following Organization for Economic Cooperation and Development (OECD) Guidelines 425 (acute toxic class method) with reference to Lorke's procedure [31] [32]. Animals received single oral doses of 100, 300, 500, 1000, and 2000 mg/kg b.wt. Following administration, the animals were observed continuously for the first 4 hours for behavioral, neurological, and autonomic changes (such as sedation, tremors, salivation, or convulsions), and subsequently monitored daily for a period of 14 days to identify any delayed signs of toxicity or mortality.

2.5. Experimental Groups and Treatments

Twenty-four mice were randomly divided into six groups, each containing four animals, to evaluate the effects of different solvent fractions of *D. candenatensis* stem bark. Treatments were administered as follows:

Group I (Control): Received vehicle (1% Tween 80 in water or 1% normal saline, 10 ml/kg body weight).

Group II (Standard): Treated with Indomethacin (oral) or Diclofenac sodium (intraperitoneal) at 10 mg/kg body weight.

Group III (HDCB): Received the n-hexane fraction orally at 200 mg/kg body weight.

Group IV (DDCB): Received the dichloromethane fraction orally at 200 mg/kg body weight.

Group V (EDCB): Received the ethyl acetate fraction orally at 200 mg/kg body weight.

Group VI (ADCB): Received the aqueous fraction orally at 200 mg/kg body weight.

The dose of 200 mg/kg was chosen based on prior literature demonstrating significant pharmacological effects with minimal toxicity [16]. This was further supported by preliminary in-house studies confirming the dose's efficacy and tolerability in our animal model. Consequently, this dose was consistently applied throughout the study to ensure comparability.

2.6. Analgesic Activity

2.6.1. Acetic Acid Induced Writhing Method

The analgesic potential of various fractions of the ethanolic extract of *D. candenatensis* stem bark was assessed using the acetic acid-induced writhing test in Swiss albino mice [33]. Pain was induced in all groups by intraperitoneal injection of 0.7% acetic acid. Test samples and the vehicle were administered orally 30 minutes prior to pain induction, whereas the standard drug, indomethacin, was given 15 minutes before the acetic acid injection. Five minutes after acetic acid administration, the number of characteristic body contractions in each mouse was recorded for a duration of 10 minutes. Complete writhing was not always finished by the mice which (incomplete writhing) was counted as half-writhing and two half-writhing were counted as one complete writhing. Reduction of writhing count was considered indicative of analgesic activity. Analgesic potential was evaluated by comparing the count of writhing showed by the samples to that of control. Calculation of percent analgesic activity was achieved using the following formula:

$$\text{Percentage analgesic activity} = [(A - B)/A] \times 100\% \text{ [33]}$$

here, A represents the mean writhing count of the control group, while B indicates the mean writhing count of the experimental group.

2.6.2. Formalin Induced Paw Licking Method

The antinociceptive activity was evaluated employing the formalin test, as outlined by Dubuisson and Dennis [34]. Each group of mice taken a 20 µL injection of 5% formalin into the right hind paw, administered 30 minutes after oral gavage of the test samples and 15 minutes following intraperitoneal administration of the standard drug (diclofenac sodium). Following formalin injection, the mice were monitored for 30 minutes to determine the total duration of licking or biting of the treated paw. The percentage reduction in pain response was calculated distinctly for the early phase (first 5 minutes post-formalin injection) and the late phase (15 - 30 minutes post-formalin injection).

2.6.3. Eddy's Hot Plate Test

The Eddy's hot plate test evaluates analgesic activity by measuring paw withdrawal responses to thermal stimuli, such as paw licking or jumping [35]. In this assay, mice were individually placed on a hot plate maintained at 55˚C ± 5˚C, and the latency of their response was recorded for each experimental group. Reaction time

(first jump or onset of licking) was measured before treatment and at 0.5, 1, and 2 hours after administering the respective treatments. Diclofenac sodium, used as the standard, was administered intraperitoneally to Group II.

2.6.4. Tail Immersion Method

In the tail immersion method [36], pain was elicited by submerging the lower 5 cm of the mouse's tail in water maintained at 55˚C ± 2˚C, with tail withdrawal serving as an indicator of nociceptive response. The study groups received vehicle and test samples 30 minutes prior, while the standard drug was administered 15 minutes before tail immersion. A cut-off time of 20 seconds was set to prevent accidental injury. The latency to respond (tail withdrawal) was recorded for each mouse.

2.7. Anti-Inflammatory Activity

2.7.1. Carrageenan-Induced Hind Paw Edema

The anti-inflammatory potential of various fractions of *D. candenatensis* was evaluated using the method described by Olaleye *et al.* [37], with slight modifications. Inflammation was induced by injecting 0.1 mL of carrageenan into the sub-plantar tissue of the right hind paw of each mouse. Treatments were administered 30 minutes prior to carrageenan injection, except for the standard group, which received indomethacin orally 15 minutes before the injection of the inflammatory agent. Paw volume was measured using a micrometer screw gauge at the 1st, 2nd, 3rd, and 4th hour after carrageenan administration. The percentage inhibition of inflammation was calculated using the following formula:

$$\text{Percentage inhibition} = (1 - D_t/D_o) \times 100$$

where, D_o represents the average inflammation of the control group at an indicated time, D_t represents the average inflammation treated by extract or reference (indomethacin) groups at the same time.

2.7.2. Formalin-Induced Mice Paw Edema

The formalin-induced paw edema method, used to assess anti-inflammatory potential, involved oral administration of various fractions over a 4-hour period, followed by formalin injection and measurement of paw swelling during the acute phase of inflammation [38]. One hour after the final dose, formalin (1% w/v in normal saline) was injected to induce paw edema, which was measured from immediately before injection up to four hours post-injection, until inflammation subsided. Statistical significance was determined by comparing all treated groups to the control. The anti-inflammatory activity was calculated using the following formula:

$$\text{Percentage inhibition} = (1 - D_t/D_o) \times 100$$

where, D_o refers to the average inflammation (hind paw edema) of the control at an indicated time, D_t represents the average inflammation treated by extract or reference (indomethacin) groups at the same time.

2.8. Statistical Analysis

All data obtained from above experiments were expressed as mean ± SEM. Statistical analysis was performed using one way ANOVA (Analysis of Variance) followed by Dunnett's test, employing GraphPad Prism 8.0.2 (263).

3. Results

3.1. Qualitative Phytochemical Analysis

Phytochemical analysis of different extractives of *D. candenatensis* was conducted to identify the presence of bioactive compounds. The analysis revealed the presence of reducing sugars, tannins, phenols, flavonoids, steroids, alkaloids, glycosides, terpenoids and proteins, and the absence of saponins, as qualitatively presented in Table 1.

Table 1. Phytochemical analysis results of different extractives of *D. candenatensis.*

Phytochemical Test	CDCB	HDCB	DDCB	EDCB	ADCB
Alkaloids	+	+	+	+	+
Flavonoids	+	−	+	+	+
Tannins	+	−	−	+	+
Phenol	+	−	+	+	+
Saponins	−	−	−	−	−
Steroids	+	+	+	+	+
Glycoside	+	+	+	+	+
Proteins	+	−	+	+	+
Terpenoids	+	−	+	+	−

Here, + = Present in mild amount, − = Absent.

3.2. Oral Acute Toxicity Test

Administration of the different fractions of *D. candenatensis* stem bark did not produce any mortality in mice at doses up to 2000 mg/kg body weight during the 14 days observation period. Furthermore, no noticeable behavioral or physiological changes were observed in the treated groups, indicating the relative safety of the extracts at the tested dose range. Based on these findings, a safe dose of 200 mg/kg b.wt. was selected for subsequent analgesic and anti-inflammatory studies to ensure therapeutic efficacy while maintaining safety.

3.3. Analgesic Activity

3.3.1. Acetic Acid Induced Writhing Method

Different extractives of *D. candenatensis* exhibited notable analgesic activity by reducing acetic acid induced writhing in mice, as shown in Table 2 and Figure 1(a). The n-hexane fraction (HDCB) at 200 mg/kg b.wt. produced the highest inhibition (50.86%), followed by the ethyl acetate fraction (EDCB) with 43.97%,

the aqueous fraction (ADCB) with 41.38%, and the dichloromethane fraction (DDCB) with 34.05%. All fractions demonstrated highly significant effects (****p < 0.001) compared to the control group.

Table 2. Analgesic activity of different extractives of *D. candenatensis* stem barks by acetic acid induced writhing method.

Groups	Treatment	Dose (mg/kg b.w.)	No. of writhing (Mean ± SEM)	% Writhing inhibition
Group-I (Control)	1% Tween 80 in water	10 ml/kg	29 ± 0.46	--
Group-II (Standard)	Indomethacin	10	18 ± 0.41****	27.59
Group-III	HDCB	200	14.25 ± 0.85****	50.86
Group-IV	DDCB	200	19.125 ± 0.77*****	34.05
Group-V	EDCB	200	16.25 ± 1.55****	43.97
Group-VI	ADCB	200	17 ± 0.71****	41.38

Values were reported as mean ± S.E.M. (n = 4). Values were analyzed as compared to control using one way ANOVA followed by Dunnett's test. Asterisks indicated statistically significant values from control, *p < 0.05, **p < 0.01, ***p < 0.001 and ****p < 0.0001.

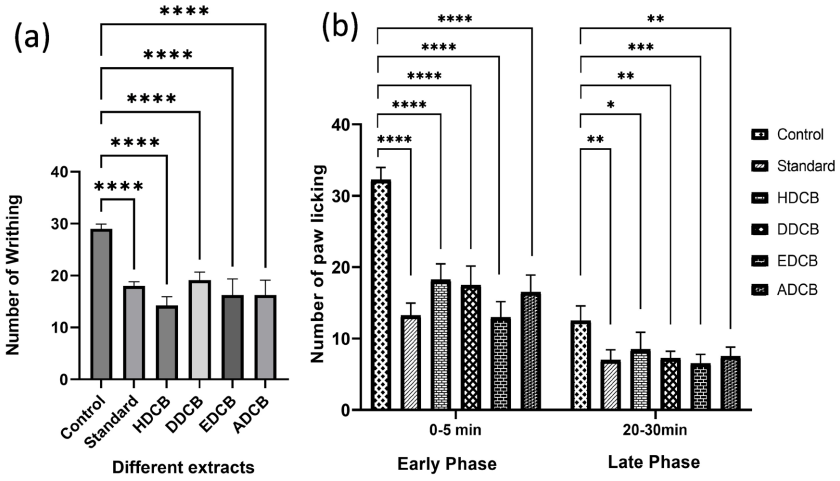

Figure 1. One way ANOVA followed by Dunnett's multiple comparisons test for analgesic activity of different extractives of *D. candenatensis* by (a) Acetic acid induced writhing method, and (b) Formalin induced paw licking method to compare each extract group with the control. Asterisks indicated statistically significant values from control, *p < 0.05, **p < 0.01, ***p < 0.001 and ****p < 0.0001.

3.3.2. Formalin Induced Paw Licking Method

Paw licking was significantly (****p < 0.0001) reduced by all extractives, as revealed in Table 3 and Figure 1(b). In the early phase (0 - 5 min), the HDCB, DDCB, EDCB, and ADCB fractions produced 43.41%, 45.73%, 59.69%, and 48.84% inhibition of paw licking, respectively. In the late phase (20 - 30 min), the respective inhibitions were 32.00%, 42.00%, 48.00%, and 40.00%. Among all fractions, the ethyl acetate fraction (EDCB) showed the highest inhibition in both phases, approaching the effect of the standard drug indomethacin (58.91% and

44.00% inhibition in the early and late phases, respectively).

Table 3. Impact of various fractions of *D. candenatensis* stem barks on paw licking inhibition during the early and late phases of the formalin induced paw licking method.

Group	Doses (mg/kg bw)	Early phase (0 - 5 min)	Inhibition (%)	Late phase (20 - 30 min)	Inhibition (%)
Group-I (Control)	1 ml/kg	32.25 ± 0.99	-	12.5 ± 1.2	-
Group-II (Indomethacin)	10	13.25 ± 0.99****	58.91	7 ± 0.82**	44
Group-III (HDCB)	200	18.25 ± 1.28****	43.41	8.5 ± 1.37*	32
Group-IV (DDCB)	200	17.5 ± 1.52****	45.73	7.25 ± 0.55**	42
Group-V (EDCB)	200	13 ± 1.24****	59.69	6.5 ± 0.75***	48
Group-VI (ADCB)	200	16.5 ± 1.37****	48.84	7.5 ± 0.75**	40

Values were reported as mean ± S.E.M. (n = 4). Values were analyzed as compared to control using one way ANOVA followed by Dunnett's test. Asterisks indicated statistically significant values from control, *p < 0.05, **p < 0.01, ***p < 0.001 and ****p < 0.0001.

3.3.3. Tail Immersion Method

In tail immersion method, all fraction of *D. candenatensis* stem barks exhibited significant reduction in pain stimulus, as illustrated in **Figure 2(a)**. One hour after extract administration of DDCB, HDCB, EDCB and ADCB reduced the painful stimulation 45.95% and 43.24% respectively illustrated in **Figure 2(b)**; it indicates that all fractions demonstrated highly significant effects (****p < 0.001) compared to the control group at the 1 hour after administration.

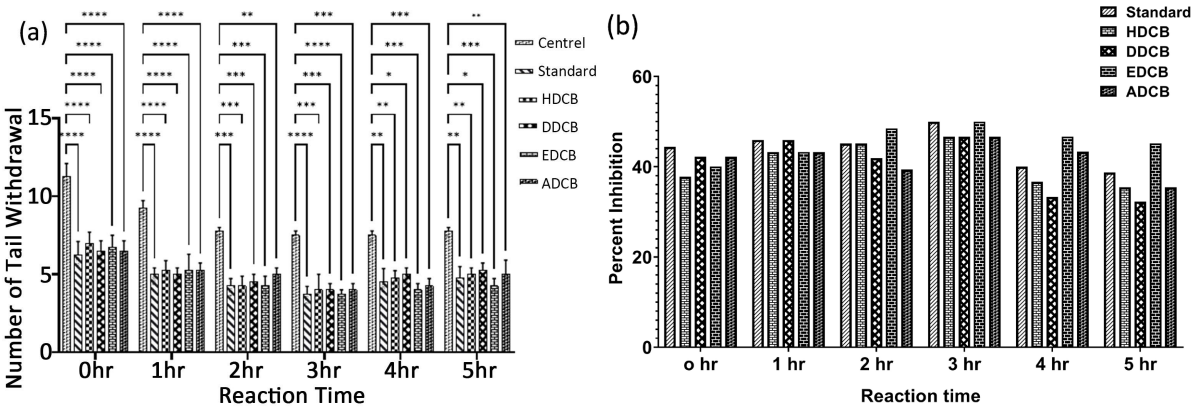

Figure 2. Impact of various fractions of *D. candenatensis* stem barks by tail immersion method. (a) One-way ANOVA followed by Dunnett's multiple comparisons test was used to compare each extract group with the control for anti-inflammatory activity for analgesic activity. Values were reported as mean ± S.E.M. (n = 4) and asterisks indicated statistically significant values from control, *p < 0.05, **p < 0.01, ***p < 0.001 and ****p < 0.0001. (b) Percentage of inhibition.

3.3.4. Eddy's Hot Plate Method

The analgesic potential obtained from various fraction of *D. candenatensis* by Eddy's hot plate method was illustrated in the **Figure 3(a)**. The maximum result was found at the ethyl acetate fraction which displayed highest reaction time for

the response against thermal stimuli 40.91% compared to standard Diclofenac 57.89% inhibition illustrated in Figure 3(b).

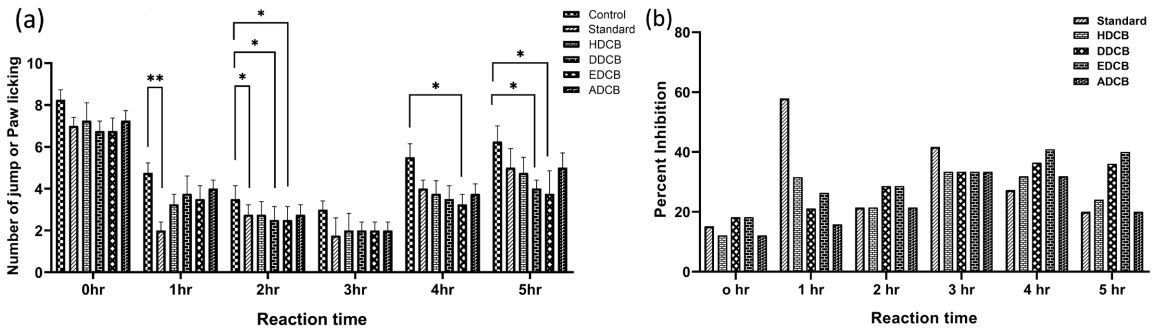

Figure 3. Effects of the different fractions of *D. candenatensis* stem barks by Eddy's hot plate method. (a) One-way ANOVA followed by Dunnett's multiple comparisons test was used to compare each extract group with the control for anti-inflammatory activity for analgesic activity. Values were reported as mean ± S.E.M. (n = 4) and asterisks indicated statistically significant values from control, *p < 0.05, **p <0.01, ***p < 0.001 and ****p < 0.0001. (b) Percentage of inhibition of pain at various time intervals.

3.4. Anti-Inflammatory Activity

3.4.1. Carrageenan Induced Hind Paw Edema

The anti-inflammatory activity obtained from various fraction of *D. candenatensis* by carrageenan induced hind paw edema method was demonstrated in the Figure 4(a). The figure shows that carrageenan-induced paw edema was significantly reduced (*p < 0.05) by the EDCB and DDCB fractions. Maximum inhibition (47.37%) was exhibited by EDCB after 4 hours of administration compared to standard Indomethacin which showed 47.37% inhibition Figure 4(b).

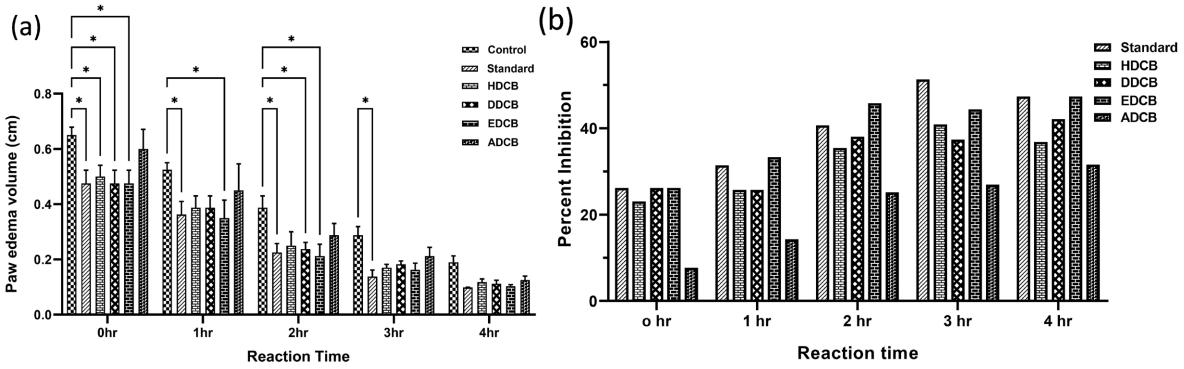

Figure 4. Effects of the different fractions of *D. candenatensis* stem barks on carrageenan induced paw edema model. (a) One-way ANOVA followed by Dunnett's multiple comparisons test was used to compare each extract group with the control for anti-inflammatory activity. Values were reported as mean ± S.E.M. (n = 4) and asterisks indicated statistically significant values from control, *p < 0.05, **p < 0.01, ***p < 0.001 and ****p < 0.0001. (b) Percentage of inhibition at various time intervals.

3.4.2. Formalin Induced Hind Paw Edema

The anti-inflammatory activity obtained from various extractives of *D. candenatensis* by formalin induced hind paw edema test was illustrated in the Figure 5(a). The figure indicates that formalin-induced paw edema was significantly re-

duced by all fractions except the aqueous fraction. The highest inhibition (51.30%) was observed with the EDCB fraction at 200 mg/kg b.w., 3 hours post-administration, which was comparable to the standard drug indomethacin (51.30% inhibition), illustrated in the Figure 5(b).

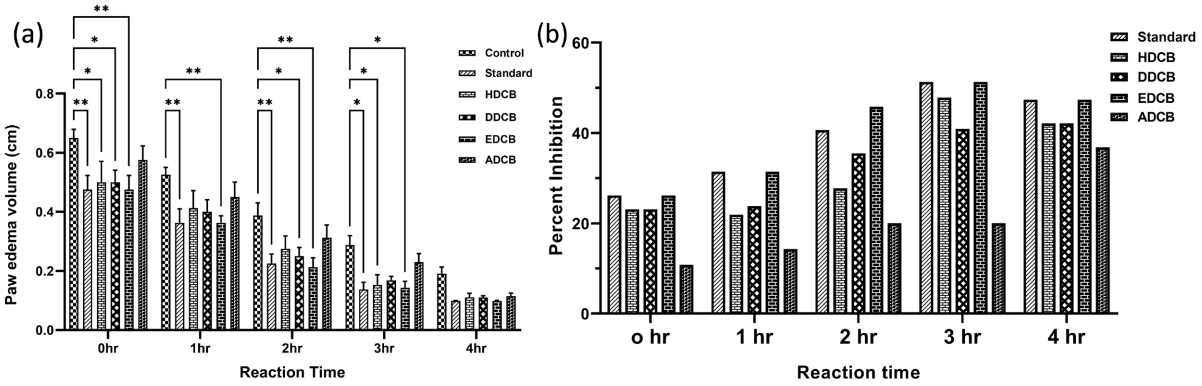

Figure 5. Effects of the different fractions of *D. candenatensis* stem barks on formalin induced hind paw edema model (a) one-way ANOVA followed by Dunnett's multiple comparisons test was used to compare each extract group with the control for anti-inflammatory activity. Values were reported as mean ± S.E.M. (n = 4) and asterisks indicated statistically significant values from control, *p < 0.05, **p < 0.01, ***p < 0.001 and ****p < 0.0001. (b) Percentage of inhibition at various time intervals.

4. Discussion

Mangrove plants are rich sources of diverse bioactive constituents, including flavonoids, tannins, and alkaloids, which possess diverse pharmacological properties. Despite this, these plants have been comparatively underexplored, and their potential in drug development remains largely untapped. Increased scientific focus on mangrove species could reveal novel therapeutic compounds, offering substantial contributions to pharmaceutical research [11] [12]. The present study aimed to assess the phytochemical composition and pharmacological potential of different solvent fractions from *D. candenatensis* stem bark. The analgesic and anti-inflammatory activities were evaluated through multiple *in vivo* models, each providing insight into the underlying pharmacological mechanisms. Phytochemical screening revealed the presence of reducing sugars, tannins, phenolics, flavonoids, steroids, alkaloids, glycosides, and proteins, while saponins were absent. These bioactive constituents are well documented for their pharmacological activities, particularly in pain modulation and inflammation control [39] [40]. Flavonoids, such as quercetin, exert analgesic and anti-inflammatory effects by interacting with the L-arginine-nitric oxide pathway, modulating serotonergic and GABAergic neurotransmission, and suppressing the release of pro-inflammatory mediators [41]. Phenolic compounds can inhibit cyclooxygenase (COX) and lipoxygenase (LOX) enzymes, reducing prostaglandin and leukotriene synthesis, while tannins may exert local anti-nociceptive effects by precipitating proteins at the sensory nerve endings, thereby reducing nerve excitability [42]-[44].

The analgesic potential of the extracts was elucidated through multiple experi-

mental pain models. In the acetic acid induced writhing test, all extracts demonstrated significant inhibition of writhing, with EDCB and ADCB showing the highest percentage of inhibition. The writhing response is primarily mediated through the release of peripheral inflammatory mediators such as prostaglandins (particularly PGE_2 and $PGF_2\alpha$), bradykinin, histamine, and serotonin, which sensitize nociceptors to pain stimuli [45]. Phytochemical analysis revealed the presence of flavonoids, tannins, and phenolic compounds, all of which are known to inhibit prostaglandin synthesis and suppress oxidative stress, thereby contributing to analgesic activity through peripheral mechanisms [40] [46]. Flavonoids, particularly quercetin-like compounds, can inhibit cyclooxygenase (COX) and lipoxygenase pathways, reducing prostaglandin biosynthesis, while tannins may exert protein precipitating effects on the peritoneal lining, thereby decreasing sensitivity to chemical pain stimuli [47].

Similarly, In the formalin induced paw licking test, all extracts reduced nociceptive behavior in the early phase (neurogenic pain), whereas inhibition in the late phase (inflammatory pain) was less pronounced. The early phase is mediated primarily by direct activation of nociceptors through substance P and bradykinin. The selective inhibition observed in the early phase suggests that the extracts may act mainly on neurogenic pain pathways, possibly through modulation of ion channels or interference with neurotransmitter release. This observation may be linked to the flavonoid content, which can modulate neurotransmitter systems and ion channel activity, while tannins may stabilize peripheral nerve endings, thereby contributing to the suppression of early phase neurogenic responses. Steroids, on the other hand, are more likely to influence the late inflammatory phase by attenuating mediator release. So the early phase effect may be associated with flavonoid-mediated modulation of peripheral nerve excitability and potential interactions with serotonergic and GABAergic systems, as previously reported for quercetin [41].

In the tail immersion method, all extracts and the standard drug exhibited significant analgesic effects for up to 4 hours. The Eddy's hot plate test further supported these findings, where the EDCB fraction showed comparable analgesia to diclofenac at the 2nd hour. These thermal nociception models evaluate centrally mediated pain responses via supraspinal pathways involving opioid receptors. Therefore, the observed effects suggest that, in addition to peripheral action, certain extract components may also influence central pain modulation pathways, potentially via endogenous opioid system activation or ion channel modulation [48].

The peripheral anti-inflammatory activity was confirmed by carrageenan and formalin-induced paw edema tests. In the carrageenan and formalin induced paw edema tests, all extracts significantly reduced paw swelling, particularly during the late phase (2 - 4 h), which is dominated by prostaglandin production and leukocyte infiltration. The EDCB and DDCB extracts showed inhibition patterns comparable to the standard drug indomethacin. This suggests that these extracts may

suppress key mediators such as histamine and serotonin in the early phase, and prostaglandins, nitric oxide (NO), and pro-inflammatory cytokines (IL-1β, IL-6, TNF-α) in the late phase [49]. Flavonoids, known for their inhibitory effects on prostaglandin synthesis, may be responsible for the attenuation of both early and late phases of nociceptive responses. Tannins and steroids, through their membrane-stabilizing and anti-inflammatory actions, are likely to contribute predominantly to the late-phase activity observed in formalin- and carrageenan-induced models. The presence of flavonoids and steroids in the extracts may contribute by inhibiting enzymes such as phospholipase A_2, COX, and glutathione S-transferase, thereby reducing the cascade of eicosanoid synthesis [47] [50] [51]. Alkaloids may further modulate inflammatory cell recruitment and vascular permeability [43] [52].

The pharmacological effects observed across these models suggest that *D. candenatensis* extracts act through a multi-target mechanism involving inhibition of pro-inflammatory mediator synthesis, reduction of peripheral nociceptor activation, and possible modulation of neurotransmitter systems [53]. This integrated mechanism explains the extracts' ability to attenuate both neurogenic and inflammatory pain pathways, as well as their capacity to inhibit late-phase inflammatory responses. The synergistic presence of flavonoids, phenolics, tannins, and steroids in the extracts likely underlies these analgesic and anti-inflammatory potentials.

While the current study provides promising evidence for the analgesic and anti-inflammatory activities of *D. candenatensis* stem bark fractions, some limitations should be noted. These results should be interpreted as preliminary. Future investigations with larger, statistically powered sample sizes are necessary to validate the observed effects. Additionally, mechanistic investigations and safety assessments will be essential to identify the active constituents responsible for the observed pharmacological effects and establish their therapeutic potential and clinical relevance.

5. Conclusion

On the basis of the present findings, it can be concluded that *Dalbergia candenatensis* stem bark extracts possess noteworthy analgesic and anti-inflammatory effects, likely mediated through both peripheral and central mechanisms. As this work represents a preliminary investigation, future studies are warranted to isolate and characterize the active compounds, elucidate their precise mechanisms of action, and evaluate their clinical safety and efficacy, thereby paving the way for the development of novel plant-based therapeutics.

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Authors' Contribution

Sayema Khanun: Conceptualization, Methodology, Investigation, Formal analysis, Data Analysis, Data curation, Writing-Original Draft, Writing-Editing.

Hemayet Hossain: Formal Analysis, Methodology, Writing-Review & Editing.

Md. Hossain Sohrab: Project administration, Conceptualization, Supervision, Resources, Validation, Writing-Review & Editing.

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Conflicts of Interest

The authors declared no conflict of interest among the authors.

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