Article

Evaluation of Therapeutic Activity of *Physalis angulata* (*In Vitro Studies*)

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Abstract: *Physalis angulata* L. family Solanaceae, commonly known as ground cherry, cape gooseberry, or bladder cherry, has a long history of traditional use in various regions around the world. The primary goal of this study is to investigate the different pharmacological effects produced by the ethanolic leaf extracts of *Physalis angulata.* The leaf extract was prepared in two different dosages: 250 mg/kg body weight and 500 mg/kg body weight, which were administered according to the body weight of the mice. In yeast-induced pyrexia in mice, after 4 h, positive control (Paracetamol 150 mg/kg), *Physalis angulata* 250 mg/kg, *Physalis angulata* 500 mg/kg expressed temperature were 98.78 ± 0.051 °F, 97.4 ± 0.213 °F and 96.56 ± 0.177 °F respectively. In the evaluation of acetic acid-induced peripheral analgesic activity, *P. angulata* extract exhibited 43% and 63% inhibition of writhing at 250 mg/kg and 500 mg/kg body weight, respectively. Whereas the standard Diclofenac-Na inhibited 76% at a dose of 25 mg/kg body weight. In castor oil-induced diarrhea, plant extract inhibited defecation by 59.65% at 250 mg/kg body weight and 72.45% at 500 mg/kg b.w., whereas standard loperamide at a dose of 3 mg/kg b.w. inhibited 83.50% of defecation. Ethanolic extract of *Physalis angulata* at the dose of 300 mg/kg, 2000 mg/kg and 5000 mg/kg showed average weight 21.2 ± 1.56 gm, 21.8 ± 0.82 gm and 24.45 ± 1.51 gm respectively at 2nd day. The disc diffusion method has been adopted for the evaluation of antimicrobial activity. The ethanolic extracts of *Physalis angulata* leaf exhibited inhibitory activity against fourteen strains, including Bacillus megaterium, Salmonella paratyphi, Candida aibicans, Vibrio mimicus, and Staphylococcus aureus.

Keywords: *Physalis angulata*; analgesic; antipyretic; antidiarrheal; acute toxicity; antimicrobial

1. Introduction

Since the dawn of civilization, people have relied heavily on the therapeutic qualities of medicinal plants. The World Health Organization estimates that even now, almost 80% of the world's population, particularly in less developed countries uses traditional medicine as their major source of healthcare [1]. There are roughly 2000 different ethnic groups in the world, and nearly all of them have unique traditional medicinal practices [2]. *Physalis angulata* (Family: Solanaceae) commonly referred to as the cut-leaf ground cherry or bladder cherry, is an herbaceous shrub that is native to tropical America but is currently found as a weed around the world. This erect herb boasts smooth leaves with deeply cut edges. *Physalis angulata*, is a bit of a bully in the garden. It spreads easily, handles most weather conditions, and shrugs off weed killers. This bushy annual herb stands roughly 50 cm tall and has smooth or slightly hairy stems [3]. Beyond its well-known ability to strengthen the immune system, this plant surprisingly finds a role in the kitchen, particularly when making sauces [4]. Standardised medicinal plant extracts are used in a variety of traditional and mainstream medications across the globe. Natural remedies have been shown to regulate the aberrant cell division process and have a very good therapeutic index against various tumour cell. *Physalis angulata* boasts a rich history of medicinal use in Japan, particularly for fevers. This plant's extracts or infusions are used as dermatitis, asthma, and anti-malarial remedies in many parts of the world

[5,6]. Scientific investigations have revealed a diverse range of molecules within the plant, including carbohydrates, minerals, vitamins, and fats. Interestingly, the entire plant contains various steroidal lactones that belong to physaline and withanolide, such as physalins A–I, physagulin A–G, withangulatin A, and withanolide T. Withanolides have a C-28 ergostane-type steroid structure with a δ-lactone group at C-22 and C-26. It also contains a flavonol glycoside named myricetin 3-*O*-neohesperidoside [7–10]. Interestingly, people in some places actually eat the leaves and fruits, and parts of the plant might even have medicine in them. Scientists are looking into these special molecules called anolides to see if they can fight cancer and inflammation [11]. Furthermore, in vitro studies have shown promise for the plant's potential as an anticancer agent. Specifically, *P. angulata* leaf extracts have demonstrated cytotoxic effects against various cancer cell lines, including Y79, HeLa, DLD-1, MCF-7, and HGC-27 [12]. These findings instigate further investigation to explore the potential therapeutic applications of *P. angulata* extracts and their isolated bioactive compounds. *P. angulata* leaves have a rich history of traditional use as medicine in Southeast Asia, North America, and South America, particularly for treating bacterial infections. This widespread use suggests the potential antibacterial properties of the plant. However, this potential remains largely unexplored by modern science. Crucially, no in vivo studies, meaning studies conducted within living organisms like mice or humans, have been documented to definitively confirm the effectiveness of *P. angulata* against bacteria. Further scientific exploration through in vivo research is necessary to bridge the gap between traditional knowledge and validate the potential of *P. angulata* as a bactericidal agent [13]. Ingesting significant amounts of *Physalis angulata* might be harmful to one's health because it contains poisonous chemicals, mainly glycoalkaloids and steroidal lactones like physalins. In particular, the unripe fruits and leaves contain glycoalkaloids, which have been linked to neurological side effects including headaches and dizziness as well as gastrointestinal symptoms like nausea, vomiting, and diarrhoea. High doses or extended exposure can, in extreme circumstances, cause toxicity to the kidneys and liver. The cytotoxic effects of physalins can be hazardous, particularly at elevated dosages, despite their therapeutic benefits. It can be dangerous to consume too much of the plant or to eat its unripe sections, even though it has medical uses [14–16].

Thus, we have tried to investigate the pharmacological antipyretic, analgesic, antidiarrheal, acute toxicity, and antimicrobial activity, of the plant extracts that could be beneficial in further drug discovery.

2. Materials and Methods

2.1. Flowchart outlining the experimental steps

The flowchart below outlines the complete experimental workflow, beginning with plant collection and concluding with the various testing methods (Figure 1).

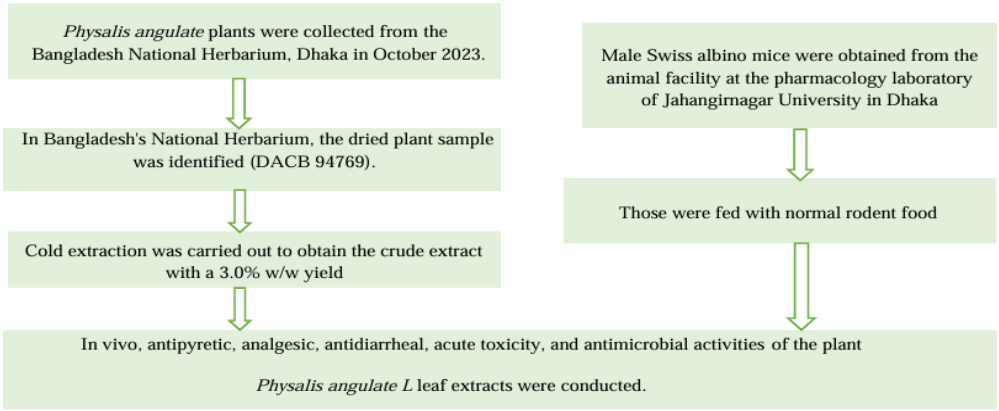

Figure 1. Diagrammatic representation of the experimental protocol.

2.1.1. Plant Material Collection

Physalis *angulata* (Voucher specimen: 94769 DACB) was collected in October 2023 from the Bangladesh National Herbarium, Dhaka. After removing unwanted materials, the plant's leaves were dried in the shade and ground into a coarse powder.

2.1.2. Extraction

For 14 days, 350 g of the coarse powder was soaked in of ethanol with a 10:1 solvent to dry weight ratio in sealed glass containers, and shaken intermittently [17,18]. The mixture was filtrated by a clean cloth followed by

cotton, and then filter paper [19]. The ethanol extract was filtered and concentrated through a rotary evaporator. 10.2 g of crude extract from 350 g of dry powder with a yield rate of approximately 3%.

2.2. Experimental Animals

The trials were performed on young Swiss Albino mice that were 4–5 weeks old and weighed between 20–30 gm. The animals were obtained from the animal facility at the pharmacology laboratory of Jahangirnagar University in Dhaka. The subjects had one week of acclimatization in the animal facility of the Department of Pharmacy at Dhaka International University, located in Bangladesh. The mice were provided with regular laboratory food and water without any restrictions, and their natural day-night cycle was preserved. The research was conducted with proper ethical approval (Ref No: CPP/DIU/EC/005) at Dhaka International University.

2.3. Experimental Design for Antipyretic Activity

To assess the antipyretic properties of *Physalis angulata* extract, a previously established method using yeast-induced fever in mice was employed described by Subedi et al. [20]. The experiment involved four groups of five mice each; Group I (control) received saline (10 mL/kg), Group II (standard) received paracetamol (150 mg/kg), Group III received *Physalis angulata* extract (250 mg/kg), and Group IV received the same extract (500 mg/kg). All preparation was made by dissolving the materials in distilled water.

The mice were weighed to establish the appropriate dosage, and their baseline body temperatures were measured using a digital thermometer. Fever was induced by administering a subcutaneous injection of a 15% Brewer's yeast suspension at a dose of 10 mL/kg. Following an overnight fast with unrestricted access to water, rectal temperatures were recorded 24 h post-injection [21]. Animals with a temperature rise of less than 0.5 °C were excluded, while those with a rise above 0.5 °C were confirmed to have pyrexia. Each group received its respective treatment, and rectal temperatures were measured 1,2,3, and 4 h post-medication.

2.4. Experimental Design for Analgesic Activity

Using the model of acetic acid-induced writhing in mice, the analgesic efficacy of *Physalis angulata* extract was investigated according to the method described by Debnath et al. [22,23]. The study included four groups with every group containing 5 mice; Group I (negative control): 1% Tween-80 in distilled water, 10 mL/kg orally given, Group II (positive control): Diclofenac Na in distilled water, 25 mg/kg orally given, Group III: *Physalis angulata* leaf extract in distilled water, 250 mg/kg d orally given, Group IV: *Physalis angulata* leaf extract in distilled water, 500 mg/kg orally given.

Mice were administered test and control solutions using a feeding needle and injected intraperitoneally with 0.7% acetic acid at 30-min intervals. Writhing frequency was recorded over 15 min as a measure of distress [24,25]. Any unfinished writhing was regarded as partial writhing; hence, two partial writhing were seen as one complete writhing.

The percentage of writhing inhibition relative to the control group was used as a measure of analgesia and was determined using the following formula:

$$\text{Inhibition of writhing \%} = [(W_c - W_t) / W_c)] \times 100$$

where, W_t is the average number of writhing in the test group and. W_c is the average number of writhings in the control group.

2.5. Evaluation of In-vivo Antidiarrheal Activity

The mice were divided into four groups to assess the antidiarrheal effects of *Physalis angu*lata extract in a model of castor oil-induced diarrhea described by Jahan et al. [26,27]. Each group contained five animals. The groups were as follows: Group I (control group, treated orally with 1% Tween-80 in distilled water), Group II (standard group, administered Loperamide at a dose of 3 mg/kg body weight), Group III (test group-I, given *Physalis angulata* leaf extract at 250 mg/kg body weight), and Group IV (test group-II, given *Physalis angulata* leaf extract at 500 mg/kg body weight). One hour prior to receiving a 0.5 mL oral dose of castor oil, each group was given their respective treatments. The mice were then individually housed in separate cages lined with blotting paper to observe for signs of diarrhea. After administering the castor oil, the occurrence of diarrhea was monitored and recorded hourly for four hours. The number of fecal deposits or any other fluid that stained the blotting paper was counted and noted for each mouse. The latency period for the onset of diarrhea was also recorded for each mouse. Fresh blotting papers were replaced every hour.

2.6. Evaluation of Acute Toxicity Test

The oral acute toxicity study of ethanolic extract of *Physalis angulata* was evaluated according to Organization for Economic Cooperation and Development (OECD) guideline 423 on BALB/c mice (20–30 g) [28,29]. Prior to the experiment, all the animals were housed in an overnight fasting state with unrestricted access to water. There were four groups, each consisting of five animals. Group I received an oral administration of a normal saline solution containing 0.9% sodium chloride (NaCl). The mice in this group were given a solution of (0.01 times their body weight) milliliters on the first day. Groups II and III and, IV received the extract orally at doses of 300, 2000, and 5000 mg/kg body weight (dissolved in purified water). The mice in this group were given a solution of (0.01 times their body weight) milliliters on the first day. The animals were monitored for any toxicological impact throughout the initial 4-h period following treatment [30]. In addition, animals were examined for a period of 3 days to determine if there were any harmful consequences [31]. Furthermore, alterations in behavior and several factors like body mass, urine patterns, food consumption, body temperature, and alterations in eye and skin pigmentation were observed.

2.7. Determination of Antimicrobial Activity

The minimum inhibitory concentration of the *Physalis angulata* extract was evaluated using the broth dilution method following Clinical and Laboratory Standards Institute (CLSI) guidelines (document M26-A). *Pseudomonas aureus*, *Bacillus megaterium*, *Salmonella paratyphi*, *C. albicans*, *Salmonella typhi*, *Staphylococcus aureus*, *Vibrio Parahaemolyticus* and *E. coli* were used in the experiment.

To prepare bacterial suspensions for testing, a small quantity of each organism in its active growth stage was transferred to vials with fresh, nutrient-rich liquid and incubated in a controlled environment to enhance growth. After several hours, a tiny amount from each vial was moved to fresh broth in test tubes and mixed thoroughly. The bacterial suspensions were then aseptically transferred to individual Petri dishes, ensuring uniform distribution by gently rotating the dishes. Five petri dishes were prepared for each of the five bacterial strains, and all dishes were appropriately labeled.

Ten 5 mm filter paper discs were placed on the agar surface of each petri dish using sterile forceps. The discs were positioned at least 15 mm from the edge and spaced to avoid overlapping inhibitory zones. In a sterile laminar flow hood, 10 µL of extract solutions (250 µg/10 µL and 500 µg/10 µL) were added to the discs with a micropipette, resulting in final concentrations of 250 µg and 500 µg per disc for testing [32].

Kanamycin antibiotic discs (30 µg/disc) were used as a positive control, placed on each petri dish to confirm test organism susceptibility and compare responses with the test samples. After applying the discs, the plates were inverted and incubated at 37 °C for 16–18 h. Post-incubation, the antibacterial activity was assessed by measuring the diameter of the inhibition zones in millimeters with a calibrated scale [32].

2.8. Statistical Analysis

Every test parameter's mean and SEM were utilized to calculate the data. All of the study's data were analyzed using GraphPad Prism 9 and Microsoft Excel, utilizing a one-way ANOVA and the Dunnett test. All differences were considered for statistical significance at $p < 0.05$.

3. Results

3.1. Evaluation of Antipyretic Activity

There is a significant change in rectal temperature (In Fahrenheit) over time. The changes in body temperature for negative control, positive control (Paracetamol 150 mg/kg), *Physalis angulata* 250 mg/kg, and *Physalis angulata* 500 mg/kg extracts were shown in at different time intervals (Figure 2).

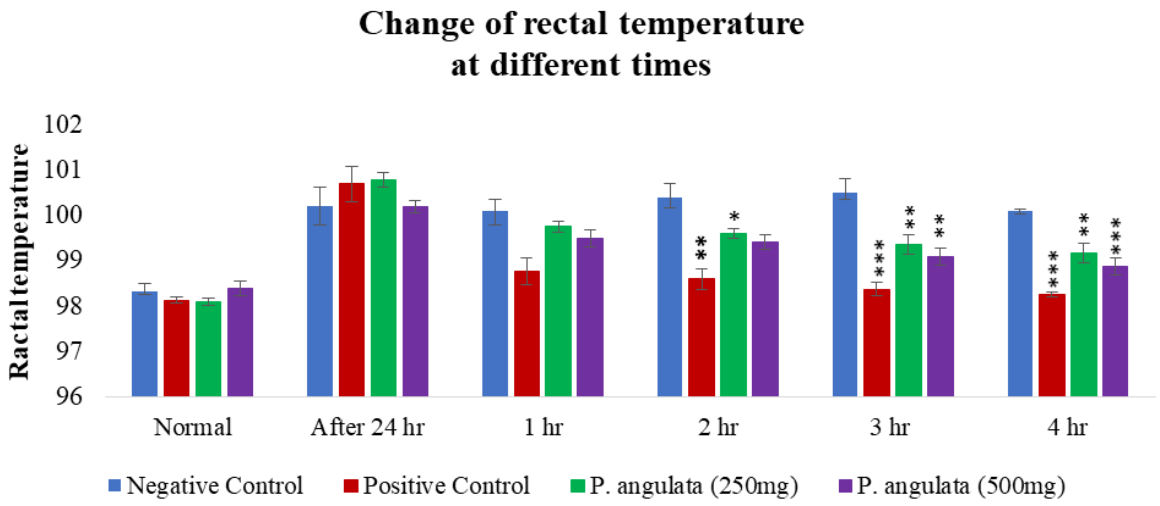

Figure 2. Change of rectal temperature at different times in different samples of *P. angulata* (Significance: * P<0.05, ** p<0.01, ***p<0.001).

3.2. Determination of Analgesic Activity

In a model of the writhing reflex, *Physalis angulata* extract showed analgesic properties. The analgesic effect of different doses, standard and negative control groups are shown in Figure 3. Comparing the two doses (250 mg/kg and 500 mg/kg) to the negative control group revealed a significant ($p < 0.05$) decrease in writhing. Furthermore, the traditional drug diclofenac sodium shown a highly substantial effect ($p < 0.0001$) (Figure 3).

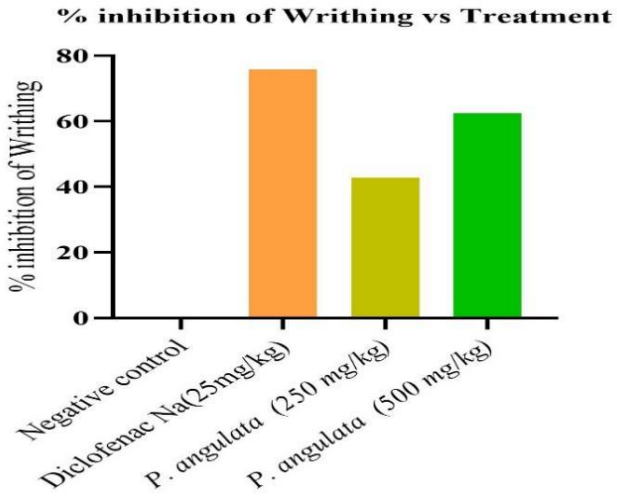

Figure 3. Percentage inhibition of writing of different groups on acetic acid-induced writing in mice (standard was Diclofenac Na).

3.3. Evaluation of In-Vivo Antidiarrheal Activity

The latent durations for the control, standard (Loperamide 3 mg/kg), plant extract 250 mg/kg, and 500 mg/kg were 48 min, 172.2 min, 118.8 min, and 130.2 min, respectively (Figure 4). The percentages of defecation inhibition were 83.50, 59.65, and 72.45% for the standard (loperamide 3 mg/kg), the *Physalis angulata* 250 mg/kg, and the *Physalis angulata* 500 mg/kg (Figure 5).

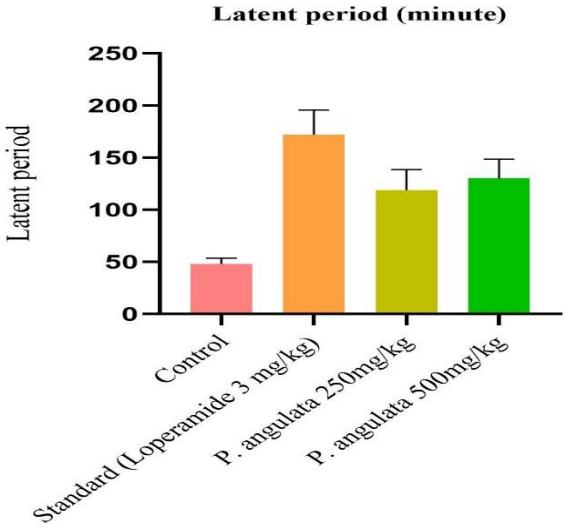

Figure 4. Effect of the extract of *Physalis angulata* on prolongation of the latent period in castor oil-induced diarrheal episodes in mice.

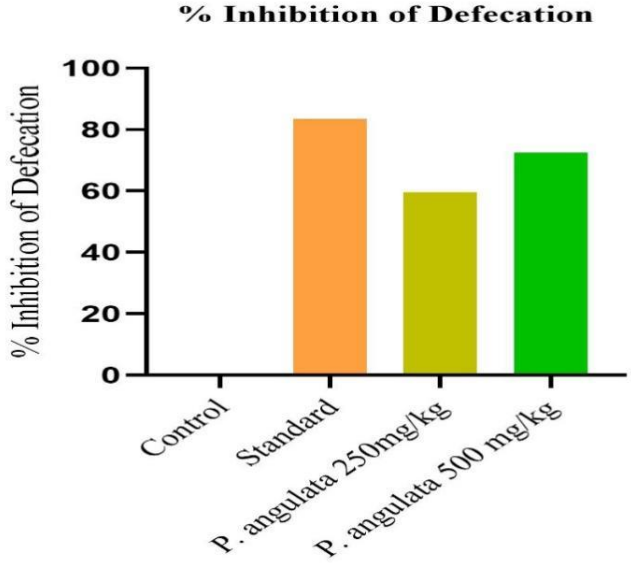

Figure 5. Percentage inhibition of defecation of four different groups**.**

3.4. Evaluation of Acute Toxicity Test

The mean weight of mice in the control group (Group-I) experienced a modest increase from 26.6 ± 1.76 gm to 28.3 ± 1.82 gm, indicating normal growth. However, Group-II (300 mg/kg) exhibited a weight gain that was comparable to the control group. On the other hand, Groups III (2000 mg/kg) and IV (5000 mg/kg) experienced lesser weight gains, ranging from 15.8 ± 0.92 gm to 21.8 ± 0.82 gm and 22 ± 1.48 gm to 24.45 ± 1.51 gm, respectively (Figure 6).

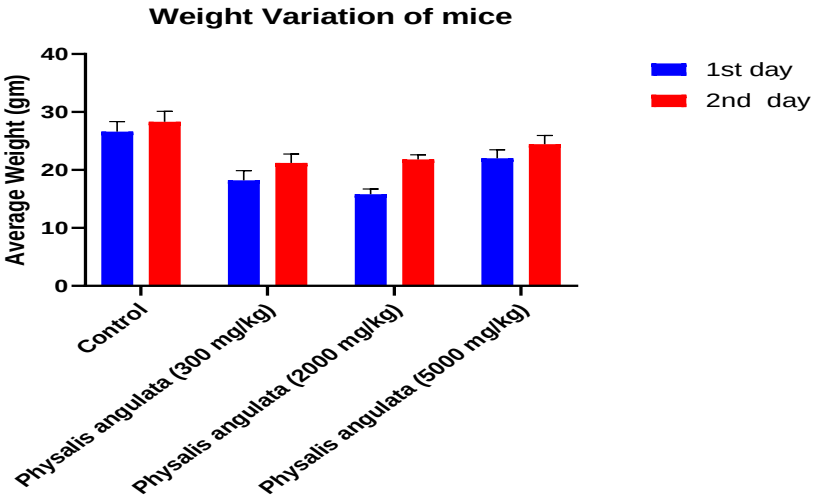

Figure 6. Weight variation of different groups.

3.5. Determination of Antimicrobial Activity

The crude 250 µg/disc plant extract exhibited a good zone of inhibition against *Pseudomonas aureus* (10 mm), *Bacillus megaterium* (15 mm), *Salmonella paratyphi* (10 mm), *C. albicans* (10 mm), *Salmonella typhi* (13 mm), *Staphylococcus aureus* (12 mm), but *Vibrio Parahaemolyticus* and *E. coli* showed no zone of inhibition while the dose of *Physalis angulata* 500 µg/disc sample showed good to moderate zone of inhibition against most of the strains notably *Shigella boydii* (11 mm), *Shigella dysenteriae* (12 mm), *Pseudomonas aureus* (11 mm), *Bacillus megaterium* (19 mm), *Bacillus subtilis* (12 mm), *Salmonella paratyphi* (20 mm), *Candida aibicans* (13 mm), *Aspergillus niger* (11 mm), *Salmonella typhi* (11 mm), *Vibrio mimicus* (13 mm), *Staphylococcus aureus* (15 mm) (Figure 7).

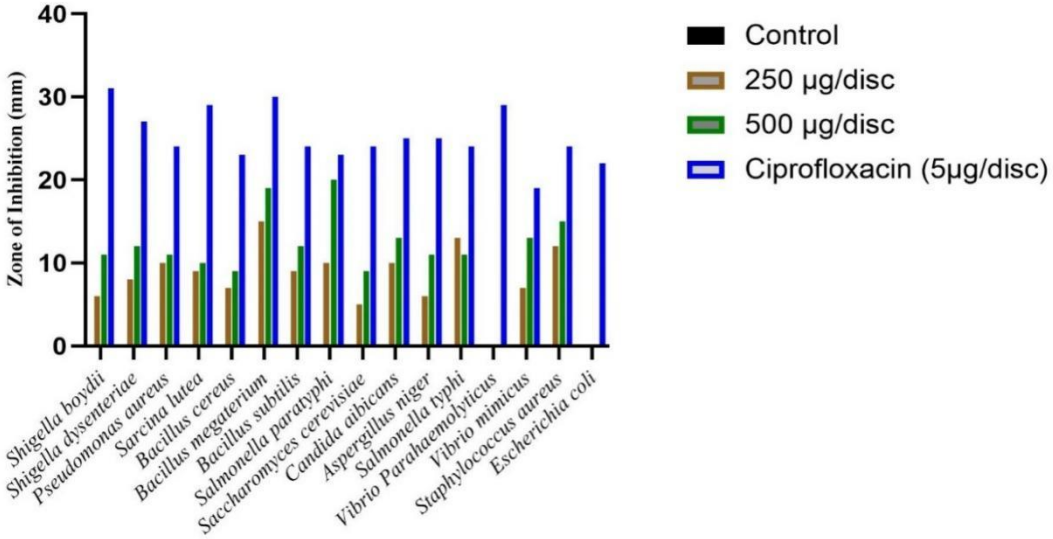

Figure 7. Zone of inhibition of *P. angulata* leaf extracts against sixteen strains.

4. Discussion

A number of plants have been utilized in traditional medicine for many years. Certain treatments appear to be effective even if there may not be enough scientific evidence (double-blind trials, for instance) to support this claim. These plants ought to be considered medicinal herbs [33]. The leaves of *Physalis angulata* possess antimicrobial, cytotoxic, and antioxidant activity. This study gives information about the antipyretic, analgesic, antidiarrheal, antimicrobial, and anti-toxic effects of *Physalis angulata* leaves.

Using ethanol, *Physalis angulata* leaves were extracted, producing a yield of about 3% by weight. Many existing anti-inflammatory and fever-reducing medications (both steroid and non-steroid based) cause a variety of

side effects. This is mainly because these drugs block the activity of two enzymes, COX-1 and COX-2, in a non-specific way [34]. As a result, researchers are actively searching for drugs that selectively target COX-2 or work through entirely different mechanisms to minimize side effects [35,36]. In this study, *Physalis angulata* leaf extract exhibited a statistically significant effect in antipyretic, analgesic, antidiarrheal, acute toxicity and antimicrobial tests. After 4 h of extract administration, *Physalis angulata* 250 mg/kg, *Physalis angulata* 500 mg/kg expressed temperatures of 97.4 ± 0.213 °F and 96.56 ± 0.177 °F, respectively. When comparing *Physalis angulata* to other medicinal plants like; *Launaea sarmentosa* and *Aegialitis rotundifolia*, *Physalis angulata* shows a clear dose-dependent antipyretic effect. At 250 mg/kg, *Physalis angulata* reduced the temperature to 97.4 ± 0.213 °F after 4 h, while at 500 mg/kg, it further decreased to 96.56 ± 0.177 °F. In contrast, *Launaea sarmentosa* at 400 mg/kg lowered the temperature to 97.48 °F after 5 h, comparable to the 250 mg/kg dose of *Physalis angulata*. *Aegialitis rotundifolia*, on the other hand, showed a more moderate effect, reducing the temperature to 97.9 °F, which is higher than both doses of *Physalis angulata*. Therefore, *Physalis angulata* at 500 mg/kg demonstrates stronger antipyretic activity, achieving a temperature close to the standard acetyl salicylic acid [29].

Analgesics are substances that, either centrally or peripherally, act on the sensory nerve system to lessen or remove pain without appreciably changing awareness. Two categories of analgesics exist: non-opioid/non-narcotic/antipyretic/anti-inflammatory analgesics, and opioid/narcotic/morphine-like analgesics, which depress the central nervous system. Non-opioids mainly affect peripheral pain pathways and increase the pain threshold in the central nervous system [37]. Any substance that reduces the amount of writhing will exhibit analgesia through the peripheral route of prostaglandin production suppression. Our findings from the experiment for acetic acid-induced belly constriction showed a significant decrease in writhing reflux. The analgesic effect seen at doses of 250 mg/kg and 500 mg/kg was similar to that of the NSAID diclofenac sodium standard medication. The peripheral analgesic efficacy of *Physalis angulata* leaf extract may be mediated by cyclooxygenase inhibition, which inhibits local peritoneal receptors. *Physalis angulata* showed dose-dependent analgesic effects, inhibiting writhing by 43% at 250 mg/kg and 63% at 500 mg/kg. This is comparable to medicinal plants like; *Launaea sarmentosa* (AELS) at 400 mg/kg, which also inhibited writhing by 63.1%, while *Aegialitis rotundifolia* (AEAR) showed 57.1% inhibition at the same dose. At lower doses, AELS (200 mg/kg) and AEAR showed 55.36% and 47.86% inhibition, respectively, which aligns with Physalis angulata at 250 mg/kg. The standard drug at 50 mg/kg showed the highest inhibition of 69.23% [29].

Castor oil causes diarrhea due to its active metabolite, ricinoleic acid, which stimulates intestinal peristalsis and fluid secretion [38]. This causes alterations in the intestinal mucosa's electrolyte permeability and increases peristaltic movement in the small intestine [39]. Castor oil has also been shown to boost the release of endogenous prostaglandin [40]. *Physalis angulata* extract possesses significant anti-diarrheal activity, decreasing the rate of defecation, and increasing the percentage inhibition of defecation. Consequentially, the *Physalis angulata* extract at 500 mg/kg showed a better effect than the 250 mg/kg extract dose. The extract reduced the amount of defecation by castor oil-induced diarrhea by 69.65% at 250 mg/kg body weight and 72.45% at 500 mg/kg body weight.

An essential component of determining the safety of synthetic or natural compounds meant for ingestion by humans or animals—as well as any potential environmental effects—is the assessment of acute toxicity. Determining the harmful effects of a material after a single or brief exposure, usually over a period of 24 to 48 h, is known as acute toxicity testing [31]. The average body weight of the control group considerably dropped while the average body weight of the *Physalis angulata* leaf extract group showed no alteration in appearance or behavior and increased average body weight, indicating that it had no harmful effect. On the second day, the average weight of the *Physalis angulata* ethanolic extract at doses of 300 mg/kg, 2000 mg/kg, and 5000 mg/kg was 21.2 ± 1.56 gm, 21.8 ± 0.82 gm, and 24.45 ± 1.51 gm, respectively. An earlier investigation demonstrated the *Physalis angulata* leaves's antitoxin properties by treating HeLa cell cultures with an ethanolic extract. In addition, the anticancer cell's notable decline and the cell's morphological alterations [5].

An ethanolic extract from *Physalis angulata* has shown efficacy against various bacterial strains. It previously demonstrated antibacterial activity against *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923). The minimum bactericidal concentration (MBC) and minimum inhibitory concentration (MIC) values for *E. coli* were 5 mg and 10 mg, respectively, while for *S. aureus*, the MBC and MIC were 2.5 mg and 5 mg, respectively [5]. A further study assessed the antibacterial activity of *Physalis angulata* leaf extracts against ATCC strains of *Pseudomonas aeruginosa* (27853), *Klebsiella pneumoniae* (13883), and *Staphylococcus aureus* (25923), and demonstrated the extracts' efficacy against these organisms [3]. Furthermore, experiments revealed that *P. angulata* leaf extracts were efficient against a variety of bacteria, such as Salmonella species, *Staphylococcus aureus*, and *Escherichia coli*. [41]. This study differs from prior research in that it showed better antibacterial activity against the strains mentioned: *Bacillus megaterium, Salmonella paratyphi, Candida aibicans, Vibrio*

mimicus, and *Staphylococcus aureus*. All microorganisms except *Escherichia coli* and *Vibrio Parahaemolyticus* were susceptible to the effects of *Physalis angulata* plant extracts at doses of 250 mg/kg and 500 mg/ kg.

The study on *Physalis angulata* ethanolic leaf extracts has several limitations that can be addressed in future research. Only two dosages were tested, so exploring a wider range of doses could provide a better understanding of the dose-response relationship. Long-term toxicity studies should be conducted to assess the safety of prolonged use. Investigating the specific molecular mechanisms and identifying the bioactive compounds responsible for the observed effects would deepen understanding of its pharmacological properties. Expanding the antimicrobial evaluation to include more microbial strains, especially drug-resistant ones, could offer a broader insight into its therapeutic potential. Additionally, a detailed phytochemical analysis and comparison with other medicinal plants could help highlight the uniqueness and efficacy of *Physalis angulata* extracts.

5. Conclusion

This work involved the effective extraction of *Physalis angulata* leaf and subsequent pharmacological tests utilizing the ethanolic extract of dried leaf. The materials examined exhibited noteworthy analgesic, antipyretic, antidiarrheal, antibacterial, and cytotoxic actions during our analysis. It is crucial to emphasize that the pharmacological activity detected in the plant extract is entirely dependent on the dosage. Further investigation is necessary to determine and separate the active components accountable for these actions, which could potentially result in the creation of innovative medications aimed at treating a range of disorders and advancing the biomedical sciences. A biochemical analysis might be performed to assess plasma levels of AST, ALT, and ALP in mice with CCl_4-induced hepatotoxicity. Plasma levels of oxidative stress indicators, including MDA, NO, and APOP, can be assessed to determine the analgesic activity in mice.

Author Contributions: MS conducted the animal and laboratory work under the guidance of A.S.M. R.D.B., D.D., R.R.R. carried out writing the manuscript, literature review and data analysis. A.S.M., B.S.N., R.A., T.R., S.S. and A.K.C. helped in manuscript writing and data analysis. A.S.M., R.D.B. helped in conceptualization, writing, review, and editing the manuscript. A.S.M. supervised the project. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: This research project has received ethical approval from the Ethical Committee of the Department of Pharmacy, Dhaka International University, Dhaka 1212, Bangladesh according to their ethical guideline (Ref No: CPP/DIU/EC/005).

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

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Conflicts of Interest: The authors declare no conflict of interest.

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