Original Research Article

Evaluation of In-vitro Antimicrobial, Anti-Arthritis and In-vivo Analgesic and Neuropharmacological Investigation of the Bark Extract of *Solanum Americanum Milli*

ABSTRACT

Purpose: The objective of this study was to investigate the impact of methanol-prepared bark extract of *Solanum Americanum Milli.* Bark on several in vitro activities, including antimicrobial and anti-arthritis effects. Additionally, the study aimed to assess the analgesic and neuropharmacological properties of the extracts in an animal model.

Place and Duration of Study: The research study was carried out from March 2023 to July 2023 at the Bangladesh Council of Scientific and Industrial Research (BCSIR) and the Laboratory of Phytochemistry and Pharmacology, Department of Pharmacy, as well as the Laboratory of Microbiology at Stamford University Bangladesh, Dhaka.

Methodology: The methodology involved various doses of methanolic bark extract of *Solanum Americanum Milli* (MESAB), and employed techniques such as the Disk Diffusion Method for antimicrobial test and the Inhibition of Protein Denaturation Assay for anti-arthritic test. The analgesic efficacy was assessed by the hot plate test and administration of acetic acid induced writhing responses in *Swiss albino* mice. The mechanism test is conducted through the cyclic guanosine monophosphate (cGMP) test. The neuropharmacological inquiry included the measurement of locomotor activity through the utilization of open field and hole cross tests.

Result: The Disk Diffusion Test of MESAB yielded findings indicating its antimicrobial properties against all strains used in this research. The findings from the study examining the anti-arthritis properties indicate that MESAB exhibits a significant efficacy of 94.59% in inhibiting arthritis at a concentration of 1000 µg/mL. This level of efficacy is comparable to that of diclofenac sodium, which also has a similar efficacy of 98.19% at the same concentration. The findings of the present study demonstrated that MESAB displayed analgesic properties in both analgesic models, suggesting the involvement of both central and peripheral pathways. Based on the results of the study, it was observed that the administration of MESAB at a dosage of 400 mg/kg had a significant analgesic effect. The application of MB before to treatment resulted in a further enhancement of this effect, leading to an increase in the percentage of inhibition from 66.75% to 79.70%, respectively. The consideration of the involvement of the GABA-benzodiazepine receptor in neuropharmacological activity holds significant importance. In the Open Field and Hole Cross Tests, it was shown that the extract exhibited significant result compared to Diazepam in terms of increasing motor coordination.

Conclusion: In the future, plant-derived pharmacological compounds may offer potential benefits for various clinical disorders, such as neurodegenerative illnesses. These substances possess neuropharmacological properties, antibacterial effects, and anti-arthritic activity, which could potentially serve as alternatives to non-steroidal anti-inflammatory drugs (NSAIDs).

Keywords: Antimicrobial, anti-arthritis, analgesic, denaturation, neuropharmacological.

1.0 INTRODUCTION

The enduring use of medicinal plants, documented throughout several historical periods, is crucial to addressing a diverse range of ailments. Based on the available research, it seems that a significant proportion of Ethiopia's population resists on traditional medicine. The presence of bioactive compounds in medicinal herbs is pivotal in determining their therapeutic efficacy. Biomolecules such as essential oils, saponins, alkaloids, terpenoids, phenolic compounds, flavonoids, and tannins are known to contribute to the therapeutic properties shown by many plant species (Shaira et al., 2023). *Solanum americanum* is classified under the taxonomic family Solanaceae. The organism in question fulfills a significant ecological function within the taxonomic family Solanaceae. *Solanum americanum*. The perennial botanical specimen, with a potential height range of 25 to 100 centimeters, is adorned with simple trichomes. The dark-hued fruits possess a diameter ranging from around 8 to 10 millimeters and exhibit a lackluster visual quality. The leaves exhibit a variety of shapes, ranging from oval to heart-shaped, with dimensions of from 4-10 cm in length and 3-7 cm in width. They possess a dense covering of fine hairs and have prominently serrated edges (Shomudro et al., 2023). During antiquity, *Solanum americanum* was used for medicinal purposes. This plant is extensively used in traditional medicine because of its potent analgesic and sedative properties, which are accompanied by narcotic effects. Additionally, it is often administered topically for the treatment of measles, itching, and dermatitis. To address hepatic ailments and mitigate the occurrence of jaundice, the leaves and berries of the plant are subjected to a cooking process to extract their therapeutic attributes. *Solanum americanum*, sometimes referred to as American black nightshade or Glossy nightshade, is a well-recognized plant species. The use of the plant in some areas of North-Eastern Nigeria for the treatment of diarrhea and dysentery has inspired the pursuit of empirical evidence to substantiate this traditional assertion (Usman et al., 2018).

The primary objective of this study was to assess the in-vitro anti-arthritis, anti-microbial, and in-vivo analgesic and neuropharmacological properties of the barks derived from *Solanum americanum Mill*.

2.0 MATERIALS AND METHODs

2.1 Plant Material

In July 2022, a specimen of *Solanum americanum Mill*, a plant species, was collected from West-Delpara, Kutubpur, Narayanganj, Dhaka, Bangladesh. The plant (accession number: DACB 87210) was accurately identified by the experts at the Bangladesh National Herbarium located in Mirpur, Dhaka. The powder was derived from the desiccated barks of the plant, which had been carefully preserved in a shaded environment.

2.2 Reagents

Methanol, sodium hydroxide (NaOH), diluted hydrochloric acid (HCl), concentrated sulfuric acid (H_2SO_4), acetic acid, and methyl blue were all procured from Sigma Chemical Co., located in the United States. The procurement of morphine sulfate was conducted via the acquisition from Gonoshasthaya Pharmaceuticals Ltd., located in Dhaka, Bangladesh. We bought a quantity of sterile saline solution from Orion Infusion Ltd. Square Pharmaceuticals Ltd. served as the producer for both diclofenac sodium injection and diazepam injection. The DMSO was provided by the German business Merck. The Bovine Serum Albumin (BSA) used in this study was acquired from Polysciences, Inc., India.

2.3 Preparation of Plant Extract

A total of 300 grams of plant bark underwent a process of washing with distilled water, followed by a period of drying in a shaded environment for 15 days. Following the drying process, the barks were pulverized using an electric blender. The solid substance was then combined with 1200 mL of methanol in a hermetically sealed container for 10 days, during which it was regularly agitated. The combination underwent filtration using cheesecloth, followed by further filtration of the acquired filtrate using Whatman

filter paper. The filtrate underwent concentration using a water bath and subsequent drying in an oven at a temperature of 40°C. The resulting extract was then transferred to an air-tight container and kept in a refrigerator(Uwaya et al., 2022).

2.4 In-Vitro Antimicrobial Test

2.4.1 Test organisms

A total of four bacterial strains, namely *Bacillus cereus* (JN 797797), *Klebsiella pneumoniae* (CP05288), *Vibrio cholerae* (FJ 462446), *Streptoccus aureus* (CP003357), were obtained from the microbiology laboratory of the Bangladesh Council of Scientific and Industrial Research. Additionally, four fungal strains, namely *Penicillium chrysogenum* (WIS 54-1255) *Aspergillus niger* (PRJNA19275) *Yeast budding* (238059) and *Mucor hiemalis* (NR_152948) were also collected from the same laboratory.

2.4.2 Antimicrobial susceptibility test

The Mueller Hinton Agar (MHA) was used as the medium for the cultivation of microorganisms. Sterilized discs, measuring 6 mm in diameter, were thereafter introduced into the agar plates as a component of the disc diffusion technique (Al-askar et al., 2023). MESAB was dissolved in predetermined quantities of solvents to provide solutions with known concentrations (300, 500, and 700 µg/mL). Subsequently, the petri dishes were subjected to incubation at a temperature of 4 °C for 2 hours, facilitating the diffusion of the extracts into the agar medium. Subsequently, the measurement of the zones of inhibition around the discs was conducted following the incubation of the Petri dishes at a temperature of 37 °C for one day. The measurement of the zone of inhibition was conducted after 24 hours, and the results were recorded in centimeters (Valgas et al., 2007).

2.5 In-Vitro Anti-arthritis Test

A volume of 0.5 mL of a reaction mixture was prepared, consisting of 0.45 mL of bovine serum albumin (5% aqueous solution), 0.05 mL of MESAB crude extract at concentrations of 62.5, 125, 250, 500, and 1000 µg/mL, and 0.05 mL of salicylic acid as the reference drug. The pH of each solution was adjusted to 6.3 by the addition of 1 N HCl. Following a 20-minute incubation period at a temperature of 37°C, the samples underwent a further heating process lasting 30 minutes at a temperature of 57°C. Following the addition of a 2.5 mL phosphate buffer, the spectrophotometer measurement was recorded at a wavelength of 660 nm. Instead of using Bovine Serum Albumin (BSA) in the product control, a volume of 0.05 ml of distilled water was used in the test control (Sharma & Goel, 2023). Equation used for calculating percentage of inhibition of protein denaturation:

$$\% \text{ Inhibition} = \frac{(\text{OD of Control} - \text{OD of sample})}{\text{OD of control}} \times 100$$

Here, OD means optical density.

2.6 In-Vivo Analgesic Test

2.6.1 Hot Plate Test

The experimental procedure followed the standard hot-plate approach developed by Eddy and Leimbach (Tita et al., 2001). In this study, a total of five animals were subjected to different treatments. Specifically, the animals were administered either M at a dosage of 10 mg/kg subcutaneously or MESAB at varying dosages of 0, 47.5, 95, 190, and 380 mg/kg subcutaneously. After 30 minutes, the animals were placed on a hot plate from Basile, Varese, Italy, which was heated to a temperature of 55°C. The researchers then recorded the time it took for the animals to respond to the painful stimulus, which included behaviors such as paw elevation and licking. The designated time limit was set at 30 seconds. The formula for the analgesic effectiveness of treatment was as follows:

Percent Analgesic Score = $\frac{Ta-Tb}{Ta}$ X100.

Time (in seconds) to react (before medication administration): Tb; Time (in seconds) to react (after drug administration): Ta.

2.6.2 Writhing Test

The analgesic efficacy was assessed in mice using the writhing test, which was produced by administering 0.6% acetic acid (0.1 mL/10 g; intraperitoneally).

The administration of each dosage of the extract occurred 30 minutes before the injection of acetic acid. The quantification of writhes and stretching movements, characterized by the contraction of the abdominal muscles and extension of hind limbs, was conducted 5 minutes after the injection of the acid. This counting process was performed for a duration of 30 minutes, with observations recorded every 5 minutes. The degree of the induced analgesic effect was evaluated in comparison to an effective dosage of acetylsalicylic acid (ASA) at 200 mg/kg administered intraperitoneally (i.p.) (Farouk et al., 2008). The proportion of writhing restraint was determined by using the following formula:

% Of writhing = $\frac{VC-VT}{VC}$ X 100

VT = number of writhing motions in extract-treated mice. VC = number of writhing motions in the control group of mice.

2.7 Investigation for analgesic activity mechanism (s)

2.7.1 Involvement of cyclic guanosine monophosphate(cGMP) pathway

The animals were given a pre-treatment of methylene blue, a substance known to block guanylyl cyclase and/or nitric oxide, at a dosage of 20 mg per kilogram of body weight through intraperitoneal injection. Following a 15-minute interval, mice were administered either a vehicle solution (consisting of 0.2% tween 20, orally) as the control group or MESAB at a dosage of 400 mg/kg. In order to examine the potential role of the cyclic guanosine monophosphate (cGMP) pathway in the observed analgesic effects of MESAB, the experimental animals were administered 0.6% acetic acid (v/v, 10 mL/kg, intraperitoneally) 30 minutes following the administration of MESAB. The number of abdominal writhes was then recorded for a duration of 30 minutes, commencing 5 minutes after the injection of acetic acid. (Shomudro & Chowdhury, 2023).

2.7 In-Vivo Neuropharmacological Test

2.7.1 Open Field Test

The experimental methodologies used in this study have been adapted from the previous research work (Barragan-galvez et al., 2023). A one-square-meter open area was divided into many smaller squares using tiling. The whole arrangement was enclosed by a wall with a height of 40 cm. Following the administration of an oral test drug treatment for both MESAB, as well as an intraperitoneal dosage of Diazepam, the mice's exploratory behavior was assessed by tallying the number of squares visited during a period of 5 minutes at 30, 60, 90, and 120 minutes.

2.7.2 Hole Cross Test

The experimental participants were placed into a cage of 30 × 20 × 14 cm, which had a divider with a centrally located hole measuring 3 cm in diameter (Eunice et al., 2023). The animals are subjected to a vehicle, a medication, or MESAB prior to their release for passage into the subsequent compartment. The mice were subjected to observation for a duration of 3 minutes at four different time intervals: 30, 60, 90,

and 120 minutes after the administration of medications. The recorded data pertained to the number of passages made by the mice.

2.8 Statical Analys

The bioassay readings were performed in triplicate, and the tabular data is shown as the mean ± standard deviation. The statistical studies were performed with the software program Microsoft Excel.

3.0 RESULTS and DISCUSSIONS

3.1 Antimicrobial Assays

The antibacterial and antifungal activity of various doses of plant extract was assessed against gram-positive and gram-negative bacteria, as well as four different species of fungi, by measuring the zone of growth inhibition in millimeters (mm). The findings of the study indicated that there was a positive correlation between the concentration of plant extract and the size of the inhibitory zone, as seen in Table 1 and Table 2. The observed zone of inhibition for antibacterial activity ranged from 7 to 19 mm, whereas the zone of inhibition for fungus ranged from 6 to 13 mm. The presented findings show that MESAB has the greatest antibacterial efficacy against *Escherichia coli* and *Bacillus cereus*, both of which are responsible for causing severe ailments such as urinary tract infections, respiratory illnesses, pneumonia, gastrointestinal illnesses, and diarrhea.

Table 1. Inhibition zone of MESAB against different bacteria

Diameter of Zone of Inhibition (mm)				
Test organisms	MESAB (300 µg/disc)	MESAB (500 µg/disc)	MESAB (700 µg/disc)	Ciprofloxacin
Gram Positive Bacteria				
Bacillus cereus	13	15	19	25
Staphylococcus aureus	11	14	16	26
Gram Negative Bacteria				
Vibrio cholerae	12	14	16	27
Klebsiella pneumonia	09	15	16	24

Table 2. Inhibition zone of MESAB against different fungi

Diameter of Zone of Inhibition (mm)				
Test organisms	MESAB (300 µg/disc)	MESAB (500 µg/disc)	MESAB (700 µg/disc)	Griseofulvin (50 µg/disk)
Fungi				
Penicillium chrysogenum	06	11	14	19
Aspergillus niger	07	09	11	20
Yeast budding	10	11	14	21
Mucor hiemalis	07	11	13	21

Table 1 and 2 displays the inhibitory regions formed as a result of the presence of MESAB. The research has shown that the MESAB has antibacterial properties against all strains used in this investigation. The findings have suggested that MESAB had a more potent antibacterial activity compared to MESAL. The aforementioned statistics closely corresponded to the antibacterial activity of MESAL as reported in earlier investigations (Shomudro et al., 2023). The observed impact of the MESAB may be ascribed to its high

concentration of medicinal phytochemicals, including flavonoids and phenolic compounds, which possess antimicrobial, anti-inflammatory properties (Shomudro et al., 2023).

3.2 In Vitro Anti-Arthritic Activity

The denaturation property of Bovine Serum Albumin (BSA) was compared to that of the reference medication, as seen in Table 3 and Figure 1.

Table 3. Percentage inhibition in protein denaturation of MESAB

Samples	Concentrations (µg/mL)	% of inhibition
Diclofenac Sodium	62.5	84.64
	125	87.38
	250	89.18
	500	93.69
	1000	94.59
MESAB	62.5	69.36
	125	72.37
	250	85.58
	500	92.79
	1000	95.49

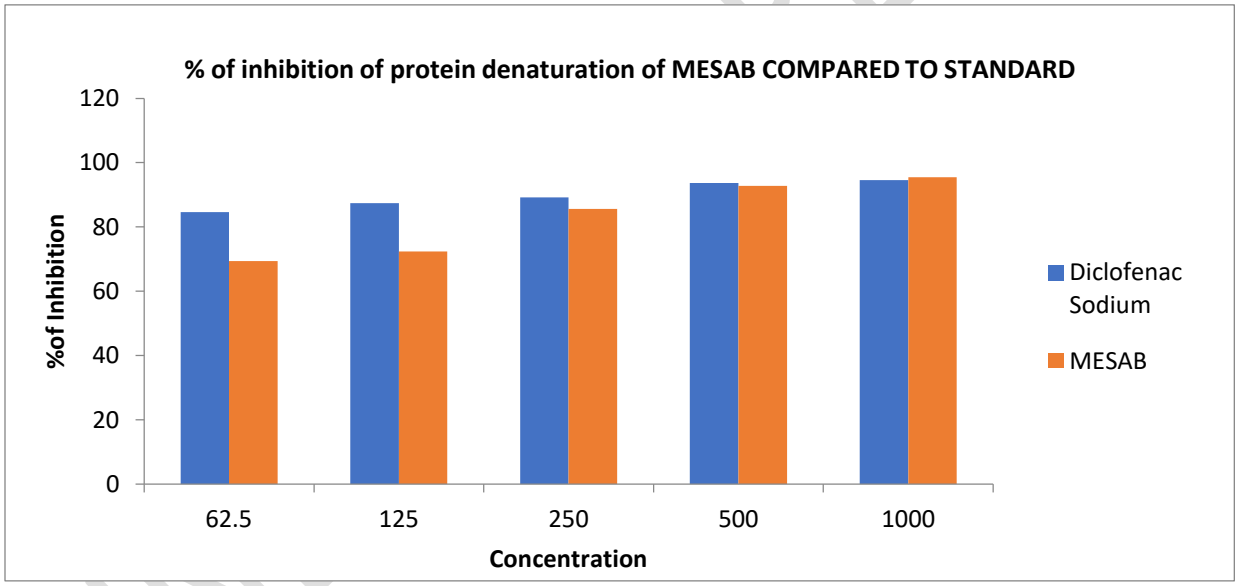

Figure 1. Percentage of inhibition of MESAB compared to standard

Rheumatoid arthritis (RA), a kind of inflammatory illness, affects around 1% of the population in economically advanced nations. The indications indicative of acute rheumatoid arthritis (RA) includes reduced mobility, heightened sensitivity to pain (hyperalgesia), and a halt in the increase of body weight (Amresh et al., 2007). The findings of this study demonstrate that the MESAB exhibits a noteworthy anti-arthritic efficacy of 94.59% at a concentration of 1000 µg/mL, which is comparable to the effectiveness of Diclofenac sodium at the same quantity (98.19%). These results are shown in Table 3 and Figure 1. Due to its notable therapeutic potential in the context of arthritic conditions, it is plausible that it may be used as a treatment for Rheumatoid arthritis in further clinical applications.

3.3 In-Vivo Analgesic Activity

3.3.1 Hot plate test

The results of the hot plate test after the use of MESAB at the doses of 200 and 400 mg/kg i.p. showed an increase in the time of reaction to the thermal stimulus compared to control is shown in the Table 4.

Table 4. Primary Data Table for Hot Plate Test for Plant Extract of MESAB

Reaction time at different time intervals (in sec)						
Group	**Dose**	**Average wt. of mice (g)**	**30 min**	**60 min**	**90 min**	**120 min**
Control			6.4	7.6	6.0	5.4
Morphine	(5mg/kg)	20 to 26	8.6	9.4	11.0	6.6
MESAB	(200mg/kg)		9.2	9.4	11.6	12.0
MESAB	(400mg/kg)		7.8	8.4	8.5	14.8

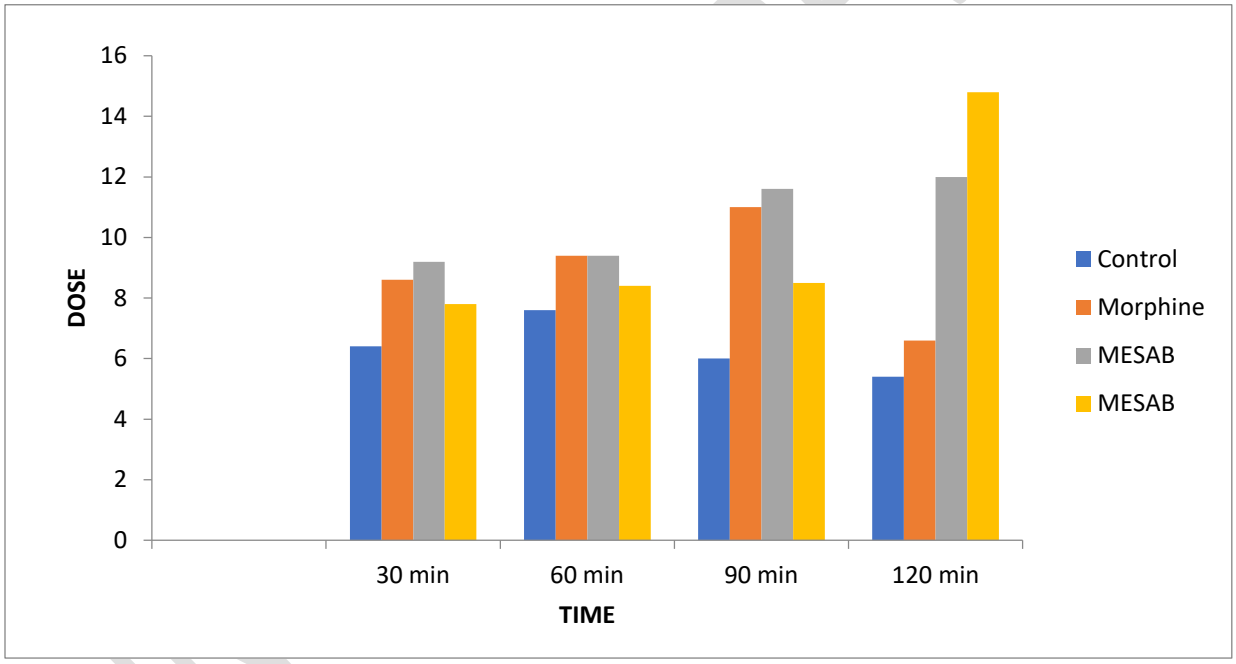

Figure 2: Analgesic effect of Plant Extract of MESAB by hot plate test

The results of experimental studies have shown that the MFSAB has a pain-reducing effect that is dependent on the administered dosage, as seen in the hot plate model. Nevertheless, the analgesic activity exhibits a 1.2-fold decrease compared to diclofenac sodium. However, further research is required to validate this hypothesis. This particular kind of association suggests that the doses used in these examinations fall within the therapeutic range of MESAB, whereby certain medications demonstrate their optimal therapeutic efficacy. The presence of certain compounds such as alkaloids, saponins, and flavonoids may give rise to these effects (Dharmasiri et al., 2003).

3.3.2 Writhing test

In the acetic acid-induced writhing test, the methanolic extracts of the bark of *Solanum americanum milli* exhibited inhibitory effects of 66.75% and 53.62% respectively. In comparison, the reference medication Diclofenac-Na had an inhibition rate of 80.36%. In comparison to established benchmarks, the findings exhibited noteworthy analgesic efficacy that is contingent upon the dosage administered.

Table 5. Analgesic Activity of MESAB on Mice by Writhing Test

Administered Substance	Dose	% Writhing	% Of Inhibition
Control	10mL/kg	100	0.00
Diclofenac sodium	10mg/kg	19.74	80.36
MESAB	200mg/kg	39.28	53.62
MESAB	400mg/kg	32.14	66.75

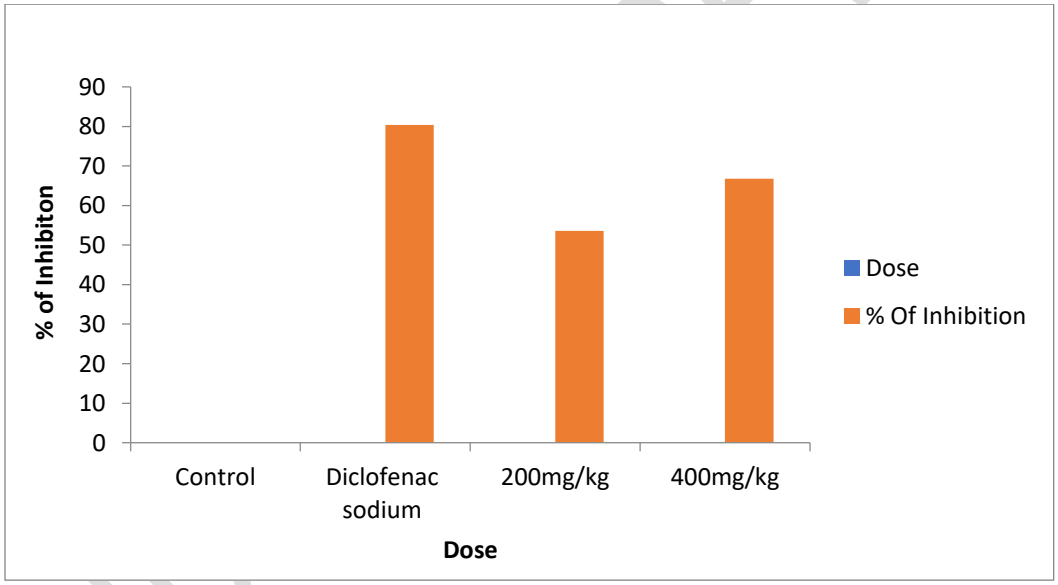

Figure 3: Analgesic effect of MESAB by acetic acid-induced writhing method

The findings of the present study demonstrated that MESAB had analgesic effects in both analgesic models, suggesting the involvement of both central and peripheral mechanisms. The administration of MESAB resulted in a significant and dose-dependent reduction in abdominal writhing, as shown in an experimental model of acetic acid-induced abdominal constriction. The efficacy of the test in assessing the effectiveness of moderate analgesic non-steroidal anti-inflammatory drugs (NSAIDs) is attributed to the indirect mechanism of acetic acid. Acetic acid is believed to induce the release of prostaglandins and lipo-oxygenase products into the peritoneum, which subsequently activate the nociceptive neurons that are responsive to NSAIDs. The findings from the experiment involving acetic acid-induced writhing provide compelling evidence that the observed action is likely associated, at least partially, with the inhibition of lipo-oxygenase and/or cyclooxygenase in the peripheral tissues. This inhibition subsequently leads to a reduction in prostaglandin synthesis and disrupts the transduction mechanism in primary afferent nociceptors (Yukio Sato, Hiroshi Yamakose, 1970).

3.4 Involvement of cyclic guanosine monophosphate (cGMP) pathway

Table 6 presents the results obtained from administering the cGMP inhibitor methylene blue as a pre-treatment to the mice. This experimental approach was used to investigate the potential involvement of the cGMP pathway (MB). While the application of acetic acid resulted in the manifestation of nociceptive behavior, the administration of 20 mg/kg of MB in isolation did not exhibit any discernible impact on this behavior.

Table 6. Analgesic Activity of MESAB through cGMP pathway by Writhing Test

Administered Substance	Dose	% Writhing	% of Inhibition
Control	20mg/kg	100	0.00
MESAB	400mg/kg	20.30	79.70

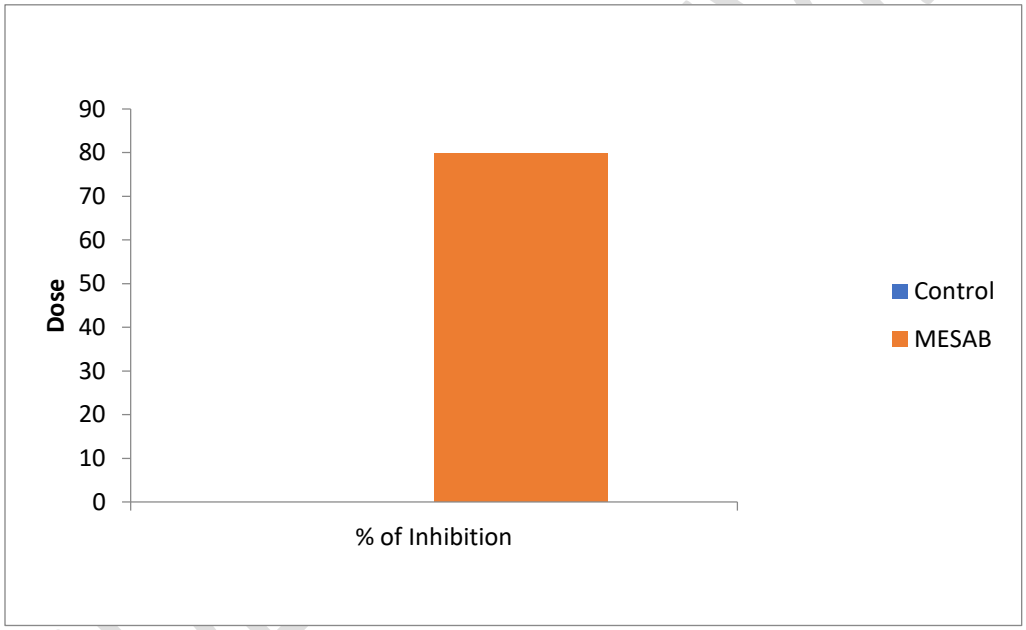

Figure: 4 Analgesic Activity of MESAB through cGMP pathway by Writhing Test

The study provided evidence that administering MESAB at a dosage of 400 mg/kg resulted in a very effective analgesic effect. This effect was further enhanced by pre-treatment with MB, leading to an increase in the percentage of inhibition from 66.75% to 79.70% respectively. The analgesic properties of cGMP-blocking drugs have been extensively acknowledged in previous studies (Ferdous et al., 2020). The research findings demonstrated that the administration of methylene blue, a substance that inhibits the cyclic guanosine monophosphate (cGMP) pathway, resulted in an enhanced effectiveness of nociception elicited in ants by MESAB. The interaction between MESAB and the nitric oxide (NO) and cyclic guanosine monophosphate (cGMP) pathway has been demonstrated. The modulation of different potassium (K+) channels by the nitric oxide (NO)/cyclic guanosine monophosphate (cGMP) pathway has been extensively studied. It has been observed that the activation of ATP-sensitive K+ channels through the NO/cGMP pathway leads to the suppression of action potential generation. This effect is achieved by hyperpolarizing the peripheral terminal of afferent neurons.

3.6 Neuropharmacological Activity

3.6.1 Open Field Test

The results of the open field test indicated that the depressive effects of the extracts were apparent during the second observation time in the test subjects, at both the 200 mg/kg and 400 mg/kg body weight levels.

Table 7. The primary data table for the open field test for the both MESAB

Number of movements					
Group	Average wt. of mice (g)	0 min	30 min	60 min	90 min
Control	24	89.6	39	32.4	22.8
Diazepam (10mg/kg)		87.2	62	36.2	18.8
MESAB (200mg/kg)		71.6	59.2	49.4	30.1
MESAB (400mg/kg)		58.8	40.4	31.1	15.8

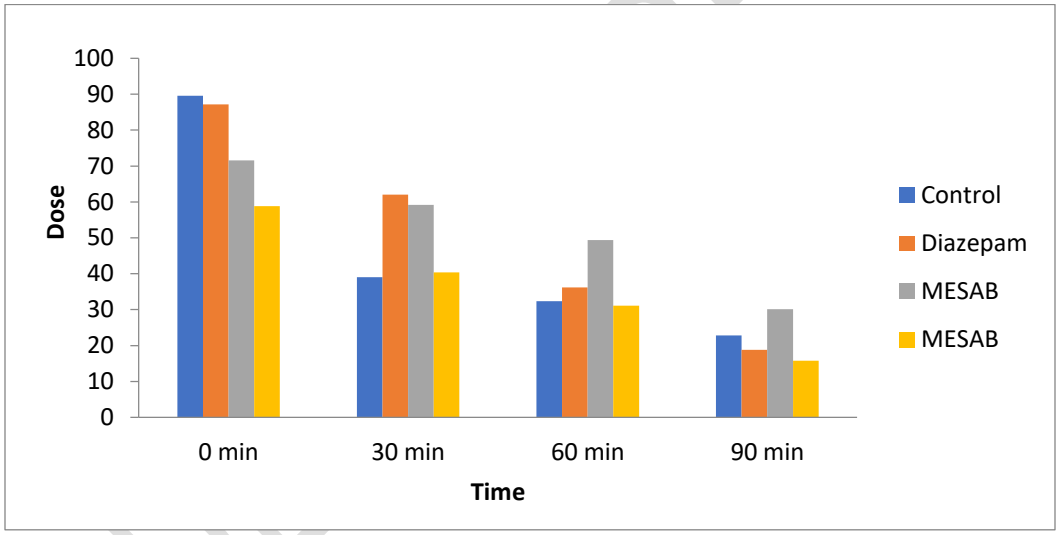

Figure 5: Neuropharmacological activity of *Swiss albino* mice by open field test

3.6.2 Hole Cross Test

After 30 minutes, it was seen that the mice entered a state of sleep, leading to a significant decrease in their overall activity levels. The subjects exhibited prolonged somnolence for 90 minutes after the administration of the extract. The results obtained from the open field test and the hole cross test revealed a significant decrease in locomotor activity induced by the extracts. During the second observation period, which lasted for 60 minutes, a discernible effect on locomotor activity reduction was seen for both the 200 mg/kg and 400 mg/kg body weight concentrations. The aforementioned impact was consistently observed throughout the third and fourth observation periods, which lasted for 90 and 120 minutes, respectively. Furthermore, the assessment of anxiety was conducted by examining observable manifestations using hole-cross tests.

Table 8. The primary data table for the Hole Cross Test for the MESAB

Number of movements					
Group	Average wt. of mice (g)	30 min	60 min	90 min	120 min
Control	24.5	23.8	24.4	23.8	19.4
Diazepam (10mg/kg)		9.67	6.67	3.67	2.33
MESAB (200mg/kg)		12	9.4	6.21	0.4
MESAB (400mg/kg)		7.4	6.6	0.00	0.00

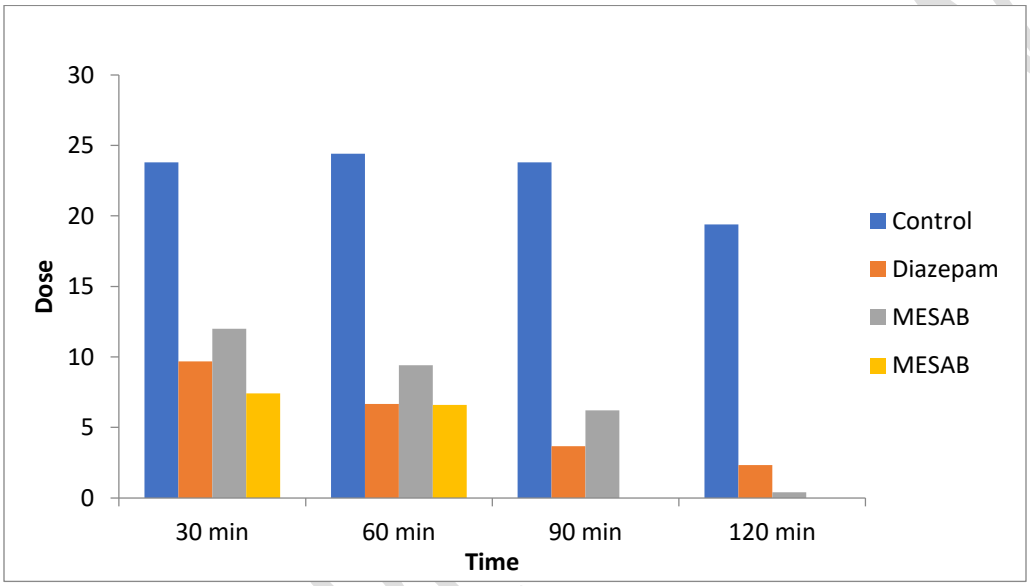

Figure 6: Neuropharmacological activity of by of Swiss albino mice hole cross test

The test included quantifying the spontaneous migration of the animals between chambers via the hole over a period of 5 minutes. The observations were conducted at time intervals of 30, 60, 90, and 120 minutes after intraperitoneal administration of the MESAB. No discernible effects were seen in the test animals at the 0-minute mark. After duration of 30 minutes, it was noticed that the mice entered a state of sleep, resulting in a significant decrease in their overall locomotor activity. Despite the passage of 90 minutes after the administration of the extract, the subjects remained in a state of sleep. The locomotor activity was greatly reduced by the extracts, as shown by the outcomes of the open field and hole cross tests. The observed decrease in locomotor activity was seen for both the 200mg/kg and 400mg/kg dosages of body weight at the second observation (60 minutes) and persisted through the third and fourth observations (90 and 120 minutes). Furthermore, the assessment of anxiety was conducted by quantifying observable indicators, namely by the administration of hole-cross tests. Assessing the effect of a medication on an animal's locomotor activity is an essential component in assessing the drug's influence on the central nervous system. Activity is seen as a quantifiable indicator of central nervous system (CNS) excitability (Pal et al., 2010). Consequently, a decrease in activity levels might potentially serve as an indication of CNS depression, which in turn may imply drowsiness. To assess the sedative effects, this study used the open field and hole cross tests. The findings presented showed a correlation between the dosage of the extract and a decrease in locomotor activity. The neuropharmacological activity of the plant may be attributed to the presence of alkaloids, glycosides, flavonoids, and tannins since any of these compounds might potentially be responsible (Thirupathy et al., 2011). The

observed decrease in the number of holes traversed by mice between the second and fourth observations during the hole cross-test suggests the potential tranquilizing impact of EEGP. The potential involvement of the GABA-benzodiazepine receptor in the process is worth considering (Shaira et al., 2023).

4.0 CONCLUSION

The findings of this study revealed that MESAB exhibits noteworthy antibacterial and anti-inflammatory activities. The findings of this study provide support for the ethnomedical assertions made on MESAB, validating its purported anti-arthritic, anti-inflammatory, and antibacterial characteristics. There exists a considerable body of research indicating that the methanolic bark extract of this plant possesses neuropharmacological properties. Therefore, it is plausible that the observed pharmacological effects can be attributed to phytochemicals such as alkaloids, tannins, flavonoids, glycosides, phenols, and other similar compounds. These ingredients were identified by the process of phytochemical screening. In further investigations, the utilization of GC-MS analysis, column chromatography, and nuclear magnetic resonance (NMR) could potentially facilitate the identification of the specific phytochemical compound accountable for the observed pharmacological effects.

8.0 ETHICAL APPROVAL

The authors affirm that all studies underwent a thorough evaluation by an appropriate ethics committee and were conducted in accordance with established ethical standards.

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