Original Research Article

TITLE:ASSESSMENT OF PHARMACOLOGICAL ACTIONS OF *BRASSAIOPSIS HAINLA* LEAVES IN THE CONSTRAINT OF HELMINTHS, THROMBOLYTIC, CYTOTOXIC, DIARRHEA, PYREXIA: IN VIVO, IN VITRO APPROACHES.

Comment [a1]: The scientific term is written in italics.*Brassaiopsishainla*

ABSTRACT:

Aims: This study is intended to evaluate the methanol extract of Brassaiopsishainlaleaves (MEBH) in the parameter of helminths, thrombolytic, cytotoxic, diarrhea&pyrexia by using In vivo and in vitro tactics.

Comment [a2]: The scientific term is written in italics. *Brassaiopsishainla*

Methodology: In vivo tests, including those for anti-diarrheal and antipyretic effects, were conducted using mice that were orally dosed with MEBH, while in vitro tests were conducted using aquarium worms, human blood, and brine shrimp for a specific purpose, such as anti-helminthic analysis, clot lysis analysis, and cytotoxicity.

Results: The anthelmintic potential of MEBH was demonstrated in a dose-dependent approach on aquatic worms (Tubifex Tubifex) at different concentrations. Still, the effective one is MEBH 100 (µg/ml) = 29.749 ± 1.444 times of paralysis, 15.256 ± 6.118.The MEBH demonstrated a high level of thrombolytic activity at 48.358% of clot lysis.Compared to negative control but lower than standard drug.Following 24 hours, the LC_{50} was assessed in a brine shrimp lethality test. The LC_{50} value of the extract was 328.02 µg/mL, which is relatively toxic to brine shrimp, predominantly when associatedwith the LC_{50} value of 3.8 µg/mL for typical vincristine sulfate. In the research examining its efficacy towards diarrhea, Gastrointestinal motility, and peristalsis indexes among 200 and 400 mg/kg were identified to be significantly ($P < 0.005$) hindered by MEBH. The highest diminution in fever elicited by MEBH at 400 mg/kg demonstrates its promising antipyretic potential.

Conclusion: Our study confirmed that MEBH possesses anthelmintic, antipyretic, cytotoxic, thrombolytic, and anti-diarrheal effects, which makes it an intriguing source for innovative treatments.

Keywords: Anthelmintic, Anti-diarrheal, Brassaiopsishainla (MEBH),Brine shrimp lethality bioassay, methanolic extract, Thrombolytic.

1 Introduction

A majority of the current drugs used to treat a wide range of chronic and severe illnesses were first isolated from plants that humans discovered. Allopathy has been largely supplanted by natural medicine in the treatment of numerous ailments as of late because of its apparent therapeutic efficacy. This is true both in developed and emerging nations. Such mainstream medications are used for primary healthcare due to their broad biological and medicinal pursuits, increased safety, and cheap costs. *Brassaiopsishainla* is a plant of the Araliaceae family used for medicinal purposes. Boiled blossoms and leaves are consumed against hypertension& leaf decoction is administered for urinary problems.Originates from monsoonal forests in Asia. Large palmate papery leaves are held on spiny stems and branches. Many medicinal plants are utilized in traditional medicine. These medicinal plants can be utilized to make breakthrough medicines. Active ingredients in many medications come from plants. Medical plants offer several benefits for treating the ailment. All plant components interact instantaneously.

Consequently, symbiotic medicine is based on the premise that some medications can increase or harm the effects of others or even negate their potential adverse effects. Defending medical status quo Plant-based medications can aid in cancer treatment. Plants are used in defensive medicine to prevent disease transmission. This will reduce the necessity for chemical therapy or the detrimental consequences of synthetic remedies if the disease has already shown itself**[1]**. Bangladesh possesses more than a thousand plant species, giving its medicinal plants a rich source of pharmacologically active traits**[2]**.

Most Bangladeshis reside in rural, hilly areas. Despite this, woodlands, meadows, roadsides, orchards, and streams are full of potential remedies. This underscores the need to analyze

indigenous flora used in traditional treatments for diverse biochemical pathways, which may lead to new therapies. *Brassaiopsishainla*leaves had the crudest fiber (39.44%)**[3]**.

Parasitic nematodes, trematodes, cestodes, and other larval insects can infest persons and other animals, causing bronchitis, malnutrition, eosinophilia, anemia, and much more. It's called helminthiasis.It exacerbates social and economic issues in the regions**[4]**.Nonetheless, anthelmintic resistance in parasitic worms has become a major issue throughout the developing world as a result of negligent anthelmintic administration. In terms of public health, this is a significant issue**[5]**.

Thrombolytic medications convert the precursor protein, plasminogen, to the active form, plasmin, which then dissolves the fibrin mesh. As a direct consequence, the clot dissolves, and the normal flow of blood is restored**[6]**.

Diarrhea generates intestinal emptying, including stool water retention. A substantial rate of excretion is indicated if a person defecates more than three times per day, has squishy or liquid feces, or perhaps both**[7]**. In developing countries, the primary factors of diarrhea are bacterial infections triggered by microorganisms, including Salmonella, Escherichia coli, Vibrio cholera, and Shigella**[8]**.And although though there is a broad selection of treatment choices available for diarrhea, the majority of individuals in third-world nations still resort to herbal medicines as their preferred method of diarrhea management**[9-11]**.

Infection, tissue damage, inflammation, transplant rejection, malignant tumors, or other pathologies can cause hyperpyrexia. Pro-inflammatory mediators, such as interleukin 1β, α, β and TNF-α, are often released by infected or injured tissue, and these cytokines stimulate the manufacture of prostaglandin E2 (PGE2) in the preoptic hypothalamus region, causing the hypothalamus to boost the physique's temperature**[12]**.

The investigation aims to determine the extract's capabilities. Anthelmintic research examines a material's ability to paralyze or kill parasitic worms and other parasites. A thrombolytic test lyses blood clots in the arteries. The cytotoxic test determines an effective dose of a drug or plant extract. Screening for anti-diarrheal and antipyretic properties determines whether the chemical can alleviate diarrhea and lowering body temperature. In developing Bangladesh, indigenous plant therapies are essential. Several ailments were treated using plants**[13-15]**.*Brassaiopsishainla*is one of these uninvestigatedplants. It's crucial to find new drugs for ailments.For its potential health benefits, this plant was considered.

2 MATERIALS AND METHODS

2.1 Chemicals and drugs:

Tween 80, Aspirin, Ethylenediaminetetraacetic acid (EDTA), Loperamide, castor oil, activated charcoal, sodium chloride, and phosphate were purchased from Sigma Chemical Co. (St. Louis, MO, USA), and Albendazole 10 mg/ml, streptokinase (Lyophilized Alteplase) vial of 15,00,000 IU & vincristine sulfate (1 mg/vial) was accumulated from Beacon Pharma Ltd., Bangladesh. A specified term referring to components was utilized in this study effort: a systematic grade chemical.

2.2 Collection and identification of the plant

A local expert helped preference the plant from Shitakundu (Chittagong Hill Tracts Area) in December 2021. Bangladeshi Botanist and taxonomic expert Dr.SheikhBakhtearUddin has achieved the correct identification.

2.3 Preparation of plant material

These plant specimens are usually taken from very different environments. Afterward, they are reduced in size so they may be ground up. With the use of a grinder, the components were reduced to a stiffprecipitate and then placed in an airtight container for future usage.

2.4 Extraction

For 14 days, the leaves were steeped in a diffidentvolume of methanol at standard room temperature. They were infrequently shaking and stirring. Through the use of a cotton plug as well as some Whitman filter paper, the fluid was filtered (primary). A slurry or viscous mass was generated by evaporating the solvent with water at 60°C to remove the solvent from the mixture. At room temperature, a ceiling fan was used to dry off the thick mass of matter. The prepared quotation was kept in the database for future pharmacological evaluation.

Comment [a3]: ?????

2.5 Experimental animal

Jahangirnagar University's pharmacy animal facility in Dhaka, Bangladesh, provides the animals for this experiment. The Swiss albino mice were retained in a natural 12-hour light-to-dark cycle in the same animal housing. It took a week for each animal to become used to the new surroundings. The animals were maintained at a constant temperature of 25°C in a well-ventilated enclosure. They were given the bare necessities, such as pellets and safe drinking water. The use and care of animals wereaccomplished per the guidelinesofthe National Institutes of Health.

2.6 Standardization and Quality Control of the Extract

Accreditation of the methanol extract of *Brassaiopsishainla* (MEBH) was accomplished, and eminence and physiochemical control of the crude extract was carried out in order to secure the effectiveness of the investigation of the acute toxicity of animal models.

2.7 Phytochemical screening

The phytochemical analysis of the methanol extract*Brassaiopsishainla* (MEBH) leaves and stems carried out the standard method to evaluate the alkaloid, carbohydrate, glycosides, flavonoid,tannins, steroid, saponins, phenols, protein, cholesterol, gum test & tannic acid**[16-18].**

2.8 Anthelmintic Activity

We followed, with some minor adjustments, the procedure for assessing the anthelmintic efficacy of crude extracts as described by Ajaiyeoba et al**[19]**. The aquarium worm classified as TubifexTubifex was chosen for this research to evaluate the feasibility of its utilization as an anthelmintic since it shares the same anatomical traits and is representative of the same family as intestinal worms. This experiment's sludge worm was acquired from an aquarium store in Chittagong and was subsequently collected for use. The investigation was carried out with several separate groups, one of which contained the standard drug Albendazole at a concentration of 10 (µg/ml), while the other condensed crude extracts at trace levels of 10, 25, 50, and 100 (µg/mL), in ascending order. In order to carry out this experiment, ten to twelve worms were inserted in each Petri dish, which was then divided into one of five distinct groups. The Petri dish was then given an additional 3 mL of each of the varied concentrations that had been determined for the groups. The initial time, the time the worms became paralyzed, and the moment they dropped dead were all carefully observed and recorded. The time at which the worms became paralyzed and the time at which they died were taken into consideration when determining the anthelmintic activity of this trial. When the worm's motion cannot be detected after vigorously trembling it, the paralytic time and the mortality period are considered by the authorization of the worm's mobility either after wobbling or when immersed in slightly hot water. This is carried out to determine when the worm died.

Comment [a4]: ????

Comment [a5]: in vitro

2.9 Thrombolytic Activity

The in vitro thrombolytic assay was carried out through the use of a streptokinase vial in compliance with the methodology described byHasanat, A. et al.**[20]**.After drawing 0.5 ml of venous blood from healthy participants, their blood was deposited in ten microcentrifuge tubes that had been sterilized & weighed in anticipation. For clot formation, each tube was incubated for forty-five minutes at a temperature of 37°C. After the clot had utterly formed, a small volume of serum was carefully withdrawn from each test tube to prevent disrupting the clot. The clot was weighed beforehand, and afterward, 100 µl of methanol extract with a 10 mg/ml concentration was additional to it. Within the positive control group, there was an addition of 100 µL of streptokinase; within the negative control group, there was an addition of 100 µL of distilled water. Clot lysis necessitated a further incubation of the tubes at 37°C for one hour and ninety minutes. After the expulsion of the fluid, an additional measurement was taken to determine the weight of the clot, and the difference in weight was computed.The percentage of clot lysis was figured using the formula:

$$\%\,of\ clot\ lysis = \left(\frac{Weight\ of\ clot\ after\ removing\ the\ fluid}{Weight\ of\ clot} \right) \times 100$$

This human-related experiment was conducted rendering to the ethical morals laid down in the 1964 Declaration of Helsinki. Department of Pharmacy at International Islamic University Chittagong, Bangladesh, gave their signature of approval to this study's methodology. (Ref. Number: IIUC/PHARM-AEC-150/10-2022).

2.10 Brine Shrimp Lethality Bioassay

The technique developed by Alam et al. was utilized to evaluate the cytotoxic characteristics of MEBH, despite a few minor adjustments implemented**[21]**. To determine the level of toxicity, it was tested on Artemia salina leaches, also known as brine shrimp eggs. Artificial saltwater was created by extensively combining seawater (38 g/L) with 1N NaOH to a pH of 8. Then, the mixer was used to hatch shrimp eggs at room temperature with a steady source of oxygen. The nauplii, or shrimp larvae, emerged from eggs in about two days. In order to produce a range of concentrations from 31.25 to

1000 µg/ml, the crude extract was diluted in DMSO (5 mg/mL) with artificial seawater and utilized as a test sample. Following the same protocol as the previous experiment, vincristine sulfate was deployed as a positive control at doses ranging from 0.125 µg/ml to 10 µg/ml. Each test vial comprised ten nauplii that were maintained at room temperature and in the light for 24 hours. As immediately as the incubation period ended, an amplifying glass was used to count the number of surviving nauplii in each container. The mortality percentage of nauplii was figured out according to the equation:

$$Percentage(\%) of\ mortality = \frac{N_{\circ} - N_1}{N_{\circ}} \times 100$$

where, N_0 number of nauplii taken and $N_1$the number of nauplii alive.

2.11 Anti-diarrheal activities

a. Castor oil-induced diarrhea

The technique is accountable for the anti-diarrheal actions carried out byNwodo, O. and E. Alumanah**[22]**. Before commencing the test, the animals in the experiment were starving for a period of twenty-four hours. The mice were split equally into ten separate categories, each with a count of five. Both a negative control (1% Tween-80 solution given orally at 10 mL/kg b.w.) and positive control (loperamide administered orally at 5 mg/kg b.w.) were conducted in this experiment. Plant extract (MEBH) was treated orally with gavage at dosages of 200 and 400 mg/kg of body weight. The route of administration was oral. Oral delivery of 0.5 mL of castor oil was performed precisely one hour after the initial injection, and the animals were confined in separate cages with adsorbent paper underneath individuals. Every hour for 4 hours, the feces of each mouse were counted and inspected, and they were replaced every hour on the hour. The equation considered the level of % inhibitionof defecation:

$$\%\, inhibition\, of\ defecation = \frac{A - B}{B} \times 100$$

Where A = average eradication feces number of the control group;

B = average eradication of feces number in the test group

b. Castor oil-induced gastrointestinal motility by charcoal marker

This gastrointestinal motility experiment was supported by the method described byMascolo, N., et al**[23]** with the treatment of animals of each group (n = 5) as described in the castor oil-induced diarrhea. Animals were given a therapy of 1 mL of the charcoal meal (consisting of 10% charcoal in 5% gum acacia) one hour after the treatment group was given to the animals. This treatment was performed orally on each mice. Following the provision of charcoal for a period of one hour, the animals were then executed. A calculation was made to calculate the distance traveled by a charcoal meal from the pylorus to the caecum, and the results were reported as a proportion of the entire length of the gut. The following formulations express the percentage of inhibition and the Peristalsis index.

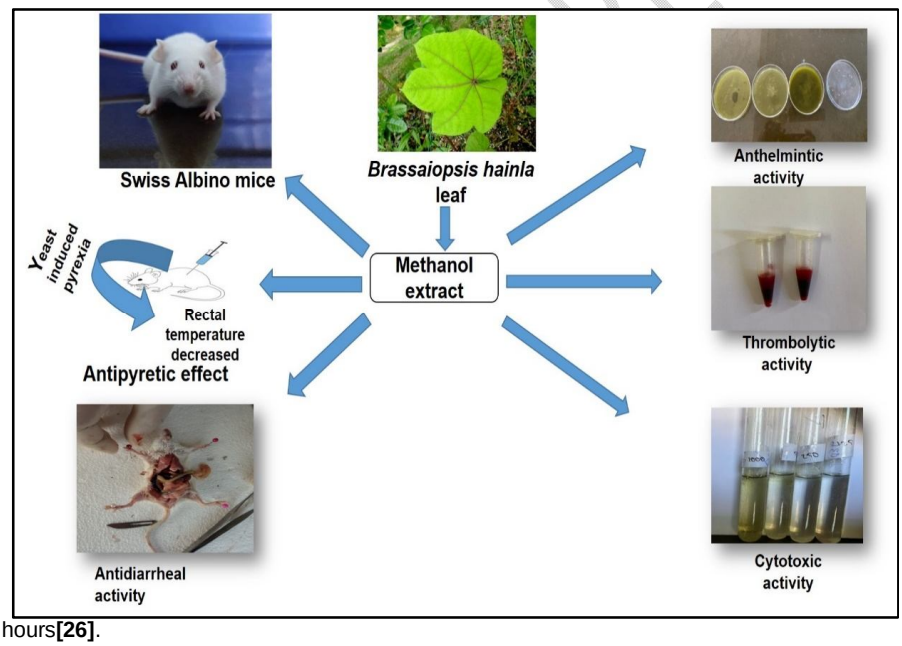
$$\%\,of\ inhibition = \frac{Distance\,travel\,by\,the\,control\,(cm) - Distance\,travel\,by\,the\,test\,group\,(cm)}{Distance\,travel\,by\,the\,control\,(cm)} \times 100$$

2.12 Statistical analysis:

The results were articulated as Mean ± SEM. One-way ANOVA with Dunnett's test was used for this experiment. GraphPad and Microsoft Excel were used for the statistical and graphical evaluations. The *P* = 0.05 were considered to be statistically significant**[24]**.

2.13 Antipyretic activity test:

Brewer's yeast-induced fever in tentative animals was used as the paradigm for determining antipyretic efficacy**[25]**. The infusion of 10 milliliters of a 20% aqueous yeast solution per kilogram of body weight subcutaneously resulted in the induction of hyperpyrexia. Before participating in the trials, the animals who were chosen went without food for one whole day but had access to water on demand. An Ellab thermometer was used to capture the first rectal temperatures of the animals. After 18 hours of subcutaneous treatment, animals were chosen for the antipyretic activity based on the extent to which they showed an increase of 0.3–0.5º degrees Celsius in their rectal temperature. Oral administration of several plant extracts at a dose of 400 mg/kg body weight was compared to oral administration of paracetamol at a dose of 150 mg/kg body weight as a reference medication. The only substance that the Control group got was 10 milliliters of distilled water per kilogram. After therapy, the patient's rectal temperature was taken once per hour for the next four



hours**[26]**.

Fig 1: Pharmacological activities of MEBH leaves extract (In vivo, in vitro approaches)

3 RESULTS

Qualitative phytochemical screening the qualitative phytochemical analysis of a methanolicextract of *Brassaiopsishainla* (MEBH)leaves showed the existence of alkaloids, glycosides, flavonoids, steroids, saponins, and protein inall extracts. The phytochemical analysis in a qualitative manner is summarized in Table 1.

TABLE 01. PROPORTIONAL PHYTOCHEMICAL SCREENING OF *BRASSAIOPSIS HAINLA* (MEBH)

Test Name	MEBH
Alkaloid	+
Carbohydrate	–
Glycosides	+ +
Flavonoids	+ +
Tannins	–
Steroid	+
Saponins	+
Phenol	–
Protein	+
Cholesterol	–
Tannic acid	–

Here, '+ +' or '+': present; '–': absent.MEBH: Methanolic extract of *Brassaiopsishainla*.

3.1 Anthelmintic Effect

The anthelmintic activity of MEBH was investigated on Tubifex worms, whose findingsare concise in Table 2 and figure 2. In this investigation, at the 10, 25, 50, and 100 µg/ml concentrations, the extract manifested significant paralysis time 33.194 ± 2.949, 31.590 ± 2.201, 30.400 ± 1.650, 29.749 ± 1.444 min& death time 35.936 ± 2.760, 27.634 ± 6.476, 21.005 ± 6.794, 15.256 ± 6.118 min respectively. In contrast, the standard drug Albendazole10 (µg/ml) showed paralysis and death times 29.932 ± 1.426 and 10.273 ± 4.791 min, respectively, at 10µg/ml. The result indicated that the effect of anthelmintic was directly proportionate to the concentrations of crude extract.

Comment [a6]: How do you calculate the fractions of minutes in death?

TABLE 02. THE EFFECT OF MEBH LEAVES ON TUBIFEX WORM

Treatment &Concentration	Timetaken for paralysis (minute)	Timetaken for death (minute)
MEBH 10 (µg/ml)	33.194 ± 2.949	35.936 ± 2.760

MEBH 25 (µg/ml)	31.590 ± 2.201	27.634 ± 6.476
MEBH 50 (µg/ml)	30.400 ± 1.650	21.005 ± 6.794
MEBH 100 (µg/ml)	29.749 ± 1.444	15.256 ± 6.118
Albendazole 10 (µg/ml)	29.932 ± 1.426	10.273 ± 4.791

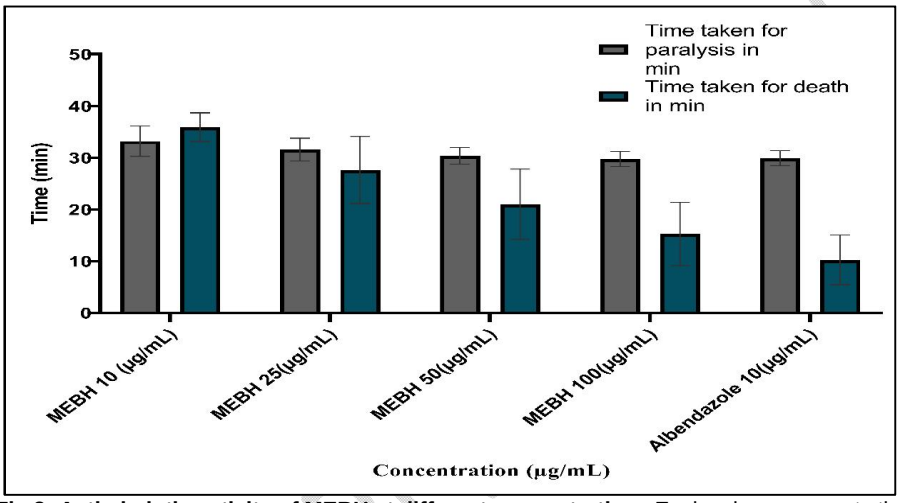


Fig 2: Anthelmintic activity of MEBH at different concentrations. Each value represents the mean ± SEM (n= 5).

3.2 Thrombolytic Effect

MEBH percentages were not far behind the standard drug in terms of comparison to the negative control (Figure 3). MEBH Clotting time could be better depending upon blood variations given by volunteers. Here, Standard drug Streptokinase produced 75.03% clot lysis whereas MEBH produced 48.49% of clot lysis compared to 4.84% of negative control (distilled water).

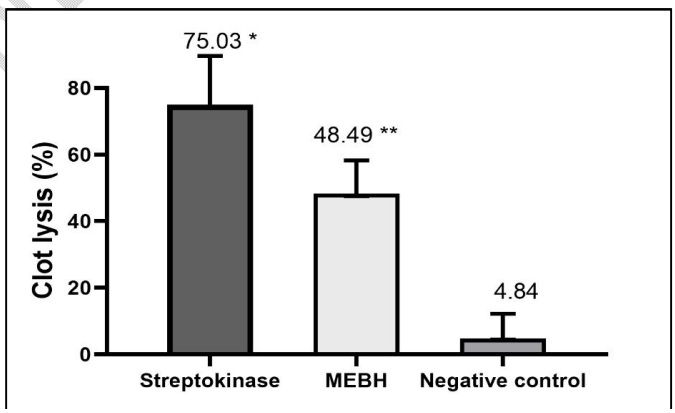


Fig 3: Percentage of clot lysis of human blood by MEBH and standard drug. Values are represented in mean ± SEM (n = 3). * *P*< 0.01 and ** *P*< 0.001 compared with the control group (Dunnett's test) Here, Streptokinase used as standard drug. MEBH: methanolic extract of *Brassaiopsishainla*.

3.3 Cytotoxic Activity

MEBH displays a 50% mortality rate as an outcome of its constituent chemical. Plant extract deaths are viewed as relatively unlikely. The latent cytotoxicity of crude extract was evaluated via brine shrimp cytotoxic assay. The fatality result of plant extract was assessed in Figure 4.

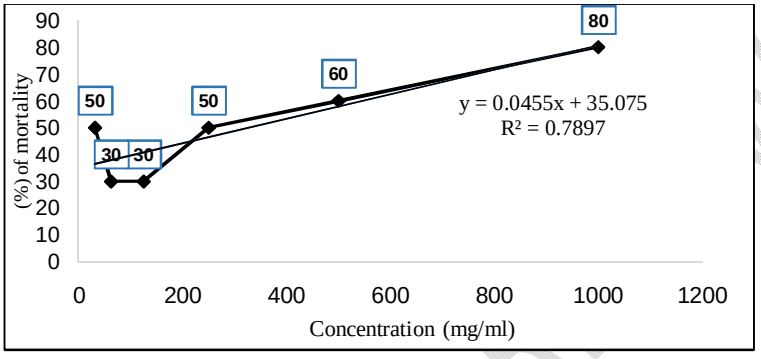


Fig 4: Cytotoxicity of MEBH. The LC50 value of MEBH was 328.02 µg/ml, verified via a linear regression equation (y= 0.0455x + 35.075), and the typical percentage of mortality was 50%.

3.4 Anti-diarrheal activity

a Castor oil-induced diarrhea

The castor-oil-induced diarrhea was assayed by methanolic extract of *Brassaiopsishainla* (MEBH) for four hours, whereas a significant dose-dependent manner activity was depicted (Table 3& figure 5). Diarrheal episodes were predominantly condensed by the positive control loperamide (5 mg/kg) in atremendously significant (*P*< 0.0001) manner (65.63%). In contrast, the MEBH exhibited 52.41% and 62.96% by 200 and 400 mg/kg doses. In the defecation phase, 400 mg/kg exhibited maximum inhibition (66.34%, *P* < 0.001), while the positive control loperamide (67.65%, *P*< 0.0001).

TABLE 03.THE EFFECT OF MEBH LEAVES ON CASTOR OIL-INDUCED DIARRHEA IN SWISS ALBINO MICE.

Treatment (mg/kg)	TotalNumber of faeces	Inhibition of defecation (%)	Total number of diarrheal faeces	Inhibition of Diarrheal (%)
Control (10ml/kg)	13.60±0.9	0	5.40±0.6	0
Loperamide(5mg/kg)	4.40±0.85	67.65	2.1±0.9	61.11
MEBH (200 mg/kg)	7.57±1.24	44.34	2.57±0.83	52.41
MEBH (400 mg/kg)	4.57±1.54	66.34	2.06±0.70	62.96

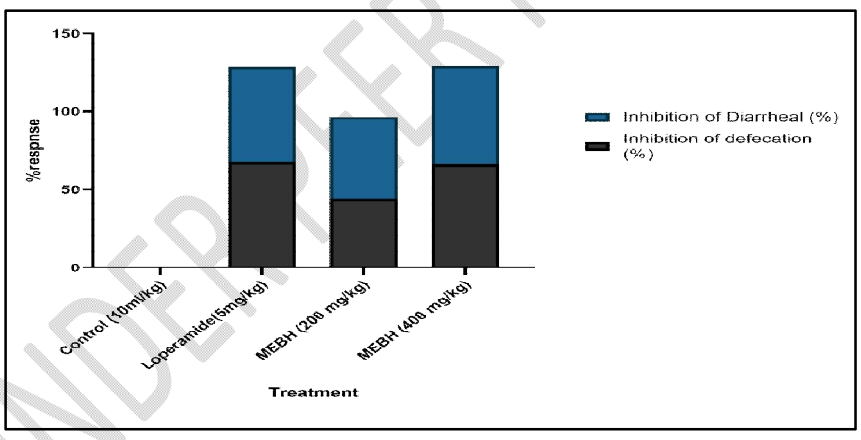


Fig 5: Anti-diarrheal activity of MEBH (Castor oil-induced diarrhea). Diarrheal episodes were predominantly condensed by the positive control loperamide (5 mg/kg) in a tremendously significant (*P*< 0.0001) manner (65.63%). In contrast, the MEBH exhibited 52.41% and 62.96% by 200 and 400 mg/kg doses. In the defecation phase, 400 mg/kg exhibited maximum inhibition (66.34%, *P* < 0.001), while the positive control loperamide (67.65%, *P*< 0.0001).

b **Castor oil-induced intestinal motility test (Charcoal marker)**

The intestinal motility by castor oil-induced followed by charcoal marker exhibited an extremely significant (*P*< 0.0001) reduction in peristalsis movement for all doses of MEBH when compared with the negative control. A disproportionate percentage of inhibition (42.4%, *P*< 0.0001) was observed in 400 mg/kg dose, followed by 37.99% in 200 mg/kg, while the standard drug loperamide was 50.41%, as shown in Table 4& figure 6.

TABLE 04. THE EFFECT OF MEBH LEAVES EXTRACT WITH REFERENCE DRUG

Treatment (mg/kg)	Total Length of Intestine (cm)	Distance Travel by Charcoal (cm)	Peristalsis Index (%)	Inhibition (%)
Control(10ml/kg)	48	41.69	84.69	0
Loperamide(5mg/kg)	50.66	20.66	59.52	50.41
MEBH (200 mg/kg)	56.1	25.83	46.1	37.99
MEBH (400 mg/kg)	53.67	24	45.47	42.4

LOPERAMIDE ON INTESTINAL MOTILITY IN MICE BY USING CHARCOAL AS A MARKER

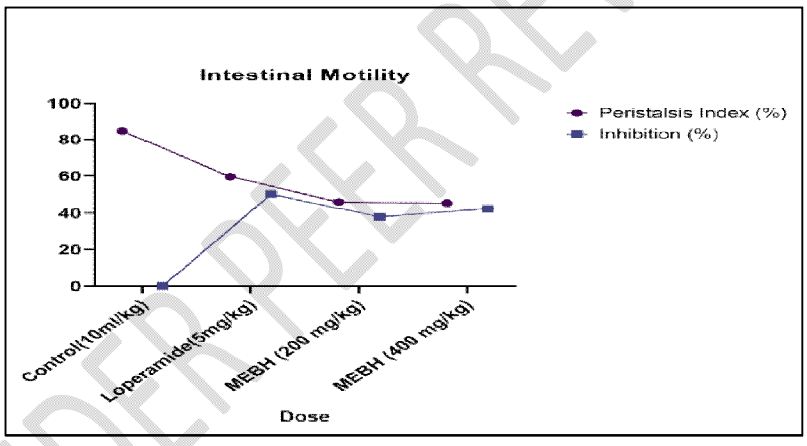


Fig 6:Anti-diarrheal activity of MEBH (Castor oil-induced intestinal motility). A disproportionate percentage of inhibition (42.4%, *P*< 0.0001) was observed in 400 mg/kg dose, followed by 37.99% in 200 mg/kg, while the standard drug loperamide was 50.41%. Results represented in Mean ± SEM (n=5) *P*<0.01, *P* <0.001, and *P*<0.0001 are statistically significant in comparison to Control.

3.5 Antipyretic activity test

TABLE 05. ANTIPYRETIC EFFECT OF METHANOLIC LEAF EXTRACT OF *BRASSAIOPSIS HAINLA* (MEBH)

Dose	Average temperature (^{0}C)	After Pyrexia (^{0}C)	Rectal temperature (^{0}C) after drug administration		
			60 min	120 min	180 min

Control	*37.02±0.17*	*39.17*±0.42	*39.15*±0.5	*39.19*±0.12	*39.09*±0.12
Paracetamol	*36.39*±0.39	*39.2*±0.56	*37.65*±0.17	*36.90*±0.39	*37.01*±0.1
MEBH 200	*37.0*±0.28	*40.01*±0.21	*38.99*±0.5	*39.00*±0.9	*39.02*±0.43
MEBH 400	*36.56*±0.1	*39.90*±015	*38.7*±0.22	*37.56*±0.12	*36.095*±0.13

Table 5 and figure 7 showthe rectal temperature change based on MEBH in mice. After 18 hours, the rectal temperature had risen dramatically after the injection of yeast suspension under the skin. Treatment with MEBH extract was much more efficacious than the control treatment in eliminating induced pyrexia.

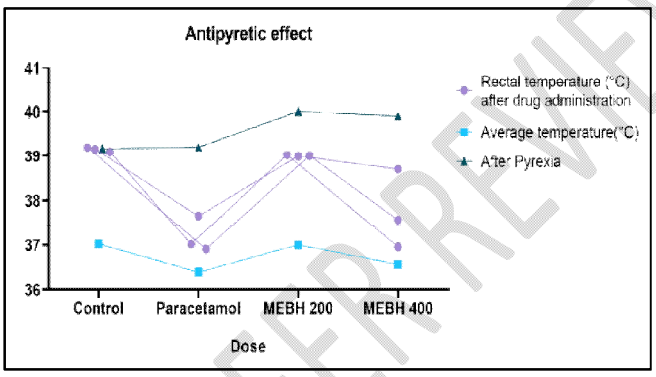


Fig 7: Antipyretic effect of MEBH

4. DISCUSSION:

Roughly two-thirds of pharmaceuticals are believed to be prepared from plant derivatives. This new course of the research, known as phytopharmacology, has started a new area to explore plant derivative substances that are efficacious in the treatment of certain chronic conditions and capture the attention of herbal remedies. Ever since the beginning of humankind's civilization, individuals have had resourcesfor medications and substances derived from plants to alleviate a wide variety of ailments. An examination into anthelmintics exposed that the crude extract generates dose-dependent immobilization, the severity of which can fluctuate from the inability to move at everyone to death. Compared to the conventional therapy with levamisole, the plant extract took significantly longer to cause paralysis and death. However, in order to achieve the same anthelmintic action as levamisole, a substantially higher concentration of the test extract was required. The pace of activity increase remained consistent throughout. An explanation for this disparity might be that the plant extract has several constituents, yet the drug only contains levamisole. An essential function for alkaloids, phenol, tannins, and terpenoids in anthelmintic action was determined from prior research**[27-29]**.

As a rule, thrombolytic drugs work by triggering the production of plasminogen, an enzyme responsible for dissolving the fibrin mesh that has been cross-linked. Because the clot has been dissolved and made more susceptible to subsequent proteolysis by other enzymes, blood can flow through previously closed arteries. Thus, thrombolytic medications are helpful in treating ischemic heart disease, ischemic stroke, deep vein thrombosis (DVT), and pulmonary embolism (PE) (e.g., Myocardium, brain, and leg). Any drug that can be utilized to treat cancer should, to the greatest extent possible, not harm healthy cells. Conversely, chemotherapeutic drugs are frequently harmful to healthy cells, especially rapidly growing cells. To prove the plant's anticancer potential, investigators will need to test the extract at shallow doses against both cancer cell lines and control cell lines. This study used a crude extract of *Brassaiopsishainla* (MEBH); hence further research is

needed to determine what chemicals in the extract are responsible for the observed effects. Plants can be used as a framework for the creation of novel chemotherapy medicines. The lethality of bioassay utilizing brine shrimp(BSLB) is an important aspect. The toxicity of crude extracts, fractions, and isolated chemicals is often first screened using brine shrimp. The impetus of the extent of lethality is that it would be precisely related to the concentration contour, which shows that the rate of death climbs steadily with increasing sample concentration. They may shed light on the test materials' possible cytotoxicity.**[30]**. This approach can be regarded as a guide for detecting antitumor drugs and pesticides owing to its low rate and convenience of conduct.

In contrast, it has been shown that cytotoxic substances typically validate high activity in the BSLB**[31]**. Furthermore, this bioassay correlates well with human solid tumor cell lines. Based on the positive link between cytotoxicity and activity versus brine shrimp nauplii, plant extracts exhibiting cytotoxic effects have been adopted for further cell line evaluation**[32,33]**.

Castor oil's primary ingredient, ricinoleic acid, causes diarrhea by altering intestinal motility, elevating luminal osmolarity, and reducing electrolyte absorption. Diarrhea is a classic symptom of many ailments**[34]**.Intestinal mucosa irritation occurs when the lipase enzyme in castor oil generates ricinoleic acid. Pro- and anti-inflammatory mediators were released in response to irritation, including prostaglandins, nitric oxide, platelet-activating factors, and cyclic adenosine monophosphate. Inflammatory mediators cause an increase in bowel movement frequency in addition to electrolyte and water levels. Ricinoleic acid may have this effect, primarily activating the G protein-coupled proteinoid receptor (EP3) on intestinal smooth muscle cells**[35]**.

Because the MEBH exhibited a substantial decrease in diarrhea frequency, we assume it might be a source of phytochemicals that might limit the release of inflammatory mediators. This is the conclusion of our study. A charcoal marker test is performed to assess the intestine's peristalsis movement. This is because ricinoleic acid (a bioactive component of castor oil) irritated and inflamed intestinal mucosa and induced diarrhea. Prostaglandins are released when the intestines are inflamed, increasing both intestinal motility and electrolyte and water concentrations**[36]**. As an anti-diarrheal agent, the α–Terpineol exhibits intense action in inhibiting the PGE2 receptor. These leaves may also include the plant's rhizome-derived ingredient, α–Terpineol. Throughout our charcoal marker assay, the MEBH showed a considerable decrease in motility due to the suppression of prostaglandin synthesis.

Subcutaneous injection of Brewer's yeast causes fever via increasing prostaglandin production. This approach has great promise for screening both natural and synthetic antipyretics. Pyrexia of the fungal variety is considered a pathogenic fever, and Prostaglandins may be responsible**[37]**. The cyclooxygenase enzyme action can be blocked to inhibit prostaglandin formation, which could be the mechanism of an antipyretic effect similar to paracetamols. The appropriate suppression of pyrexia-related mediators in our bodies is more accountable for the antipyretic action than any other particular element**[38]**. Intraperitoneal injections of diverse leaf extracts dramatically reduced the rectal temperature of yeast-infected mice. ' That the extract included pharmacologically active principles preventing prostaglandins' release is a reasonable hypothesis. Based on these results, MEBH seems to have therapeutic potential for treating fever. The outcomes of the test suggest that *Brassaiopsishainla* has a beneficial component, which has been demonstrated in the findings of the experiment.

5. CONCLUSION

The bioassay of this plant established without certainty that the methanol extract of *Brassaiopsishainla* (MEBH) is reliable in providing the uppermost anthelmintic, antipyretic, thrombolytic, & anti-diarrheal value. This research provides more indication that this plant has promise as a possible reservoir for the creation of a novel pharmaceutical. In addition, the current findings demonstrate that MEBH functions as a Natural medication in every experiment conducted so far.

6. ABBREVIATIONS

MEBH: Methanol extract of *Brassaiopsishainla* (MEBH); LC50: Lethal concentration 50; SEM: Standard error mean; DNA: Deoxyribonucleic acid; GABA: Gamma-amino-butyric acid; DMSO: Dimethyl sulfoxide; brine shrimp lethality bioassay (BSLB), deep vein thrombosis (DVT).

9. ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The human-related experiment was conducted following the ethical morals laid down in the 1964 Declaration of Helsinki. All authors officially affirm that the "Principle of Laboratory Animal Care" (NIH publication No. 85-23, revised 1985) and any applicable local or national legislation were adhered to. All experiments have been examined and approved by the appropriate ethics committee.

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