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Original Article

Buchholzia coriacea Seed Methanol Extract Represses Nociception via Central Pathway Mechanisms in Swiss Mice

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Abstract

Background: Nociception is a common feature of neurological disorders. Certain edible seeds contain bioactive phytochemicals with anti-nociceptive features. *Buchholzia coriacea* seed is traditionally used in folkloric medicine to manage pain-associated ailments. Hence, this study was conducted to assess the therapeutic properties of THE methanol extract of *B. coriacea* (MEBC) on nociception and its underlying mechanisms in Swiss mice.

Methods: *B. coriacea* seeds were purchased from the indigenous market in Oje, Ibadan, and authenticated at the Forest Herbarium Ibadan (FHI: 110572). The seeds were pulverized and cold-extracted with methanol. Forty-five male Swiss mice (23-27 g) were divided into nine treatment groups (n = 5): control (10 mL/kg), MEBC (50, 100, 200 mg/kg, p.o.), and indomethacin (10 mg/kg, p.o.). Anti-nociceptive activity was evaluated one hour post-administration using the acetic acid-induced writhing test (AAWT), formalin-induced paw licking test (FPLT), and tail flick test (TFT).

Results: The gas chromatography–mass spectrometry (GC-MS) analysis revealed 31 compounds, with linoleic acid being the most abundant. In the mechanistic study, mice were pre-treated with naloxone (1 mg/kg), atropine (2 mg/kg), propranolol (10 mg/kg), or haloperidol (1.5 mg/kg), 15 minutes before MEBC (200 mg/kg) administration. MEBC (50, 100, 200 mg/kg) significantly reduced writhing in the AAWT, inflammatory pain in the FPLT, and increased latency in the TFT. The opiate blocker naloxone (1 mg/kg), cholinergic antagonist atropine (2 mg/kg), beta-adrenergic blocker propranolol (10 mg/kg), and dopamine D2 receptor antagonist haloperidol (1.5 mg/kg) were administered and significantly reversed the anti-nociception effect of MEBC when compared with the 200 mg/kg treatment in the TFT model.

Conclusion: The findings from this study demonstrate that *B. coriacea* inhibits nociception, which is attributed to the synergistic activity of its bioactive compounds as indicated by the GS-MC. The mechanisms of action are mediate via central nervous system pathways.

Keywords: Nociception, *Buchholzia coriacea,* Abdominal writhing, Latency period, Naloxone, Haloperidol

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Introduction

Pain is a functionally important concept in life sciences because it warns organisms of potential tissue damage (1). Nociceptors are peripheral terminals of the subgroup of sensory neurons whose activation produces action potentials that ultimately initiate pain (2). The nociceptive signal is then communicated to the posterior of the spine and subsequently to the occipital region of the cortex, where it is integrated and analyzed to trigger appropriate actions that prevent further damage (3). Persistent pain can pose a major public health challenge, reducing the sufferer's quality of life and creating a socioeconomic burden. According to reports, in the first two decades of the twenty-first century, over 5.5 billion people lived in countries with little exposure to pain treatment (4).

Furthermore, pain memory prevents the affected organisms from repeating actions that may result in such unpleasant experiences (5). To address this, nonsteroidal

anti-inflammatory drugs (NSAIDs) are commonly administered, which exert anti-inflammatory, analgesic, and antipyretic effects and come in various doses and formulations (6). NSAIDs can interfere with prostaglandin production by inhibiting the cyclooxygenase enzymes (COX-1 and COX-2). COX-1 can produce prostaglandins and thromboxane A2, as well as regulate the stomach mucosal barrier, platelet aggregation, and other biological functions. Opioids are another major class of painkillers that can affect the nervous system by binding to opiate receptors in the brain, gastrointestinal tract, and other organs, thereby blocking pain transmission (7).

Long-term use of anti-nociceptive drugs, however, can cause damage to the digestive system, nephrotoxicity, tolerance, respiratory depression, and dependence (8). These side effects can diminish patient adherence and often undermine effective pain management. This highlights the urgent need for novel, safe, and effective natural pharmaceuticals for pain relief.

Buchholzia coriacea (Engler) is a perennial plant that grows as a tree. It belongs to the family *Capparidaceae* and the genus *Buchholzia***.** This tree is widely distributed in several tropical countries, including Cameroon, the Central African Republic, Gabon, Congo, Angola, Ghana, and Nigeria, among others (9,10). Commonly known as the Musk tree, it was named after R.W. Buchholz, who collected plants in Cameroon in the late 19th century (9). The seeds of *B. coriacea* have tremendous medicinal value. These seeds gave the plants their common name "wonder" or "magic kola" due to their extensive use in traditional medicine for treating a wide range of ailments.

The tree is a forest tree characterized by large, glossy leaves and conspicuous cream-white flowers arranged in racemes at the end of its branches. It is an evergreen, small to medium-sized tree that can grow up to 20 meters tall, with its natural habitat in lowland rainforests. The seeds are enclosed in a purple aril, which is chewed in the Ivory Coast and southern Nigeria, imparting a sharp, pungent taste (11). In Gabon, the plant is sometimes cultivated as a medicinal and fetish plant (12).

The fruit of *B. coriacea* is large, long-stalked, ellipsoid, resembling avocado pears, and typically measures 12 by 5-8 cm. Its endocarp is woody up to 1.3 cm thick, and becomes yellowish when ripe. The fruit contains yellow, edible pulp and a few large blackish seeds, about 2.5 cm long (13). The seeds, commonly known as Musk Tree or Wonder Kola in Nigeria, belong to the family Capparaceae Juss (14), as illustrated in Figure 1. They are referred to by various vernacular names across regions: 'Ndo' in Mende (Sierra Leone), 'Doe-fiah' in Kru-basa (Liberia), 'Eson-bese' in Akan-asante (Ghana), 'Banda' in Munga (West Cameroon), 'Esson bossi' in Central Africa, 'Kola Pimente' in French, 'Owi' in Edo State, 'Uke' in Igbo (southern Nigeria), and Obi Awogbaarun (a nut that can relieve over 200 types of diseases), as well as 'Aponmu' or 'Obi-ata' in Yoruba-speaking regions of Nigeria (15-17). The seeds measure about 2.54 cm (1 inch) long and are covered in a purple aril, which is chewed in Ivory Coast like kola nuts. They have a sharp, pungent taste similar to *Capsicum frutescens* (Solanaceae), with a hot, spicy flavor. In southern Nigeria, the seeds or kernels are consumed either raw or cooked (18).

Duru et al (19) reported that the seeds of *B. coriacea* contain macronutrients such as carbohydrates, proteins, fats, and oils in varying concentrations. Preliminary phytochemical analyses of *B. coriacea* seeds have further revealed the presence of alkaloids, tannins, flavonoids, saponins, renin, cyanogenic glycosides, cardiac glycosides, steroids, and terpenoids in different concentrations (20,21). Its fruits and leaves are known to have antioxidant (22), anti-helminthic (23), antibacterial, and antimicrobial properties, while its seeds are rich in flavonoids (24) and lower glucose levels (25). Furthermore, the Wonder Kola seed (Figure 2) exhibits anti-inflammatory activity, and the stem demonstrates anti-apoptotic effects (26-27). Egba et al (28) reported that seed extracts of Wonder Kola attenuate mercury-induced cerebral and cerebellar oxidative neurotoxicity via nitric oxide signaling pathways. Ethnomedicinally, *B. coriacea* has long been used in traditional medicine for many years for the treatment of a wide range of illnesses such as insanity, syphilis, body wounds, chronic ulcers, snake bites, gonorrhoea, convulsion, chest pain, headache, sinusitis, bronchitis, kidney pains, and as both an aphrodisiac and

Figure 1. *Buchholzia coriacea* Tree

Figure 2. *Buchholzia coriacea* Seeds

anthelminthic (29,30). Our findings so far indicate that the analgesic activity and the mechanism(s) of action of *B. coriacea* seed extract have not been studied. Therefore, the current research sought to assess the anti-nociceptive potential of a MEBC seeds in mice.

Materials and Methods

Chemicals and Drugs

Unless otherwise stated, all chemicals were obtained from Sigma Chemical Co. (Germany). The Glacial acetic acid, formaldehyde, methanol, and n-hexane were supplied by S.D. Fine Chemical Pvt. Ltd. (Mumbai, India). Tween 20 was used to dissolve the extract. All chemicals were analytically graded. Naloxone hydrochloride, atropine sulphate, propranolol, and haloperidol were purchased from Alpha Pharmacy, Lagos, Nigeria.

Animals

Swiss mice (18-27 g), aged 10-14 weeks, were acclimatized for two weeks before experimentation under standard ambient conditions. The rodents were obtained from the Animal House, College of Medicine, University of Ibadan, Nigeria. They had unrestricted access to a standard pellet diet and water. Animals were housed in cages based on their treatment groups. To minimize dietary interference during the abdominal writhing test, mice were fasted for 16 hours before evaluation in the acetic acid-induced analgesic model. All protocols were approved by the University Animal Research Ethics Committee (UI-ACUREC/18/0027).

Plant Materials

Buchholzia coriacea Engl. Seeds were obtained from Oje Market, Ibadan, Nigeria. Dr. S. A. Odewo identified the seeds at the Forest Research Institute of Nigeria (FRIN), Jericho, Ibadan, on August 30, 2016. A voucher specimen was designated under the number FHI 110572.

Preparation of Extracts

The outer coats of the seeds were peeled off, cleaned, broken into pieces, and air-dried at 40-42°C before being ground into a powder. Extraction was carried out using the maceration method by soaking 100 g dried *B. coriacea* seed powder in 1 L of n-hexane for 72 hours at 25 °C. Thereafter, the extracts were first filtered using cotton wool and then through Whatman No. 42. filter paper (125 mm). Methanol solvent was used to extract the defatted dried marc. The filtrate was concentrated using a rotary evaporator at 40 °C. The percentage yields were 2.9% w/w for n-hexane and 28.9% w/w for the methanol extract.

Preliminary Phytochemical Screening

A quantitative assessment for active chemical compounds such as alkaloids, saponins, cardenolides, anthraquinone, tannins, and flavonoids was performed on the methanol extract of *B. coriacea* (MEBC) seeds according to the method described by Trease and Evans (31).

Gas Chromatography-Mass Spectrometry Analysis

A 5975E gas chromatography–mass spectrometry (GC-MS) system (Agilent Technology) was used to analyze the MEBC seeds. For qualitative identification of the chemical constituents, spectra were compared against the NIST spectral database at a scanning speed of 12,500 u/s.

Acute Toxicity Study

The acute oral toxicity of MEBC was tested in female rats and mice following the OECD Guideline (32), as depicted in Table 1. Each group consisted of three animals, as per OECD Guidance 423. The test group animals were given a fixed dose of 2000 mg/kg of MEBC, diluted in 2% Tween 20 and distilled water, while the control group received normal saline (10 mL/kg). Animals were monitored at 6 and 12 hours post-treatment and subsequently observed daily for 14 days.

Trebling, seizures, retroperitoneal contortions, movement, mucus secretion, diarrhea, and tiredness were all assessed. Throughout the trial, the rodents' body weight and food intake were recorded regularly. On day 14, all animals were fasted for 18 hours before blood samples were taken through the animals' retro-orbital Venous plexus into both heparinized and plain bottles. Following euthanasia, the kidney, liver, brain, stomach, heart, spleen, lungs, and uterus were excised, dissected, and preserved in a 10% formalin solution containing formaldehyde.

Haematological Analysis

Blood samples were collected via the retro-orbital venous plexus and dispensed into lithium heparin tubes at 24 hours in rats and 14 days in both mice and rats after MEBC fixed-dose treatment. Automated haematological procedures were used to determine packed cell volume (PCV), red blood cell (RBC) count, total leukocytes (WBC) count, haemoglobin (Hb) concentration, and platelet count (33).

Acetic Acid-Induced Writhing Model

The acetic acid-induced writhing test (AAWT) was used to determine anti-nociceptive activity in mice, following the method of Taber et al (34), as illustrated in Table 2. The evaluation was divided into two phases:

Phase I – Screening Phase: This phase was subdivided into n-hexane and methanol sub-phases. In both sub-phases, the control (Group 1) received distilled water (10 mL/kg). In the n-hexane sub-phase, groups 2 and 3 received the n-hexane extract of *B. coriacea* seeds at 20 and 200 mg/kg, respectively. In the methanol sub-phase, group 1 (control) received 10 mL/kg distilled water, groups 2 and

Table 1. Grouping of Female Mice for Acute Toxicity Testing

Treatment	Group 1 (mL/kg)	Group 2 (mg/kg)
Mice	10	2000
Rats	10	2000

Note. The number of animals per group is 3. Group 1: Control, Group 2: Treatment.

3 received MEBC at doses of 20 and 200 mg/kg, and the reference group received indomethacin (10 mg/kg) (35). In all experimental groups, writhing was induced one hour after treatment by intraperitoneal injection of 0.2 mL of 3% acetic acid, and the mice were then placed in an observation glass chamber. The number of writhes was recorded between 5 and 15 minutes post-injection.

Phase II: In the second phase, the control group received distilled water (10 mL/kg), while groups 2, 3, and 4 received MEBC orally at doses of 50, 100, and 200 mg/kg, respectively. Each group contained five animals.

Model of Formalin-Induced Nociception

The procedure described by Hunskaar and Hole (36) was followed. There were five mice per group, and the total number of groups was five. The first group received 10 mL/kg of distilled water orally, while groups 2, 3, and 4 were administered 50, 100, and 200 mg/kg of MEBC orally, respectively. In group 5, indomethacin was administered via oral gavage at a dose of 10 mg/kg. Sixty minutes later, a microsyringe was used to inject 20 µL of 1% formalin subcutaneously into the posterior region of the mice's left hind paws in all groups. The animals were then placed in a translucent 30 × 30 × 30 cm container for an unbiased observation of the rodents.

Tail-Flick Test

D'Amour and Smith (37) described the use of a radiant heat analgesiometer. After gently wrapping the mouse in a cotton towel, the mouse's tail was exposed to a light ray. The response was a tail flick away from a light source. The reaction time was measured to the nearest second using a stopwatch. The intensity of the thermal radiation ferocity was modified to produce baseline latencies of 6–8 seconds. A cutoff time of 15 seconds was chosen since the test was repeated every 10 minutes. According to Connor et al (38), the longer cutoff time was used to diminish the impact of the stimulus to prevent tissue injury. Each mouse was subjected to two tests: once before MEBC or vehicle administration and again at 10, 20, and 30 minutes post-administration. In this test, animals were grouped in the same way as in the preceding experiment.

Anti-nociceptive Mechanism(s) of the Methanol Extract of Buchholzia coriacea Seeds

Experiments were conducted to investigate the mechanisms by which MEBC seeds potentiate anti-nociception in mice using two pain models. Mice were divided into 7 groups for each test. Since the 200 mg/kg dose of the MEBC produced the most pronounced anti-nociceptive effect in the previous three pain models of nociception, this dose was evaluated further in order to investigate the mechanism of action of MEBC. Group I (control) received purified water (10 mL/kg, p.o.), group II, MEBC (200 mg/kg p.o.), group III, naloxone (1 mg/kg i.p.), group IV, atropine (2 mg/kg i.p.), group V, propranolol (10 mg/kg i.p.), Group VI, Haloperidol (1.5 mg/kg i.p.), and Group VII, indomethacin (10 mg/kg p.o.). The doses of the blockers were chosen according to Omar (39) and Onasanwo et al (40) and were administered intraperitoneally.

Evaluation of Analgesic Effects of Methanol Extract of Buchholzia coriacea in Mice Using Three Models of Nociception

Three nociceptive tests were used to assess the activity of MEBC in mice, and the dose with the highest biological activity was identified (Table 3).

Statistical Analysis

GraphPad version 7 was used to analyze the data. One-way ANOVA followed by Dunnett's post hoc test was used. Statistical significance was set at $P < 0.05$, and results were reported as mean ± SEM with n = 5.

Results

Phytochemical Screening of Crude Extracts of Buchholzia coriacea Seeds

Table 4 depicts the constituents of the crude, methanol and n-hexane extracts from *B. coriacea* (Engl.) seeds, showing the presence of terpenoids, alkaloids, and anthraquinone in all three fractions. In contrast, flavonoids, tannins, and saponins were present in the crude and methanol fractions but absent in the n-hexane fraction. Notably, saponins

Table 2. Grouping for Preliminary Nociceptive Study of n-Hexane and Methanol in Mice

AAWT	Group 1 control (mL/kg)	Group 2 (mg/kg)	Group 3 (mg/kg)	Group 4 indomethacin (mg/kg)
n-Hexane	10	20	200	10
Methanol	10	20	200	10

Note. AAWT: Acetic acid-induced writhing test. The number of animals per group is 5.

Table 3. Experimental Design for Nociceptive Tests Groupings

Nociception models	Group 1 (mL/kg)	Group 2 MEBC (mg/kg)	Group 3 MEBC (mg/kg)	Group 4 MEBC (mg/kg)	Group 5 indomethacin (mg/kg)
AAWT	10	50	100	200	10
FPLT	10	50	100	200	10
TFT	10	50	100	200	10

Note. AAWR: Acetic acid-induced writhing test; FPLT: Formalin paw licking test; TFT: Tail flick test; MEBC: Methanol extract of *Buchholzia coriacea*. The number of animals per group is 5.

Table 4. Components of Crude, Methanol, and n-hexane Extracts of *Buchholzia coriacea* Seeds (Engl.)

Constituents Compound	Crude	Methanol	n-hexane
Saponin	+	++	-
Tannins	+	+	-
Cardiac glycoside	-	-	-
Flavonoids	+	+	-
Terpenoids	+	+	+
Alkaloids	+++	+++	+
Anthraquinone	+	+	++

+mildly present, ++moderately present, +++abundantly present, - absent.

were found in abundant quantities in the methanol extract.

Methanol Extract of Buchholzia coriacea Seeds Analysis Using Gas Chromatography–Mass Spectrometry

The chromatogram of compounds in MEBC is presented in Figure 3, showing a plot of intensity against retention time. GC-MS analysis of MEBC revealed the presence of 31 compounds (Figure 3 and Table 5). The peak number, retention time, percentage abundance, and m/z values are presented in Table 5.

The most abundant compound in MEBC is Linoleic acid ethyl ester (16.74%) with an m/z value of 308, followed by 4-hydroxy-2-methylpyrrolidine-2-carboxylic acid (9.03%) with an m/z of 146. Furthermore, 2-methoxymethyl-2-dihydroxy-5-[1-hydroxy-2-fluoroethyl]- exhibited a relative abundance of 7.8% with an m/z of 128. Other identified compounds are listed in Table 5.

Effect of Methanol Extract of Buchholzia coriacea Seeds on Weight Gain in Mice and Rats in Acute Toxicity

Table 6 shows the effect of a single dose of MEBC (2000 mg/kg) on weight gain in mice and rats. There was no significant difference in weight gain between the treated groups (2000 mg/kg MEBC) and the control (10 mL/kg). No mortality or clinical signs of toxicity were observed at this dose.

Effect of Methanol Extract of Buchholzia coriacea Seeds on Relative Organ Weight of Mice in Acute Toxicity

Table 7 presents the effect of a single dose of MEBC (2000 mg/kg) on the relative organ weights in mice. MEBC did not cause significant changes in the relative weights of most organs compared with control, except for the intestine, which indicated a significant increase ($P<0.05$). No signs of toxicity were observed in the relative organs at this dose.

Effect of Methanol Extract of Buchholzia coriacea on Relative Organ Weight (%) of Rats in Acute Toxicity

Table 8 displays the effect of a single dose of MEBC (2000 mg/kg) on the relative organ weights in rats. MEBC did not cause significant changes in the relative weights of all organs compared with control, except for the spleen, which significantly increased ($P<0.05$). The dose showed no toxicity signs in the relevant organs.

Effect of Acute Administration of Methanol Extract of the Buchholzia coriacea Seeds on Haematological Parameters in Rats After 24 Hours

Table 9 presents the effect of a single dose of MEBC on haematological parameters in female rats. After 24 hours of administration, there was no significant difference between the treated group and the control. All haematological parameters, such as RBC count, WBC count, platelet count, hematocrit, and differential leukocytes, remained within the normal range for both control and treated groups during the experiment.

Effect of Acute Administration of Methanol Extract of Buchholzia coriacea Seeds on Haematological Parameters in Rats After 14 Days

The haematological profiles of treated and control groups are presented in Table 10. Administration of a single dose of MEBC (2000 mg/kg) in female rats showed no significant differences after 14 days. The results indicated that all haematological parameters, including RBC count, WBC count, platelet count, hematocrit, and differential leukocytes, remained within the normal range in both control and treated groups during the experiment.

Effect of Acute Administration of Methanol Extract of Buchholzia coriacea Seeds in Mice Haematological Parameters After 14 Days

The haematological profiles of the treated and control groups are presented in Table 11. A single oral dose of MEBC (2000 mg/kg) in female mice after 14 days of administration showed that all haematological parameters such as RBC count, WBC count, platelet count, hematocrit, and differential leukocytes were within the normal range in both control and treated groups during the experiment. No signs of toxicity were observed.

Effects of n-Hexane Extract of Buchholzia coriacea Seeds on Acetic Acid-Induced Writhing in Mice

The mean count of abdominal writhes in the treated groups administered with the n-hexane extract (200 and 20 mg/kg) was significantly reduced (18.6 ± 0.51; 35.2 ± 4.50) compared to the control (42.8 ± 2.70) ($P<0.05$). The highest inhibition of writhing was observed at 200 mg/kg (18.6 ± 0.51), which was significantly higher than the inhibition produced by the reference drug, indomethacin (10 mg/kg) (30 ± 3.20), as shown in Figure 4.

Effect of Methanol Extract of Buchholzia coriacea Seeds on the Acetic Acid-Induced Abdominal Writhing Test in Mice

In the screening study with methanol extract, the mean count of abdominal writhes in the treated groups (200 and 20 mg/kg) was significantly reduced (5.6 ± 0.20; 13.6 ± 0.41, respectively) compared with the control group (42.8 ± 2.70). The highest inhibition of the writhing was

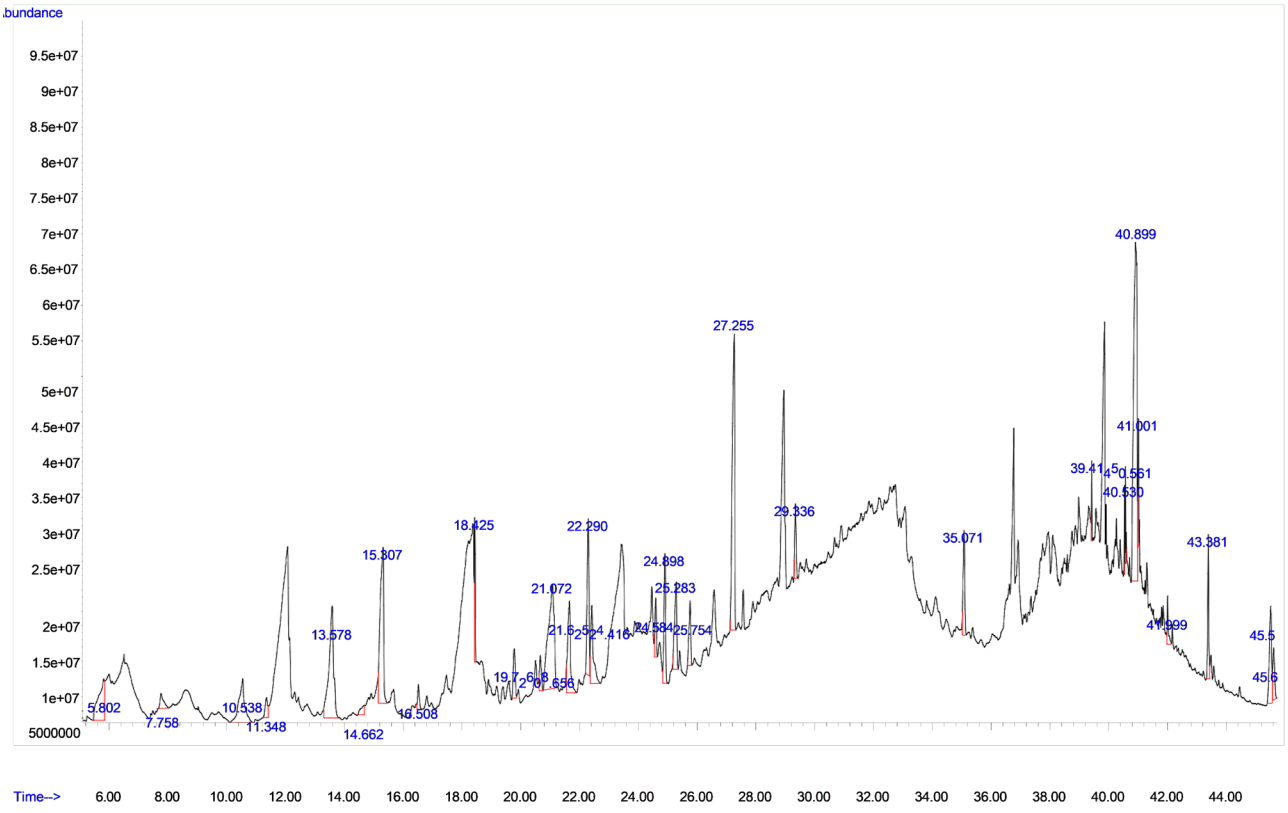

Figure 3. Gas Chromatogram Showing a Plot of Intensity Against Retention Time

observed at 200 mg/kg (5.6 ± 0.2), which was significantly higher than the inhibition produced by the reference drug, indomethacin (10 mg/kg) (30 ± 3.20), as illustrated in Figure 5.

Effect of Methanol Extract of Buchholzia coriacea Seeds on Pain-Like Behavior in Mice

Figure 6 depicts the potentials of MEBC seeds in AAWT in mice. The mean count of abdominal writhes displayed in the treated groups (50, 100, 200 mg/kg MEBC) significantly reduced compared with the control group (47.2 ± 2.50), showing a dose-dependent inhibition. The highest inhibition of the writhing was observed at 200 mg/kg (22.5 ± 0.85), but it was not higher than the inhibition produced by the reference drug indomethacin (10 mg/kg) (11.0 ± 2.40).

Effect of Methanol Extract of Buchholzia coriacea Seeds on Formalin-Induced Paw Licking Time in Mice

Figure 7 depicts the analgesic activity of graded doses of MEBC (50, 100, and 200 mg/kg) and indomethacin (10 mg/kg) on the FPLT in mice. Paw licking time was significantly reduced ($P < 05$) in both the early (0-5 minutes; neurogenic phase) and late phases (20-30 minutes; inflammatory phase). In the early phase, 200 mg/kg MEBC significantly lowered paw licking time compared with the control. In the late phase, groups administered with 100 and 200 mg/kg MEBC significantly decreased paw licking duration relative to the control. The 200 mg/kg dose of MEBC exhibited the highest protection against formalin-induced paw licking, exceeding the protection offered by 10 mg/kg indomethacin.

Effect of Methanol Extract of Buchholzia coriacea Seeds Activity on Formalin-Induced Paw Biting Time Test in Mice

Figure 8 illustrates the anti-nociceptive potential of graded doses of MEBC (50, 100, and 200 mg/kg) and indomethacin (10 mg/kg) on paw biting duration in FPLT in mice. The 200 mg/kg dose of MEBC significantly reduced paw biting time during the early phase (0-5 minutes) compared with the control. Furthermore, there was an improved reduction in paw biting time during the late phase (20-30 minutes) in all treated groups and indomethacin (10 mg/kg) compared with the control group, which received 10 mg/kg distilled water.

Effect of Methanol Extract of Buchholzia coriacea Seeds on Tail-Flick Test in Mice

MEBC administration (100 and 200 mg/kg) resulted in a significant improvement in tail-flick latency compared with control mice. The anti-nociceptive effect of MEBC was dose-dependent. Latency times at 100 and 200 mg/kg were 10.72 ± 0.85 and 14.88 ± 0.95 seconds, respectively, compared with 4.6 ± 0.25 seconds in the control group. MEBC at 200 mg/kg outperformed indomethacin (10 mg/kg; 14.62 ± 1.79 seconds) and appeared to be the most active. The longer the latency time, the greater the measure of anti-nociceptive potential, as shown in Figure 9.

Table 5. Chemical Compound of MEBC Using GC-MS

S/N	Compound	Peak No.	GC-MS- RT (min)	Percentage abundance (%)	m/z Value
01	N, N-Dimethylaminoethanol	1	5.80	3.57	89
02	1H-Pyrrole, 2,5-dimethyl-	2	7.76	0.76	94
03	2(5H)-Furanone	3	10.54	3.64	84
04	1,2-Cyclopentanediol, 3 methyl-	4	11.35	0.77	116
05	1H-Pyrrole-2-carboxaldehyde, 1-methyl-	5	13.58	7.62	109
06	Furan-2-one, 3,4-dihydroxy-5-[1-hydroxy-2-fluoroethyl]-	6	14.66	0.54	178
07	2-Methoxymethyl-2-methylpyrrolidine	7	15.31	7.80	128
08	N-[3-[N-Aziridyl]propylidene]-3-dimethylaminopropylamine	8	16.51	0.60	140
09	Phenylethyl Alcohol	9	18.43	1.96	122
10	Benzenamine, 2,4,6-trimethyl-	10	19.77	1.42	135
11	4-(2,5-Dihydro-3-methoxyphenyl)butylamine	11	20.66	0.90	121
12	4-Hydroxy-2-methylpyrrolidine-2-carboxylic acid	12	21.10	9.03	146
13	8-Azabicyclo[3,2,1]oct-6-en-3-one,8-methyl	13	21.65	3.75	137
14	N,2,4,6-Tetramethylbenzenamine	14	22.29	4.36	149
15	2,5-Methanopyrano[3,2-b] pyrrole, hexahydro-1-methyl-	15	22.42	2.95	153
16	Pyrrolidine, 1-(3-cyclohexen-1-ylidenemethyl)-	16	24.58	1.17	163
17	Pyridine,4-ethyl-2,6-dimethyl-	17	24.89	3.04	135
18	2-methoxy-4-vinylphenol	18	25.28	2.89	150
19	3-Ethyl-5,6,7,8-tetrahydroquinoline	19	25.75	1.69	161
20	3,5-Diethyl-2-n-propylpyridine	20	27.26	10.22	177
21	Pyridine,2-amino-3-(1- methylpyrrolidin-2-yl)	21	29.34	1.51	177
22	4-Hydroxy-3-nonyl-1H-quinolin-2-one	22	35.10	2.50	175
23	Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentylcyclopropyl) methyl]cyclopropyl]methyl]cyclopropyl]methyl]-,methyl ester	23	39.42	0.58	74
24	Linoleic acid ethyl ester	24	40.53	0.65	308
25	16-Octadecenoic acid, methyl ester	25	40.56	0.67	296
26	Linoleic acid ethyl ester	26	40.90	16.74	308
27	Octadecadienoic acid, 2-hydroxy-1,3-propanediyl ester	27	41.00	1.06	73
28	Octadecanal, 2-bromo-	28	41.99	0.75	346
29	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	29	43.38	2.41	330
30	9-Octadecenoic acid (Z)-, 2-hydrox y-1-(hydroxymethyl) ethyl ester	30	45.51	3.20	354
31	9-Octadecenoic acid (Z)-, 2-hydrox y-1-(hydroxymethyl) ethyl ester	31	45.61	1.28	354

Note. MEBC: Methanol extract of *Buchholzia coriacea*; GC-MS: Gas chromatography–mass spectrometry; RT: Retention time.

Effects of Naloxone, Atropine, Propranolol, and Haloperidol on the Anti-nociceptive Activity of Methanol Extract of Buchholzia coriacea Seeds in Acetic Acid-Induced Writhing Test in Mice

Figure 10 shows the effect of naloxone, atropine, propranolol, and haloperidol on the anti-nociceptive potential of MEBC in the AAWT. The most active dose (200 mg/kg) significantly reduced the number of writhes (22.50 ± 0.85) in the mice compared with the control group (47.17 ± 2.50). The nociceptive threshold was elevated about twofold (44.83 ± 4.88) in the 2 mg/kg atropine group compared with the 200 mg/kg MEBC-treated group (22.50 ± 00.85). Atropine (a cholinergic blocker) reversed the anti-nociceptive effect of 200 mg/kg, whereas the opioid, β-adrenergic, and dopaminergic receptor antagonists did not produce significant changes relative to the 200 mg/kg dose.

Table 6. Effect of MEBC Seeds on Weight Gain in Mice and Rats During Acute Toxicity

Group	Before administration (kg)	Week one (kg)	Week two (kg)
Mice			
Control 10 mL/kg	24.0 ± 1.73	26.67 ± 2.33	23.33 ± 1.33
2000 mg/kg MEBC	26.0 ± 0.57	29.0 ± 0.00	28.0 ± 0.00
Rat			
Control 10 mL/kg	116.3 ± 1.20	129.3 ± 2.91	111 ± 4.36
2000 mg/kg MEBC	113.7 ± 3.71	124 ± 4.16	113 ± 4.51

Note. MEBC: Methanol extract of *Buchholzia coriacea*. Values are expressed as mean ± SEM (n = 3). Data were analyzed using Student's test.

Effects of Naloxone, Atropine, Propranolol, and Haloperidol on the Anti-nociceptive Activity of Methanol Extract of Buchholzia coriacea Seeds in the Tail-flick Test

Table 7. Effect of MEBC Seeds on Relative Organ Weight (%) of Mice in Acute Toxicity

Organ	Control 10 mg/mL	MEBC 2000 mg/kg
Kidney	0.4 ± 0.15	0.45 ± 0.05
Liver	1.1 ± 0.21	1.25 ± 0.05
Intestine	0.5 ± 0.03	0.75 ± 0.05*
Spleen	0.2 ± 0.00	0.25 ± 0.05
Lungs	0.33 ± 0.14	0.5 ± 0.30
Heart	0.13 ± 0.03	0.15 ± 0.05
Brain	0.27 ± 0.03	0.35 ± 0.05

Note. MEBC: Methanol extract of *Buchholzia coriacea*. Values are expressed as mean ± SEM (n = 3), *$P < 0.05$ compared with control. Data were analyzed using Student's test.

Table 8. Effect of MEBC Seeds on the Relative Organ Weight (%) of Rats in Acute Toxicity

Organ	Control 10 mg/mL	MEBC 2000 mg/kg
Kidney	0.73 ± 0.09	0.7 ± 0.00
Liver	3.53 ± 0.27	3.67 ± 0.18
Intestine	1.37 ± 0.14	1.13 ± 0.14
Spleen	0.43 ± 0.03	0.67 ± 0.03**
Lungs	0.97 ± 0.03	1.07 ± 0.06
Heart	0.43 ± 0.03	0.33 ± 0.03
Brain	1.50 ± 0.00	1.43 ± 0.03
Uterus	0.47 ± 0.07	0.47 ± 0.03

Note. MEBC: Methanol extract of *Buchholzia coriacea*. Values are expressed as mean ± SEM (n = 3), **$P < 0.01$ compared with control. Data were analyzed using Student's test.

Table 9. Haematological Parameters After 24 Hours of Administration of MEBC Seeds in Rats

Variables	Control (10 ml/kg)	MEBC (2000 mg/kg)
PCV (%)	38.0 ± 3.20	42 ± 2.91
Hb (g/dL)	12.0 ± 1.80	14 ± 0.89
RBC (x10^6/µL)	6.30 ± 057	7.0 ± 0.33
WBC (x10^3/µL)	7500 ± 680.7	7633 ± 829.8
Platelet Count (x10^3/µL)	$2.4 \times 10^5 \pm 25403$	$2.3 \times 10^5 \pm 1.6 \times 10^4$
Lymphocytes (%)	67.00 ± 2.52	74.00 ± 4.93
Neutrophils (%)	21.00 ± 69	22.67 ± 4.81
Monocytes (%)	2.00 ± 1.00	2.00 ± 0.58
Eosinophils (%)	2.33 ± 0.88	1.33 ± 0.33
MVC (fL)	60.71 ± 1.10	60.78 ± 1.6
MCHC (g/dL)	32.62 ± 0.33	33.10 ± 0.21
MCH (pg)	19.80 ± 0.14	20.11 ± 0.45

Note. MEBC: Methanol extract of *Buchholzia coriacea*; PCV: Packed cell volume; Hb: Haemoglobin concentration; RBC: Red blood cell; WBC: White blood cell; MCV: Mean corpuscular volume; MCHC: Mean corpuscular hemoglobin concentration; MCHC: Mean corpuscular hemoglobin concentration. Values are expressed as mean ± SEM (n = 3), compared with control. Data were analyzed using Student's test.

in Mice

Figure 11 presents the effects of naloxone (1mg/kg), atropine (2 mg/kg), propranolol (10 mg/kg), and haloperidol (1.5 mg/kg) on the anti-nociception of MEBC in TFT in mice. MEBC (200 mg/kg) significantly elevated the latency time (14.88 ± 0.95) compared with the control group (4.60 ± 2.50). All the antagonists tested reversed the anti-nociceptive effect of MEBC. This effect was inhibited by prior administration of the opioid receptor blocker naloxone, the cholinergic antagonist atropine, the β-adrenergic receptor antagonist propranolol, and the dopamine D2 receptor blocker haloperidol. Significant reductions in latency time were observed with all blockers compared with the MEBC-treated group (200 mg/kg), with values of 3.47 ± 14.88 ± 0.95, 2.32 ± 14.88 ± 0.95, 2.52 ± 14.88 ± 0.95, and 3.30 ± 14.88 ± 0.95, respectively.

Table 10. Haematological Parameters After 14 Days of Administration of MBCE Seeds in Rats

Variables	Control (10 mL/kg)	MEBC (2000 mg/kg)
PCV (%)	46.33 ± 1.86	43.67 ± 3.18
Hb (g/dL)	14.73 ± 1.03	15.2 ± 0.76
RBC (x10^6/µL)	7.30 ± 0.71	7.83 ± 0.43
WBC (x10^3/µL)	8750 ± 1028	5733 ± 2295
Platelet count (x10^3/µL)	207667 ± 4978	207004 ± 8963
Lymphocytes (%)	68.3 ± 3.90	70 ± 2.10
Neutrophils (%)	28.33 ± 4.20	25.67 ± 2.03
Monocytes (%)	2.0 ± 0.58	1.67 ± 0.33
Eosinophil (%)	1.33 ± 033	2.67 ± 0.33
MCV (fL)	59.24 ± 0.85	60.12 ± 1.55
MCHC (g/dL)	32.87 ± 0.31	33.75 ± 0.25
MCH (pg)	19.42 ± 0.09	20.29 ± 0.52

Note. MEBC: Methanol extract of *Buchholzia coriacea*; PCV: Packed cell volume; Hb: Haemoglobin concentration; RBC: Red blood cell; WBC: White blood cell; MCV: Mean corpuscular volume; MCHC: Mean corpuscular hemoglobin concentration; MCHC: Mean corpuscular hemoglobin concentration. Values are expressed as mean ± SEM (n = 3), compared with control. Data were analyzed using Student's test.

Table 11. Haematological Parameters After 14 Days of Administration of MEBC Seeds in Mice

Variables	Control	2000 mg/kg
PCV (%)	46.33 ± 0.88	43.5 ± 2.50
Hb (g/dL)	15.47 ± 0.58	14.1 ± 0.60
RBC (x10^6/µL)	7.11 ± 0.21	6.96 ± 0.46
WBC (x10^3/µL)	3717 ± 169.1	4625 ± 25
Platelet Count (x10^3/µL)	275000 ± 16	1325000 ± 81
Lymphocytes (%)	69 ± 2.10	67 ± 6.00
Neutrophil (%)	27.67 ± 2.91	27.67 ± 7.00
Monocytes (%)	1.67 ± 0.33	1.5 ± 0.50
Eosinophil (%)	1.67 ± 0.67	1.5 ± 1.50
MCV (fL)	60.1 ± 0.56	66.7 ± 4.18
MCHC (g/dL)	33.4 ± 0.68	32.0 ± 0.59
MCH (pg)	20.5 ± 0.39	21.2 ± 1.0

Note. MEBC: Methanol extract of *Buchholzia coriacea*; PCV: Packed cell volume; Hb: Haemoglobin concentration; RBC: Red blood cell; WBC: White blood cell; MCV: Mean corpuscular volume; MCHC: Mean corpuscular hemoglobin concentration; MCHC: Mean corpuscular hemoglobin concentration. Values are expressed as mean ± SEM (n = 3), compared with control. Data were analyzed using Student's test.

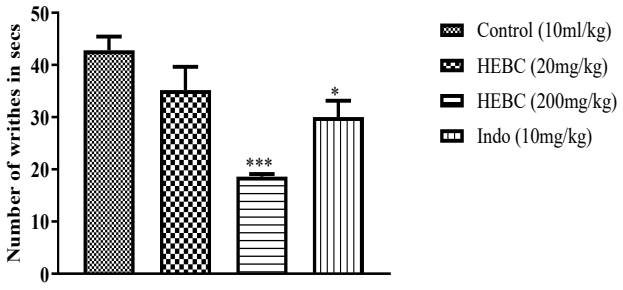

Figure 4. Effect of n-Hexane Extract of *Buchholzia coriacea* Seeds on AAWT in Male Mice. *Note*. AAWT: Acetic acid-induced abdominal writhing test. The results are expressed as means ± SEM (n = 5). Data were analyzed using one-way ANOVA followed by Dunnett's post hoc test. **P* < 0.05 and ****P* < 0.001compared with the control

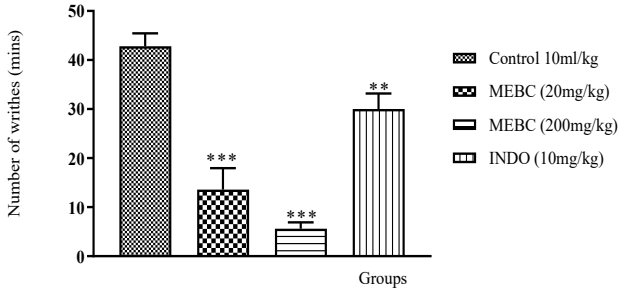

Figure 5. Effect of MEBC on AAAWT in Male Mice. *Note.* MEBC: Methanol extract of *Buchholzia coriacea;* AAWT: Acetic acid-induced abdominal writhing test. The results are expressed as means ± SEM (n = 5). Data were analyzed using one-way ANOVA followed by Dunnett's post hoc test. ***P* < 0.01 and ****P* < 0.001 compared with control

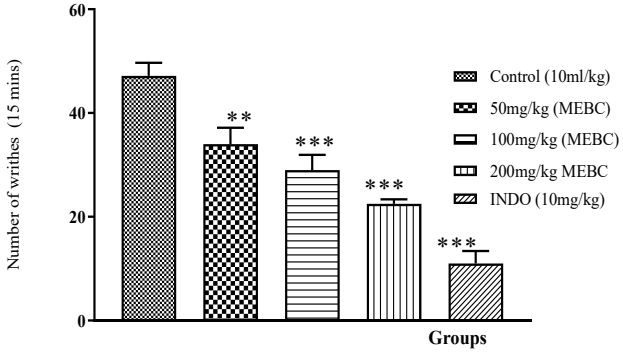

Figure 6. Effect of MEBC on AAWT in Mice. *Note.* MEBC: Methanol extract of *Buchholzia coriacea;* AAWT: Acetic acid-induced writhing test. The results are expressed as means ± SEM (n = 5). Data were analyzed using one-way ANOVA followed by Dunnett's post hoc test. ***P* < 0.01 and ****P* < 0.001 compared with the control

Discussion

Preliminary qualitative phytochemical screening of crude, n-hexane, and MEBC seeds revealed the presence of terpenoids, alkaloids, and anthraquinone in all fractions. Flavonoids, tannins, and saponins were found in the crude and methanol fractions but were absent in the n-hexane extract. The methanol extract, however, contained higher levels of saponins than the crude extract. These findings support Adediwura and colleagues' claim (41).

The importance of secondary plant metabolites to both plant and animal health cannot be overemphasized, as these chemical compounds are known to have specific pharmacological properties. Flavonoids, for example, have been shown to exhibit anti-nociceptive and anti-inflammatory potentials, and the molecular pathways underlying these effects have been proposed

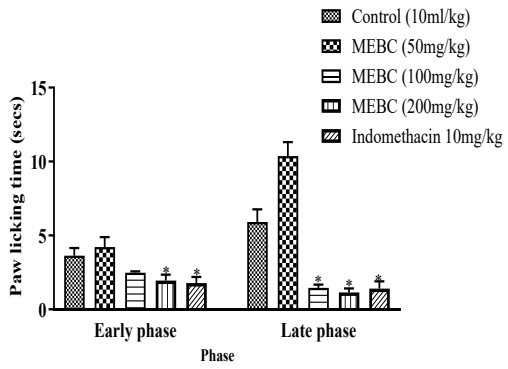

Figure 7. Effect of MEBC on FPLT in Mice. *Note.* MEBC: Methanol extract of *Buchholzia coriacea;* FPLT: Formalin-induced paw licking time. The results are expressed as means ± SEM (n = 5). Data were analyzed using one-way ANOVA followed by Dunnett's post hoc test. **P* < 0.05 compared with the control

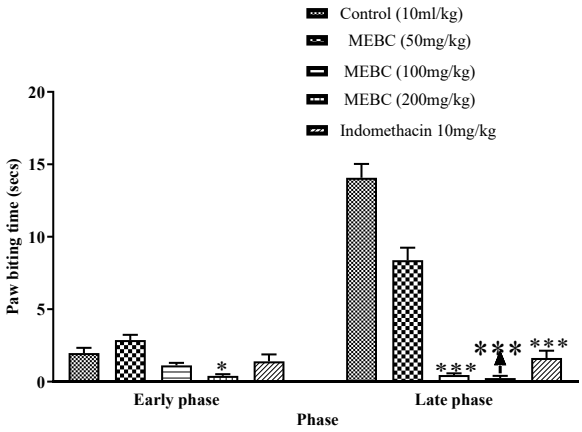

Figure 8. Effect of MEBC on Formalin-Induced Paw Biting Time. *Note.* MEBC: Methanol extract of *Buchholzia coriacea*. The results are expressed as means ± SEM (n = 5). Data were analyzed using one-way ANOVA followed by Dunnett's post hoc test. **P* < 0.05 and ****P* < 0.001 compared with the control

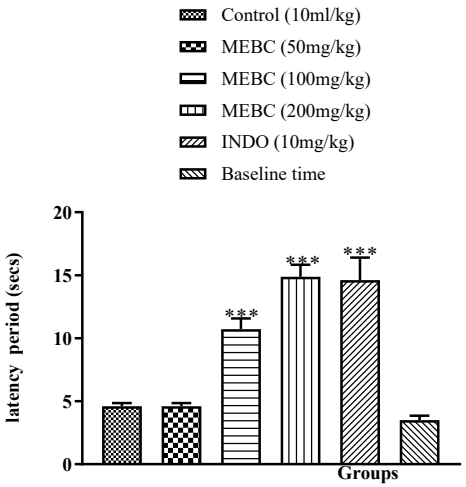

Figure 9. Effects of MEBC Seeds on TFT in Mice. *Note.* MEBC: Methanol extract of *Buchholzia coriacea;* TFT: Tail-flick test. The results are expressed as means ± SEM (n = 5). Data were analyzed using One-way ANOVA followed by Dunnett's post hoc test. ****P* < 0.001 compared with the control

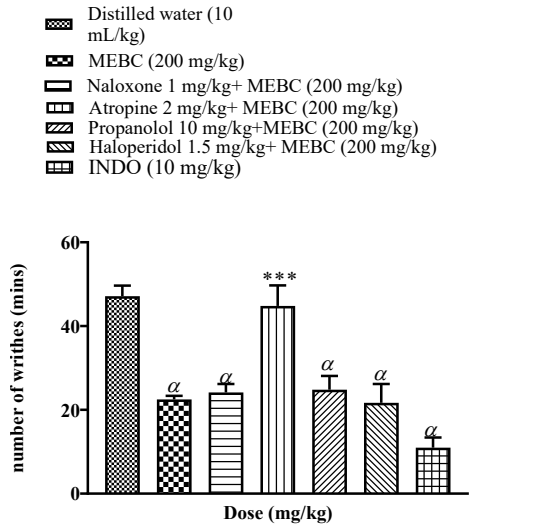

Figure 10. Effects of Naloxone, Atropine, Propranolol, and Haloperidol on Antinociception Induced by MEBC (200 mg/kg) in the AAWT in Mice. Note. MEBC: Methanol Extract Buchholzia coriacea; AAWT: Acetic acid-induced writhing test; INDO: Indomethacin. The results are represented as means ± SEM (n = 5). ***$P < 0.001$ and $^{\alpha}P < 0.05$ compared with MEBC (200 mg/kg) and distilled water, respectively

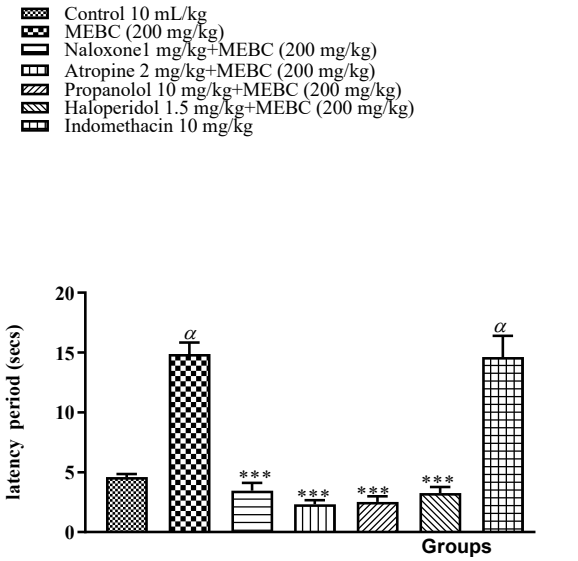

Figure 11. Effects of Naloxone, Atropine, Propranolol, and Haloperidol on Anti-nociception Induced by MEBC Seeds (200 mg/kg) on TFT in Mice. *Note*. MEBC: Methanol Extract *Buchholzia coriacea*; TFT: Tail-flick test. The findings are expressed as means ± SEM (n = 5). ***$P < 0.001$ compared with MEBC (200 mg/kg). $^{\alpha}P < 0.05$ compared with distilled water

to include inhibition of COX and lipoxygenase enzymes, degranulation of neutrophils, and reduction of eicosanoid generation (42). Tannins also possess astringent, anti-inflammatory, and anticancer properties that have been widely linked to their presence in plants. Several studies have also demonstrated that alkaloids contribute significantly to the analgesic potential of medicinal plants (43).

Since the presence of these phytoconstituents with established medical benefits may validate the use of *B. coriacea* in ethnomedicine, further therapeutic studies on the species are required. Furthermore, this study on *B. coriacea* has unfolded the nociceptive mechanisms of pain modulation via various pathways in the central nervous system. *B. coriacea* seeds were extracted with n-hexane and methanol to identify potential pharmaceutical candidates. The GC-MS analysis of MEBC revealed the presence of 31 different compounds along with their chemical structures and retention times. The most abundant compound in MEBC was linoleic acid ethyl ester (16.74%) with m/z 308, followed by 4-hydroxy-2-methylpyrrolidine-2-carboxylic acid (9.03%) with m/z 146. Furthermore, 2-methoxymethyl-2-dihydroxy-5-[1-hydroxy-2-fluoroethyl]- was present at 7.8% with m/z 128. These compounds seem to contribute to the anti-nociceptive potential of plant-derived therapeutic agents and have been reported to belong to various classes of secondary metabolites, exhibiting a broad spectrum of activity that depends on the species, climate of the country of origin, topography, and may contain various active components (44). The MEBC GC-MS chromatogram confirmed the presence of several components belonging to different classes of compounds, featuring functional groups such as phenol, furanone, and pyrrole.

No morbidity or mortality was observed in the mice and rats during the 14-day observation period. The current study found no adverse effects in the treated dose group of 2000 mg/kg, indicating that the LD50 of MEBC was greater than 2000 mg/kg. Treatment-related signs were only observed in groups administered doses greater than 2000 mg/kg, likely due to the presence of toxic substances, causing changes in body and internal organ weights, consistent with findings reported by Teo et al (45).

Changes in body weight are indicators of drug and chemical side effects, with losses exceeding 10% of the initial body weight, it is considered significant. Organ weight is also an important indicator of an animal's physiological and pathological status, and relative organ weight is critical for determining potential organ injury. The primary organs affected by toxicant-induced metabolic reactions include the heart, liver, kidneys, spleen, and lungs (46).

There were no statistically significant differences in body weight gain between the control and treated groups. Except for the intestine in mice and the spleen in rats, organ weights in the treated group did not significantly differ from those in the control group in the current study, indicating that MEBC is non-toxic. Blood parameter analysis is critical for assessing the risks associated with test compounds because changes in the haematological system have high predictive value for human toxicity when animal study data are converted.

The study's findings showed that treatment of mice and rats with MEBC for 24 hours and 14 days produced no changes in haematological parameters (i.e., Hb, haematocrit, platelet count, WBC count, differential leukocytes, and RBC counts), indicating that the extract did not affect blood cellular counts or their production.

N-hexane and MEBC seed extracts significantly reduced pain responses in chemically induced writhing potentiated

by glacial acetic acid in the preliminary screening study. The AAWT is a sensitive model of abdominal nociception commonly used in drug development (47).

Intraperitoneal injection of acetic acid caused visceral writhing, characterized by whole-body rhythms, twisting of dorso-abdominal muscles, and minimal motor activity, likely due to activation of chemo-sensitive nociceptors.

In this study, methanol and N-hexane filtrate of *B. coriacea* seeds were used to determine the most active extract. The methanol component was more potent than the N-hexane extract at a dose of 200 mg/kg. The inhibition provided by this dose was significantly greater than that of indomethacin. The inhibition of AAWT was dose-dependent, indicating a peripheral effect.

The study found that oral gavage of 50, 100, and 200 mg/kg of MEBC significantly antagonized writhing in the nociceptive study, with the highest dose of MEBC producing the greatest suppression. The number of writhes decreases when prostaglandin synthesis is inhibited (48). Furthermore, the FPLT was used to create a prototype for regional tonic and inflammatory pain (49).

There are two phases of the formalin test. The early phase (0-5 minutes) represents neurogenic pain caused by C-fiber activation following peripheral stimulation by formalin, while the late phase (15-30 minutes) is characterized by inflammatory pain resulting from the release of serotonin, histamine, and prostaglandins (50).

MEBC (200 mg/kg) significantly reduced paw licking time in the neurogenic phase, while both 100 and 200 mg/kg reduced licking time in the late phase. Paw biting analysis showed that 200 mg/kg of MEBC significantly reduced in the neurogenic phase compared with the control. However, there was a significant reduction in paw biting time in the late (inflammatory) phase in all MEBC and indomethacin-treated groups compared with the control. Notably, MEBC at 200 mg/kg provided greater protection against formalin-induced paw licking and biting than indomethacin at 10 mg/kg.

MEBC demonstrated a stronger effect in the late phase of both paw licking and biting. This reduction suggests that MEBC exerts its anti-nociceptive potential by antagonizing the peripheral release of serotonin, prostaglandins, and bradykinin, which characterize the inflammatory phase. Thus, MEBC's analgesic activity appears to be mediated by peripheral mechanisms.

In addition, heat stimulation activated peripheral nociceptors, resulting in reflexive tail removal. Prolongation of reaction time is widely regarded as a key indicator for evaluating central anti-nociceptive potentials (51). The anti-nociceptive effects of MEBC in this model confirmed its central activity.

AAWT and TFT were used to investigate the involvement of pain pathways in the anti-nociceptive potential of MEBC. AAWT can distinguish between strong and weak analgesics and can also reveal non-analgesics such as antihistamines, monoamine oxidase inhibitors, and muscle relaxants (52). Glacial acetic acid stimulates prostaglandin synthesis, resulting in the release of bradykinin, a poisonous intrinsic substance within the peritoneum that causes visceral writhing (53).

The current findings showed that atropine reversed anti-nociceptive effects on MEBC in the AAWT, whereas naloxone, propranolol, and haloperidol did not block MEBC-induced analgesia in the AAWT. This suggests that MEBC may inhibit nociception via the cholinergic pathway in the AAWT model.

In the TFT, nociception is mediated via a central pathway (54), which is involved in the anti-nociceptive effects of MEBC. The opioidergic system is a complex intercellular signaling network composed of endogenous receptors and ligands (55). To investigate this, the opiate receptor blocker (naloxone), cholinergic antagonist (atropine), the β-adrenergic blocker (propranolol) (56), and the dopamine D_2 receptor antagonist (haloperidol) were used (57). These antagonists significantly reversed the anti-nociceptive effects of all MEBC doses, indicating that the nociception stimulated by noxious heat is centrally mediated through direct activation of afferent fibers.

According to Mishra et al (58), centrally modulated analgesia is a multifaceted process involving opioidergic, dopaminergic, serotonergic, and adrenergic mechanisms. In the tail flick model of nociception, prolonged reaction time indicates central analgesia and may involve opioidergic, adrenergic, dopaminergic, and cholinergic receptors. The absence of MEBC-induced analgesia in the presence of these antagonists further confirms the central mechanisms of MEBC's antinociception. To our knowledge, this is the first study to show that *B. coriacea* seed extract exhibits centrally acting potentials via nociceptive pathways in mice. These findings suggest that the analgesic properties of MEBC may be attributed to its chemical constituents.

Conclusion

The present study demonstrated that the MEBC seeds possess significant anti-nociceptive potential. This anti-nociceptive activity seems to act via the opioidergic, adrenergic, dopaminergic, and cholinergic receptors in central pain pathways. Linoleic acid, the most abundant compound in MEBC, seems to contribute to the analgesic properties of *B. coriacea* seeds observed in the present study. Therefore, the plant's seeds can be considered a potential therapy for centrally mediated nociception. These findings support the species' analgesic use in traditional practice, which lays the foundation for future research on anti-nociceptive mechanisms.

Authors' Contribution

Conceptualization: Adedayo LD and Onasanwo SA.
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Formal analysis: Adedayo LD, Aitokhuehi NG.
Funding acquisition: Adedayo LD, Adekeye AO, Aitokhuehi NG, Onasanwo SA, Morakinyo OA.
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Methodology: Adedayo LD, Aitokhuehi NG, Ajayi AM, Bamidele O, Onasanwo SA.

Project administration: Onasanwo SA, Adedayo LD.
Resources: Adedayo LD, Aitokhuehi NG, Onasanwo SA, Morakinyo OA, Ajayi AM.
Software: Adedayo LD, Aitokhuehi NG, Ajayi AM.
Supervision: Onasanwo SA, Ajayi AM, Bamidele O.
Validation: Onasanwo SA, Ajayi AM, Bamidele O, Adekeye AO.
Visualization: Onasanwo SA, Adekeye AO, Adedayo LD, Morakinyo OA.
Writing–original draft: Adedayo LD, Aitokhuehi NG, Bamidele O.
Writing–review & editing: Adedayo LD, Onasanwo SA, Adekeye AO, Ajayi AM.

Competing Interests

The authors declare no conflict of interests and take sole responsibility for the accuracy and integrity of the research article content.

Data Availability Statement

The data supporting the findings of this study are available from the corresponding author (LA) upon reasonable request.

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