Original Research Article

In-Vitro Antioxidant, Anti-Arthritis, Anti-inflammatory, Thrombolysis, Anti-Bacterial, and In-Vivo Neuropharmacological activities of Bioactive Metabolites of *Solanum Americanum Mill*.

.

ABSTRACT

Aims: The purpose of this research was to examine the effects of methanol-prepared leaf extracts from the *Solanum americanum Mill*. plant on a variety of in vitro activities, including antioxidant, anti-Arthritis, anti-inflammatory, thrombolysis, anti-bacterial, and in-vivo neuropharmacological activities.

Study Design: Methanolic extract of *Solanum americanum* leaf (MESAL) was primarily evaluated via phytochemical screening. The potential for in vitro activities, including antioxidant, anti-arthritis, anti-inflammatory, thrombolysis, and anti-bacterial activity were determined to study due to the pharmacological interest in the plant's chemical constituent. Whether there is statistical significance to the changes seen in its in vivo neuropharmacological characteristics when tested in experimental animals.

Place and Duration of Study: This research was carried out between November 2022 to January 2023 at the Laboratory of Phytochemistry and Pharmacology in the Department of Pharmacy and Laboratory of Microbiology, Stamford University Bangladesh and Bangladesh Council of Scientific and Industrial Research,Dhaka.

Methodology: The plant was subjected to phytochemical screening utilizing a variety of test reagents and potential antioxidant, anti-arthritis, anti-inflammatory, thrombolytic, anti-bacterial, and neuropharmacological activities of MESAL were investigated. Antioxidant, anti-arthritic, anti-inflammatory, thrombolytic, and antibacterial activities were tested at different doses utilizing the DPPH Free Radical Scavenging Assay, Protein Denaturation Assays, Clot Lysis, and the Disk Diffusion Method. *Swiss albino* mice were tested using open-field and hole-cross methods to measure their locomotion as part of the neuropharmacological study.

Results: The MESAL phytochemical screening findings demonstrated that the plants' chemical compositions varied. Most antioxidant activities were found in MESA, with an IC_{50} of 11.73 µg/mL compared to Ascorbic acid's IC_{50} of 28.86 µg/mL. When compared to the standard, the percent inhibition value of MESAL's anti-arthritis activity was significantly higher. With a maximum protein denaturation value of 94.03% at a concentration of 1000µg/mL, MESAL possesses potent anti-inflammatory activity. The value of MESAL in terms of clot lysis is very significant which is 90.257%. While MESAL's antibacterial value is moderate, it is still worth considering. However, the extract was proven to be less effective than Diazepam in improving motor coordination in the Open Field and Hole Cross Tests.

Conclusion: Several pathological conditions, including neurodegenerative diseases, may benefit in the future from the use of plant-derived pharmacological agents due to their neuropharmacological, antioxidant, anti-inflammatory, and anti-arthritic activity, which can replace the use of NSAIDs.

Keywords: Antioxidant, anti-arthritis, anti-inflammatory, thrombolysis, anti-bacterial, neuropharmacological.

1. INTRODUCTION

Since ancient times, people have relied on natural items like plants, animals, microorganisms, and marine species to help alleviate symptoms of illness and treat disease. Humans have been using plants as medicines for at least 60,000 years, as evidenced by fossils[1][2]. The use of natural products as medicines offered enormous difficulty to early humans. It is quite certain that early humans frequently swallowed poisonous plants while foraging for food, resulting in vomiting, diarrhea, stupor, or other deadly effects, and possibly death. In this way, however, early people were able to obtain knowledge of food items and natural medicines [3]. Afterward, humanity invented fire, discovered how to produce alcohol, developed religions, achieved technological advances, and discovered how to create new medications.

Traditional medicines utilize natural substances and are of vital value. Traditional Chinese medicine, ayurveda, traditional Korean medicine, and unani all utilize natural ingredients and have been practiced worldwide for hundreds or even thousands of years & they have evolved into well-organized medical systems. They may have flaws in their many incarnations, but they remain a significant reservoir of human knowledge [2][4].

*Solanum americanum*belongs to the family Solanaceae. It plays an important ecological role in the family of Solanaceae. *S. americanum*. The annual herb, which can grow between 25 and 100 cm in height, is covered in simple hairs. The black fruits have a diameter of about 8-10 mm and have a dull appearance. The leaves, which range in form from oval to heart and are 4-10 cm long and 3-7 cm wide, are thickly pubescent and coarsely dentate [5]. In ancient times, *Solanum americanum* was already being used as a medicine. Being a powerful painkiller and sedative with narcotic effects, this plant is widely used in traditional medicine; it is also applied topically to treat measles, itching, and dermatitis. To treat liver problems and prevent jaundice, the leaves, and berries are cooked to extract their medicinal properties. *Solanum americanum*, commonly known as American black nightshade, Glossy nightshade. In some regions of North-Eastern Nigeria, the herb has been used to treat diarrhea and dysentery, prompting the search for scientific proof of this traditional claim [6].

The main aim of the research was to determine in-vitro antioxidant, anti-Arthritis, anti-inflammatory, thrombolysis, anti-Bacterial, and in-vivo neuropharmacological activities of bioactive metabolites of the leaves of *Solanum americanum Mill*.

2. MATERIAL AND METHODS

2.1 Plant material

The sample plant *Solanum americanum Mill* was taken in July 2022 from West-Delpara, Kutubpur, Narayanganj, Dhaka, Bangladesh. The professionals at the Bangladesh National Herbarium in Mirpur, Dhaka, were able to correctly identify the plant (accession number: DACB 87210). The powder was made from the plant's dried leaves, which had been stored in the shade.

2.2 Reagents

Sigma Chemical Co., USA provided methanol, NaOH, diluted HCl acid, concentrated H_2SO_4, and acetic acid. It was through Orion Infusion Ltd. that we acquired the sterile saline solution. Diazepam and diclofenac sodium injections were produced by Square Pharmaceuticals Ltd. The DMSO came from the German business Merck. Bovine Serum Albumin was purchased from Polysciences, Inc. India. Streptokinase was brought fromIncepta Pharmaceuticals Ltd, Bangladesh.

2.3 Preparation of plant extract

The cold maceration method was used for the extraction procedure [7]. The crude extract was obtained by soaking 400 g of plant’s leaf powder in 800 mL of methanol for 10 days and

then filtering the mixture. There was an additional 7 days of air drying after the solution was filtered. After everything was said and done, the total amount of crude extracts was 31g.

2.4 In-Vitro Analysis

2.4.1 Phytochemical identification test

Steroids, carbohydrates, alkaloids, reducing sugar, gum, flavonoids, saponins, tannins, and phenol were all checked for throughout the screening process [8]. In these tests, the degree of color intensity or the production of precipitates was utilized as an analytical response.

2.5 Antioxidant Assay

2.5.1 DPPH free radical scavenging assay

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method was used to find out how effective the extract was as an antioxidant. 2mL of a methanol solution with different concentrations of the extract were mixed with 2mL of a methanol solution with 20 µg/mL of DPPH. To finish the reaction, the samples were kept for 30 minutes in a cool, dark place at room temperature. Using a UV spectrophotometer using the technique outlined by Brand Williams [9], the absorbance of the sample was determined at a wavelength of 517 nm and compared to a blank of methanol. For each sample, the IC_{50} value was worked out and shown. Ascorbic acid (AA), an antioxidant, was used as a positive control.

Percent of inhibition was calculated using the following formula:

$$\% \text{ Inhibition} = \frac{(\text{Absorbance of blank} - \text{Absorbance of Test sample})}{\text{Absorbance of blank}} \times 100$$

2.6 Anti-Arthritis test

A total of 0.5 mL of a reaction mixture including 0.45 ml of bovine serum albumin (5% aqueous solution), 0.05 mL of MESAL crude extract (62.5, 125, 250, 500, 1000 µg/mL), and 0.05 mL of aspirin (reference drug) were used. By adding 1 N HCl, the pH of each solution was adjusted to 6.3. After 20 minutes at 37°C, the samples were heated for 30 minutes at 57°C. After adding phosphate buffer (2.5 mL), the spectrophotometer reading was taken at 660 nm. Instead of using BSA in the product control, we utilized 0.05 ml of distilled water in the test control [9].

Protein denaturation's percent of inhibition was measured by using the following formula:

$$\% \text{ Inhibition} = 100 - \frac{(\text{Absorbance of blank} - \text{Absorbance of the Test sample})}{\text{Absorbance of blank}} \times 100$$

2.7 Anti-inflammatory test

5 mL of a reaction mixture was used, with 0.2 mL of egg albumin (from a fresh hen's egg), 2.8 mL of phosphate-buffered saline (PBS, pH 6.4), and 2 mL of MESAL at final concentrations of 62.5, 125, 250, 500, and 1000 µg/mL, respectively. The quantity of double-distilled water used as a control was almost the same. The mixtures were first heated at 70° C. for 5 minutes before being incubated at (37±2)° C. in a BOD incubator (Labline Technologies) for 15 minutes. They were then cooled, and their viscosity was measured using an Ostwald viscometer, while their absorbance was measured at 660 nm (SHIMADZU, UV 1800) using a vehicle as blank. As a standard for comparison, we measured the absorbance and viscosity of diclofenac sodium at the same concentration [10]. We used the following formula to determine the percentage of protein denaturation inhibition:

$$\%\text{Inhibition} = \frac{Abs\ of\ control - Abs\ of\ test\ sample}{Abs\ of\ control} \times 100$$

Here, Abs = Absorbance

2.8 Thrombolysis test

2.8.1 Blood Specimen

Human volunteers (n = 15) who had never taken oral contraceptives or blood thinners,from them 4 mL of whole blood from a vein was collected. This was done following a procedure

approved by the Institutional Ethics Council of Stamford University Bangladesh. A medical professional helped draw blood from a vein. After the blood was taken, 500 µL was put into each of the eight microcentrifuge tubes.

2.8.2 Statement on informed consent of the donors

The researchers provided the volunteer participants with a consent form that explained the objective of the investigation, as well as the title of the research project, the names of the investigators, and their contact information. A description of the research that includes the inclusion and exclusion criteria of the donors, whether or not the donors will receive any therapy, the volume of blood that will be taken, the potential discomfort of the puncture sites, and the amount of time that will be required for the blood sampling.

2.8.3 Thrombolysis

After expressing clot lysis as a percentage of total clot lysis, the experiments for clot lysis were carried out as described in the previously reported research paper [11]. In summary, 15 separate pre-weighed sterile microcentrifuge tubes (0.5 mL/tube) were filled with 2.5 mL of venous blood collected from healthy participants and incubated at 37°C for 45 minutes. After clot formation, serum was carefully drained out of the tubes without disturbing the clot, and the tubes were weighed again to calculate the clot weight (clot weight = clot-containing tube minus the weight of tube alone). Each microcentrifuge tube containing a weighted clot must be supplemented with 100 µL of MESAL. Lyophilized streptokinase (Incepta Pharmaceutical Ltd., Dhaka, Bangladesh) was reconstituted by adding 2.5 mL of PBS and mixing it well. As a positive control, 100 µL of this suspension was added to the microcentrifuge tube. A volume of 100 µL of distilled water was used as a negative control. After incubating the tubes at 37°C for 90 minutes, we checked for clot lysis. The tubes were weighed again after incubation to measure the weight change caused by the clot's breakup. The percentage of clot lysis was calculated by taking the difference in weight before and after clot lysis.

2.9 Antibacterial Test

2.9.1 Experimental microorganisms

The Department of Microbiology at Stamford University Bangladesh supplied pure cultures of Gram-positive (*Staphylococcus aureus, Bacillus megaterium*) and Gram-negative (*Klebsiella pneumonia, Escherichia coli, Pseudomonas aeruginosa*) bacterial pathogens.

2.9.2 Disk diffusion test

1 mL of each bacterial culture was spread out evenly over a solid growth medium in a Petri dish for the disk diffusion experiment [12]. Each agar plate had four sterile paper disks impregnated with 10 µL of the diluted MESAL put on top of it. These disks were 6 mm in diameter and were manufactured by Becton, Dickinson & Co. The plates were grown in a proper incubator for 24 hours. Different concentration (300,500,700µg/mL) of MESA was used for the test. Antibacterial activity was calculated by observing the size of the inhibition zone around a disk after 24 hours of incubation with bacteria [13]. Positive controls included a disk containing an antibiotic (Ciprofloxacin), whereas negative controls consisted of disks impregnated with methanol. In each concentration, duplicates were conducted.

2.10 In Vivo experiments

2.10.1 Neuropharmacological Activity

2.10.1.1 Hole cross test

Experiment subjects were confined in a 30 × 20 × 14 cm cage with a 3 cm diameter hole cut into the center of the divider [14]. The animals were given a vehicle, the drug, or MESAL before being released to pass through to the next compartment. Mice were observed for 3 minutes at 30, 60, 90, and 120 minutes after treatments, and the number of passages was recorded.

2.10.1.2 Open field test

The hole-board test was carried out in accordance with the methodology that had been described earlier by M. Moniruzzaman et.al [15], although with some minor adjustments. In

order to conduct this test, use a circular platform that was 60 x 30 cm in diameter and drilled 16 holes into it at regular intervals. In summary, 30 minutes after the vehicle or MESAL was administered, and 15 minutes after the diazepam was given, each animal was permitted to move freely on the platform, and the number of head dips into the holes was tallied over a period of 5 minutes.

3. RESULTS

3.1 Statistical analysis

All bioassay readings were conducted in triplicate, and the tabular data is provided as the mean standard deviation. Statistical analyses were conducted using Excel.

3.2Results of phytochemical identification test

A phytochemical investigation of MESAL revealed that it has phytochemicals including tannin, flavonoids, saponin, reducing sugars, alkaloids, gums, glycosides, carbohydratesand phenolics. Steroids was found to be absent in this test (**Table 1**).

Table 1. Results of qualitative phytochemical screening

Phytochemical constituent	MESAL
Flavonoid	++
Tannin	+
Alkaloid	++
Steroid	-
Saponin	+
Carbohydrate	+
Glycoside	++
Reducing Sugar	+
Gum	+
Phenolics	++

Here (++) indicates a higher amount, (+) indicates a moderate amount, and (–) indicates absence.

3.3 In vitro antioxidant activity

Antioxidant assays have demonstrated that MESAL has a moderate IC_{50} value of 11.73, whereas IC_{50} value of the standard drug ascorbic acid is 21.86.

Table 2. Results of antioxidant activity of MESAL by DPPH assay

Samples	Concentrations	% Inhibition	IC_{50} in DPPH radical scavenging analysis (µg/mL)
Ascorbic Acid	7.81	55.72	21.86
	15.625	59.67	
	31.25	70.57	
	62.5	73.70	
	125	81.60	
	250	88.55	
	500	93.86	
MESAL	7.81	52.52	11.73
	15.625	58.99	
	31.25	61.15	
	62.5	64.74	
	125	65.46	

	250	73.38
	500	88.48

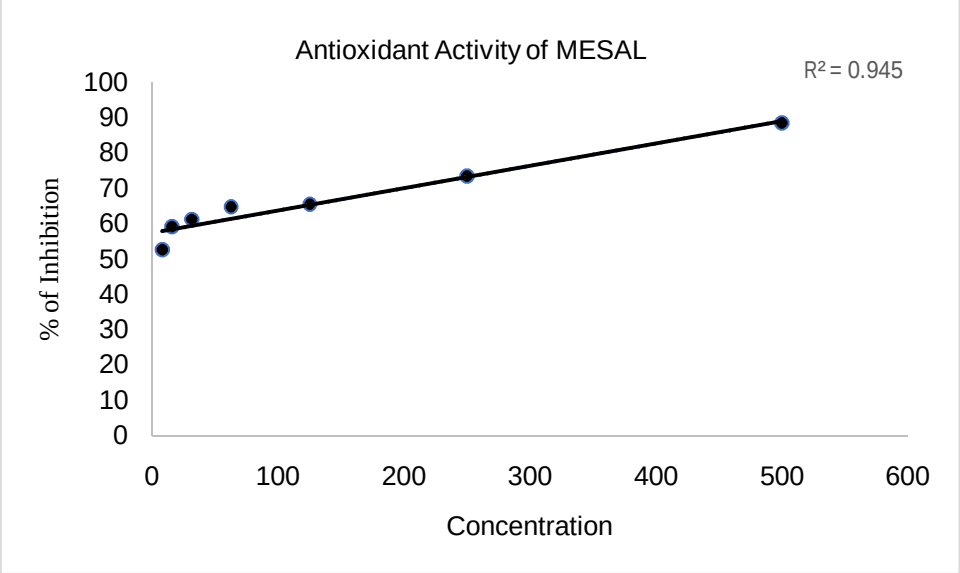

Figure 1: Graph of DPPH Scavenging activity of MESAL

3.4 In vitro anti-arthritic activity

According to **Table 3**, it has been revealed that the denaturation of BSA property is very significant compared to the standard drug

.

Table 3. Results of in vitro anti-arthritic tests.

Samples	Concentrations (µg/mL)	% of inhibition
Diclofenac Sodium	62.5	84.64
	125	87.38
	250	89.18
	500	93.69
	1000	94.59
MESAL	62.5	64.86
	125	70.27
	250	72.07
	500	81.08
	1000	90.99

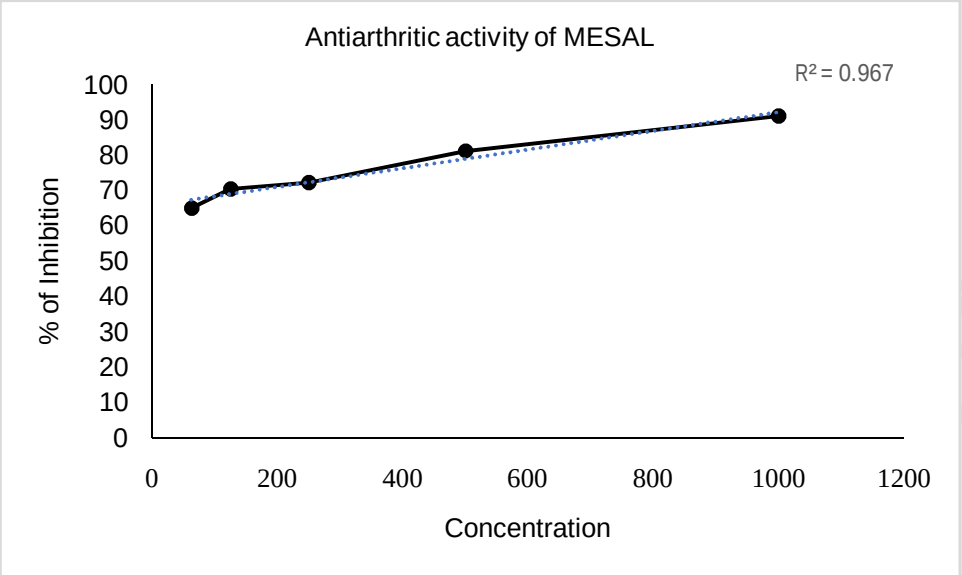

Figure 2. Graph of Antiarthritic activity of MESAL

3.5 In vitro anti-inflammatory activity

Table 4 reveals that there is a dose-dependent rise in the percentage of proteinase inhibition carried on by MESAL.

Table 4. Results of egg protein denaturation assay

Samples	Concentrations (µg/mL)	% of inhibition
Acetyl salicylic acid	62.5	20
	125	33.33
	250	66.67
	500	77.78
	1000	88.88
MESA	62.5	87.61
	125	89.54
	250	89.87
	500	90.51
	1000	93.03

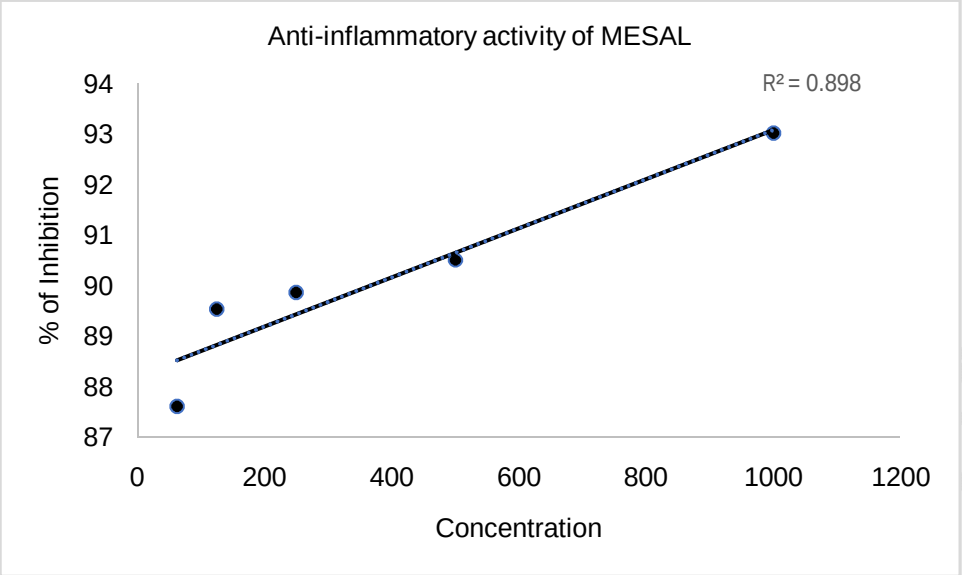

Figure 3. anti-inflammatory activity of MESAL

3.6 In vitro Clot lysis

Table 5.reveals that thrombolytic activity is significant. It is close to the standard equivalent. So, it is proved that MESAL can be used as a drug like plasmin which is an agent for reducing blood clots.

Table 5. Mean value of percent of clot lysis (N=15)

Sample	% of clot lysis
Negative control	7.296
Streptokinase	91.304%
MESAL	90.257

3.7 In vitro Antibacterial result

Table 6 demonstrated a moderate antibacterial activity compared to the standard drug.

Table 6. Antibacterial activity of MESAL

Diameter of Zone of Inhibition (mm)				
Test organisms	**MESAL (300 µg/disc)**	**MESAL (500 µg/disc)**	**MESAL (700 µg/disc)**	**Ciprofloxacin (50 µg/mL)**
Gram-Positive Bacteria				
Bacillus cereus	08	12	14	25
Staphylococcus aureus	10	13	15	26
Gram Negative Bacteria				
Escherichia coli	13	14	18	25
Pseudomonas aeruginosa	08	09	10	27
Klebsiella pneumonia	08	10	12	24

3.8 Neuropharmacological activity

3.8.1 Result of Hole cross test

Following a period of 30 mins, it was discovered that the mice started to sleep, and as a result, very little activity was seen. They continued to sleep even after the extract had been given to them for a period of 90 minutes. The findings of the open field test and the hole cross test demonstrated that the extracts caused a considerable reduction in locomotor activity. During the second observation (60 minutes), the impact of reducing locomotor activity was noticeable for both dosages of 200 mg/kg and 400 mg/kg body weight. This effect was maintained through the third and fourth observation periods (90 and 120 minutes, respectively). In addition to that, the validation of anxiety was performed by evaluating outward indicators using hole-cross tests.

Table 7. The primary data table for the Hole Cross Test for the MESAL

The mean value of the crossed-hole number				
Sample	**30 min**	**60 min**	**90 min**	**120 min**
Control	23.8	24.4	23.8	19.4
Diazepam	9.67	6.67	3.67	2.33
MESA (200mg/kg)	9.4	2.4	1.6	0.8
MESA (400 mg/kg)	8.9	1.6	0.4	0.0

3.8.2 Result of open field test

According to the results of an open field test, the depressant effect of the extracts was obser vable in the test animals as early as the second observation period at both dosages of 200 mg/kg and 400 mg/kg body weight.

Table 8. The primary data table for the open field test for both MESAL

Number of movements (mean value)				
Sample	**30 min**	**60 min**	**90 min**	**120 min**
Control	89.6	62	36.2	22.8
Diazepam	87.2	39	30.8	18.8
MESA (200mg/kg)	71.6	44.2	34.6	21.8
MESA (400 mg/kg)	62.8	37.8	26.4	11.6

3.9 DISCUSSIONS

The current research examined the effects of a methanol extract of MESAL's leaves on a variety of pharmacological tests, including antioxidant, anti-arthritic, anti-inflammatory, thrombolytic, antibacterial, and in-vivo neuropharmacological activities.

Previous research has revealed the phytochemical properties of MESAL[5]. The antioxidant potential of a compound may be reflected in its free radical scavenging activity, which is directly proportional to the concentration of polyphenolics present in the compound [16]. It has been revealed that the existence of reductones is usually linked to the reducing characteristics and that these reductones work as antioxidants by donating a hydrogen atom to break the chain of free radicals [17]. Antioxidant activity in MESAL may be due to the high levels of phenolics and flavonoids identified in the plant during phytochemical analysis. The IC_{50} value of MESAL is 11.76 whereas the IC_{50} value of ascorbic acid is 21.86. The percent inhibition of MESAL is dose-dependent. The highest percent inhibition value of MESAL is 88% with a concentration of 500 µg/mL which has been mentioned in **Table 2**.

Joint diseases such as pannus formation, severe cartilage and bone degradation, and leukocyte infiltration are characteristics of rheumatoid arthritis. The efficacy of traditional therapy for this disease has increased in recent years. Drugs including sulphasalazine, hydroxychloroquine, leflunomide, and methotrexate, as well as NSAIDs like etoricoxib and corticosteroids like methylprednisolone and prednisolone, have all been linked to unwanted side effects [18]. Current research suggests that phenolic compounds are responsible for the anti-arthritis effect [19]. This research indicates that MESAL has the highest protein

denaturation value of 90.99% while the concentration is 1000 µg/mL whereas Diclofenac sodium's value with the same concentration is 94.59% (**Table3.**). Due to its significant protein inhibition activity, it can be used as an anti-arthritic drug's active ingredient after isolating its compounds through Colum chromatography in the future.

When proteins are exposed to an outside force or substance, like a strong acid or base, a concentrated inorganic salt, an organic solvent, or heat, they lose their tertiary structure and secondary structure. This process is called denaturation. Most proteins stop being useful in biology when they lose their original shape. When tissue proteins lose their shape, this is a well-known cause of inflammation and arthritis [21]. Most research has been done on alkaloids, polyphenols, terpenoids, and flavonoids like anthocyanin and flavone because they have antioxidant and anti-inflammatory properties [20]. These phytochemicals are abundant in plants that have been used as sources of strong anti-inflammatory drugs. This research demonstrates that MESAL has a higher anti-inflammatory effect with a value of 90.51% in the concentration of 1000 µg/mL (**Table 4.**). The current research shows that crude methanolic extract of *Solanum Americanum Mill*leaves has significant anti-inflammatory activities, likely associated with the suppression of the release of mediators of the inflammatory process such prostaglandins, histamine, and neutrophils. So, it can be said that in the future the compound of *Solanum Americanum Mill* can be used as one of the NSAIDs due to its significant effect.

There have been several investigations carried out by a variety of researchers to determine which herbs, natural food sources, and their supplements have an antithrombotic (anticoagulant and antiplatelet) effect, and there is evidence that indicates that consumption of such foods leads to the prevention of coronary events and strokes [21][22][23]. Several of these thrombolytic medicines have undergone further modification via the use of recombinant technology to make them more potent and site-specific. There have been reports of adverse reactions to these medications, which may result in further difficulties [24]. In some cases, patients can die from complications like bleeding and embolism [25][26]. Many investigations have been conducted by different groups of researchers to identify novel herbs and natural foods and their supplements with antithrombotic activity (anticoagulant and antiplatelet) and establish that eating such food leads to the prevention of coronary events and stroke. In our research, it has been found that the clot lysis of MESA is higher compared to the standard drug Streptokinase with the value of 90.257 (**Table 5.**). This significant effect observed may be due to the crude extract being discovered to include tannin, alkaloid, and saponin during phytochemical screening; these phytochemicals have been suggested to be responsible for the extract's clot-lysis effect [27].

Most of the time, it appears that gram-positive bacteria are more severely affected by the inhibitory actions of plant extracts that are gram-negative bacteria. This is most evident with gram-positive bacteria. Gram-positive bacteria are thought to be more susceptible to infection because their cell walls only consist of a single layer. Cell walls of gram-negative bacteria, on the other hand, are multilayered and structurally more complex[28]. According to the results of this research, the dose had an effect on the zone of inhibition for both gram-positive and negative bacteria. As the dose is increased, the zone of inhibition also increases to a greater degree (**Table 6.**).

In this research, some neuropharmacological effects of MESAL were investigated, and it was discovered that it had antidepressant-like activities. The locomotor score for diazepam was found to be significantly lower than that of the control animals in previous studies. A reduction in locomotor activity is indicative of a sedative impact since it is seen as an indicator of alertness [29]. The intensity of locomotor activity is a measure of the excitability of the central nervous system as well as the sedation that results from depression of the central nervous system [30]. According to the results of the open field and hole cross tests, the extract produced a considerable reduction in the amount of locomotor activity that was present in the animals. The findings were also depending on the dosage being used and were statistically significant which has been demonstrated in **Table 7.** and **Table 8**.

Nevertheless, more research is required in order to identify the precise Phyto-constituents and mechanisms of action that are accountable for the biological activities shown by the methanol extract of *Solanum americanum Mill*. Fractionating the extracts to isolate the active ingredients should be the focus of future research. Further research on the plants' potential anticancer effects should include testing them in a variety of cancer cell lines.

4. CONCLUSION

The results of this investigation showed that MESAL contains significant antioxidant properties. The results of this investigation corroborate the ethnomedical claims claimed about MESAL, demonstrating its anti-arthritic, anti-inflammatory, thrombolytic, and antibacterial properties. There is substantial evidence that this plant's methanolic leaf extract also has neuropharmacological effects. Hence, phytochemicals including alkaloids, tannins, flavonoids, glycosides, phenol, and so on may be responsible for these pharmacological effects; these constituents were discovered by phytochemical screening. In the future, GC-MS analysis, column chromatography, and nuclear magnetic resonance (NMR) might help to determine which individual phytochemical is responsible for these pharmacological actions.

CONSENT

All authors declare that 'written informed consent was obtained from the patient for publication of this research report.

ETHICAL APPROVAL

The International Conference on Harmonization, the Declaration of Helsinki, and US Food and Drug Administration regulations were all adhered to in this study. The study protocol and informed consent form were reviewed and approved by the Faculty of Biological Science, University of Dhaka (approved number: 111). All participants in the research had to sign a written agreement form in order to participate, and they were free to leave at any time.

REFERENCES

[1] Q. Shi, L. Li, C. Huo, M. Zhang, and Y. Wang, "Study on natural medicinal chemistry and new drug development.," *Zhongcaoyao= Chinese Tradit. Herb. Drugs*, vol. 41, no. 10, pp. 1583–1589, 2010.

[2] D. S. Fabricant and N. R. Farnsworth, "The value of plants used in traditional medicine for drug discovery.," *Environ. Health Perspect.*, vol. 109, no. suppl 1, pp. 69–75, 2001.

[3] X. M. Gao, T. M. Zhang, J. R. Zhang, J. S. Guo, and G. S. Zhong, "Chinese materia medica," *Beijing China Press Tradit. Chinese Med.*, vol. 323, 2007.

[4] R. R. N. Alves and I. M. L. Rosa, "Biodiversity, traditional medicine and public health: where do they meet?," *J. Ethnobiol. Ethnomed.*, vol. 3, pp. 1–9, 2007.

[5] H. Usman, V. Victor, and I. Waziri, "Qualitative Phytochemical Screening and in Vitro Antimicrobial Activities of Solanum Americanum Mill," *J. Eng. Technol. Environ.*, vol. 14, no. 1, pp. 104–110, 2018.

[6] N. Joshi and D. C. Joshi, "REVIEW ON RESPONSIBLE MEDIATORS AND MECHANISM OF ACTION INVOLVED IN ANTI INFLAMMATORY ACTION OF," no. January, 2021, doi: 10.20959/wjpps202112-20823.

[7] V. Duraipandiyan, M. Ayyanar, and S. Ignacimuthu, "Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamil Nadu, India," *BMC Complement. Altern. Med.*, vol. 6, 2006, doi: 10.1186/1472-6882-6-35.

[8] D. R. Sheel, K. Nisha, and P. J. Kumar, "Preliminary Phytochemical Screening of

Methanolic Extract Of Clerodendron infortunatum," *IOSR J. Appl. Chem.*, vol. 7, no. 1, pp. 10–13, 2014, doi: 10.9790/5736-07121013.
[9] Alamgeer, A. M. Uttra, and U. H. Hasan, "Anti-arthritic activity of aqueous-methanolic extract and various fractions of Berberis orthobotrys Bien ex Aitch," *BMC Complement. Altern. Med.*, vol. 17, no. 1, pp. 1–16, 2017, doi: 10.1186/s12906-017-1879-9.
[10] S. Chandra, P. Chatterjee, P. Dey, and S. Bhattacharya, "Evaluation of in vitro anti-inflammatory activity of coffee against the denaturation of protein," *Asian Pac. J. Trop. Biomed.*, vol. 2, no. 1 SUPPL., pp. S178–S180, 2012, doi: 10.1016/S2221-1691(12)60154-3.
[11] M. K. Umesh, C. B. Sanjeevkumar, H. B. Nayaka, and R. L. Londonkar, "Evaluation of in vitro anti-thrombolytic activity and cytotoxicity potential of Typha angustifolia l leaves extracts," *Int. J. Pharm. Pharm. Sci.*, vol. 6, no. 5, pp. 81–85, 2014.
[12] A. Klančnik, S. Piskernik, B. Jeršek, and S. S. Možina, "Evaluation of diffusion and dilution methods to determine the antibacterial activity of plant extracts," *J. Microbiol. Methods*, vol. 81, no. 2, pp. 121–126, 2010, doi: 10.1016/j.mimet.2010.02.004.
[13] C. Valgas, S. M. De Souza, E. F. A. Smânia, and A. Smânia, "Screening methods to determine antibacterial activity of natural products," *Brazilian J. Microbiol.*, vol. 38, no. 2, pp. 369–380, 2007, doi: 10.1590/S1517-83822007000200034.
[14] B. D. GUPTA, P. C. DANDIYA, and M. L. GUPTA, "a Psycho-Pharmacological Analysis of Behaviour in Rats," *Jpn. J. Pharmacol.*, vol. 21, no. 3, pp. 293–298, 1971, doi: 10.1016/s0021-5198(19)36218-3.
[15] M. Moniruzzaman, M. Atikur Rahman, and A. Ferdous, "Evaluation of sedative and hypnotic activity of ethanolic extract of Scoparia dulcis Linn.," *Evidence-based Complement. Altern. Med.*, vol. 2015, 2015, doi: 10.1155/2015/873954.
[16] A. S. V. C. Rao, S. G. Reddy, P. P. Babu, and A. R. Reddy, "The antioxidant and antiproliferative activities of methanolic extracts from Njavara rice bran," *BMC Complement. Altern. Med.*, vol. 10, 2010, doi: 10.1186/1472-6882-10-4.
[17] R. Naz and A. Bano, "Phytochemical screening, antioxidants and antimicrobial potential of Lantana camara in different solvents," *Asian Pacific J. Trop. Dis.*, vol. 3, no. 6, pp. 480–486, 2013, doi: 10.1016/S2222-1808(13)60104-8.
[18] M. Manan *et al.*, "Antiarthritic potential of comprehensively standardized extract of alternanthera bettzickiana: In vitro and in vivo studies," *ACS Omega*, vol. 5, no. 31, pp. 19478–19496, 2020, doi: 10.1021/acsomega.0c01670.
[19] P. Sivasakthi, E. Sanmuga Priya, and P. Senthamil Selvan, "Molecular insights into phytochemicals exhibiting anti-arthritic activity: systematic review: John Di Battista," *Inflamm. Res.*, vol. 70, no. 6, pp. 665–685, 2021, doi: 10.1007/s00011-021-01471-0.
[20] J. Fang, "Bioavailability of anthocyanins," *Drug Metab. Rev.*, vol. 46, no. 4, pp. 508–520, 2014, doi: 10.3109/03602532.2014.978080.
[21] H. Disease, "ArticleThe Effect of Fruit and Vegetable Intake on Risk for Coronary Heart Disease Kaumudi," 2016.
[22] S. Liu *et al.*, "Fruit and vegetable intake and risk of cardiovascular disease: The Women's Health Study," *Am. J. Clin. Nutr.*, vol. 72, no. 4, pp. 922–928, 2000, doi: 10.1093/ajcn/72.4.922.
[23] C. McGraw, "Fruit and vegetable intake and risk of cardiovascular disease in US adults: the first national health and nutritional examination survey epidemiologic follow-up study," *Prim. Heal. Care*, vol. 13, no. 6, pp. 8–8, 2003, doi: 10.7748/phc.13.6.8.s11.
[24] M. Verstraete, "Third-generation thrombolytic drugs," *Am. J. Med.*, vol. 109, no. 1, pp. 52–58, 2000, doi: 10.1016/S0002-9343(00)00380-6.
[25] A. S. Gallus, "Thrombolytic therapy for venous thrombosis and pulmonary embolism," *Baillieres. Clin. Haematol.*, vol. 11, no. 3, pp. 663–673, 1998, doi: 10.1016/S0950-3536(98)80088-7.

[26] J. Wardlaw, E. Berge, G. Del Zoppo, and T. Yamaguchi, “Thrombolysis for acute ischemic stroke,” *Stroke*, vol. 35, no. 12, pp. 2914–2915, 2004, doi: 10.1161/01.STR.0000147964.46510.25.
[27] S. Masudur, R. Dewan, and A. Das, “Investigation of in Vitro Thrombolytic Potential and Phytochemical Nature of Crinum Latifolium L. Leaves Growing in Coastal Region of Bangladesh,” *Int. J. Biol. Pharm. Res.*, vol. 4(1), no. 1, pp. 1–7, 2013.
[28] A. B. Yildirim, F. P. Karakas, and A. U. Turker, “In vitro antibacterial and antitumor activities of some medicinal plant extracts, growing in Turkey,” *Asian Pac. J. Trop. Med.*, vol. 6, no. 8, pp. 616–624, 2013, doi: 10.1016/S1995-7645(13)60106-6.
[29] V. D. Thakur and S. A. Mengi, “Neuropharmacological profile of Eclipta alba (Linn.) Hassk,” *J. Ethnopharmacol.*, vol. 102, no. 1, pp. 23–31, 2005, doi: 10.1016/j.jep.2005.05.037.
[30] K. P. Thirupathy, A. Tulshkar, and C. Vijaya, “Neuropharmacological activity of Lippia nodiflora Linn.,” *Pharmacognosy Res.*, vol. 3, no. 3, pp. 194–200, 2011, doi: 10.4103/0974-8490.85007.