Phytochemical and Pharmacological Evaluation of *Parmentiera aculeata* (Family: Bignoniaceae)

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ABSTRACT: This study investigated the phytochemical constituents and pharmacological potential of *Parmentiera aculeata* a medicinal plant used traditionally in Central America. Four compounds were isolated and characterized, namely oleic acid (**1**), methyl palmitate (**2**), 7,9-pentadecanediol (**3**), and methyl 5-eicosenoate (**4**). The ethylacetate-soluble fraction (PAEA) and aqueous fraction (PAA) exhibited potent antioxidant activity (IC_{50}=12.65 µg/ml) which correlated with their high phenolic content (47.62 and 47.54 mg GAE/g, respectively). In the brine shrimp lethality assay, the PAD fraction demonstrated significant cytotoxicity (LC_{50}=0.258 µg/ml) in the brine shrimp lethality assay. PAEA showed excellent thrombolytic activity (45.99%) comparable to the standard (65.09%). All fractions exhibited anti-inflammatory effects, with PAEA and PAA achieving 95.70% and 86.97% inhibition in heat-induced hemolysis, respectively. In hypotonic solution induced hemolysis, only PAA exhibited promising result (49.6%). The plant crude extract (PA) exhibited analgesic activity in the acetic acid-induced writhing assay ($p<0.05$ for 200 mg/kg, $p<0.01$ for 400 mg/kg) and antidiarrheal effects (58.97% for both doses, $p<0.01$). Moreover, PA exhibited a dose-dependent hypoglycemic effect, with a 58.99% reduction in plasma glucose at 400 mg/kg. These findings suggest that *P. aculeata* possesses promising pharmacological properties and warrants further investigation in order to create possible therapeutic medicines.

Key words: *Parmentiera aculeata*; cytotoxicity; DPPH; antioxidant; thrombolytic; anti-inflammatory

INTRODUCTION

As the benefits of using medicinal plants in healthcare have become more widely recognized, interest in herbal medicine has increased in the twenty-first century. Often called the "Return to Nature" movement, this trend represents a global movement away from synthetic drugs and toward natural cures. Because of its enormous therapeutic potential in preventing and treating a wide range of illnesses, medicinal plants have been a mainstay of healthcare for millennia.[1] In real terms, the plant kingdom is the source of many traditional medications. In the past, most of the potent medications that were on the market a century ago came from plants. Aspirin from willow bark, digoxin from foxglove, quinine from cinchona bark, and morphine from opium poppies are traditional examples.[2] According to estimates from the World Health Organization (WHO), 80% of people worldwide use plants to treat a variety of illnesses. Accessibility and affordability are two common reasons for this preference for plant-based treatments, especially in areas where synthetic drugs may be hard to come by. Even so, there is still a great deal of room for investigation and study into natural sources for therapeutic applications.[3]

The plant *Parmentiera aculeata*, commonly referred to as Cuajilote and a member of the Bignoniaceae family, is indigenous to Mexico and Central America. Although cattle fodder is its main

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usage, humans occasionally eat the fruits, and the trees themselves double as living fences and sources of shade. The Cuajilote fruit has a substantial amount of crude fiber (3.11 to 4.25%), according to several studies, which suggests that it may have nutritional value for human consumption.[4] Indigenous communities in Mexico may have used *P. aculeata* medicinally for a very long time, according to ethnobotanical data. To cure a range of illnesses, traditional Aztec medicine used plant components such as roots, branches, bark, leaves, fruits, and flowers. Records show that it is used to treat colds, otitis (ear infections), gastrointestinal distress, edema (dropsy), and renal problems. According to reports, the Aztecs used a leaf tea as an oral beverage and as a local treatment with wet cotton for persistent ear infections. Additionally, *P. aculeata* is used in modern herbal treatments for diabetes; the fruit is said to be effective in curing colds, and the roots are used as a traditional diuretic.[5]

The present study conducted a comprehensive analysis to determine the possibility of bioactive characteristics in the examined plant extract. Experiments were especially planned to isolate phytochemicals from the plant, evaluate the extract's anti-inflammatory, anti-cancer, thrombolytic, and antioxidant characteristics *in vitro*, as well as its peripheral analgesic and antidiarrheal and antidiabetic effects in mice. The main objective was to ascertain whether the plant had bioactivities that went beyond its known traditional uses and earlier scientific discoveries.

MATERIALS AND METHODS

Sample collection and preparation. Whole plant of *P. aculeata* was collected from the Bandarban Hill Tracts in September 2019. The plant material was deposited at the Bangladesh National Herbarium in Dhaka with the voucher specimen number 65390 to ensure proper identification and record-keeping. To minimize contamination from external sources, the plant underwent a thorough cleaning process. Subsequently, the plant parts were sectioned to facilitate drying and placed in a shaded environment for a period of approximately one month. Upon complete desiccation, the entire plant material was crushed into a homogenous powder using a high-capacity grinding machine. The resultant powder was then weighed, yielding a total mass of 843 grams.

Instrumentations, drugs, and chemicals. A 400 MHz Bruker device was used to record nuclear magnetic resonance (NMR) spectra, and the solvent used was deuterated chloroform ($CDCl_3$). A Buchi Rotavapor was used to carry out the solvent evaporation process. Column chromatography (CC) used Sigma-Aldrich's Kieselgel 60 silica gel for chemical separation, and vacuum liquid chromatography (VLC) used Kieselgel 60H. Merck, Germany, supplied pre-coated silica gel 60 F_{254} plates for thin-layer chromatography (TLC) investigation. UV light and the vanillin/H_2SO_4 reagent were used to visualize the TLC spots. Additional chemicals and solvents were sourced from reputable suppliers, including Active Fine Chemicals, a pharmaceutical company based in Bangladesh.

Extraction of plant material. 843 grams of finely ground plant powder was subjected to a maceration extraction process in methanol (MeOH) within a clean, cylindrical amber-colored container. To optimize component extraction into the solvent, the container was periodically shaken and stirred for a fifteen-day period. The resulting solution was then subjected to filtration using Whatman No. 1 filter paper and fresh cotton wool to remove any particulate matter. A rotary evaporator was employed to concentrate the filtrate at reduced temperature (40°C) and pressure.[6] The final yield of the crude *P. aculeata* extract was 17.2 grams.

Preparation of different partitions. A partitioning procedure was applied to the crude MeOH extract using the methods developed by Kupchan and later improved by Van Wagenen *et al.*[7] In this process, 5 grams of crude extract were dissolved in 100 milliliters of a 10% aqueous methanol solution, and then the mixture was fractionated using sequential washes with hexane, dichloromethane (DCM), and ethyl acetate solvents

(EtOAc), yielding four distinct fractions- hexane-soluble (PAH), dichloromethane-soluble (PAD), ethyl acetate-soluble (PAEA), and aquous (PAA) fractions. Each fraction was then independently concentrated using a rotary evaporator.

Isolation of compounds. The PAH was further purified using size-exclusion chromatography. Sephadex LH-20 served as the stationary phase, while a mixture of *n*-hexane, DCM, and methanol (2:5:1) functioned as the eluent. This chromatographic technique facilitated the isolation of four compounds (**1-4**).

Properties of isolated compounds. Oleic acid (**1**): Colorless crystal and soluble in chloroform and ethyl acetate; ^{1}H NMR (400 MHz, $CDCl_3$): δ2.34 (2H, t, *J* = 7.6 Hz, H-2), 1.63 (2H, m, H-3), 1.25 (22H, m, H-4-7, H-12–17), 2.01 (4H, m, H-8,11), 5.34 (2H, m, H-9,10), 0.87 (3H, t, *J* = 7.0 Hz, H-18).

Methyl Palmitate (**2**): Colorless mass and soluble in chloroform and ethyl acetate; ^{1}H NMR (400 MHz, $CDCl_3$): δ2.34 (2H, t, *J* = 2.4 Hz, H-2), 1.62 (2H, m, H-3), 1.28 (24H, m, H-4-15), 0.88 (3H, t, *J* = 2.4 Hz, H-16), 3.66 (3H, s, $COOCH_3$).

7,9-Pentadecanediol (**3**): Colorless mass and soluble in chloroform and ethyl acetate; ^{1}H NMR (400 MHz, $CDCl_3$): δ0.87 (6H, m, H-1,15), 1.25 (16H, m, H-2-5, H-11-14), 1.63 (4H, m, H-6,10), 2.34 (2H, t, *J* = 7.6 Hz, H-8), 3.63 (1H, d, *J* = 21.2 Hz, H-7), 4.01 (1H, d, *J* = 11.6 Hz, H-9).

Methyl 5-Eciosenoate (**4**): Colorless mass and soluble in chloroform and ethyl acetate; ^{1}H NMR (400 MHz, $CDCl_3$): δ3.77 (3H, s, H-21), 5.25 (2H, m, H-5,6), 2.24 (2H, t, *J* = 12.5 Hz, H-3), 2.09 (2H, t, *J* = 7.2 Hz, H-4), 1.25 (28H, m, H-7–20), 0.85 (3H, m, H-23).

Test animal model. Male and female Swiss Albino mice weighing 25–30 grams and aged 4-5 weeks were used in the investigation. They were obtained from the International Centre for Diarrheal Disease Research, Bangladesh's animal division (ICDDR, B). The mice were kept in polypropylene cages at the State University of Bangladesh in controlled environments with a 12-hour light/dark cycle, 24±2°C, and 60–70% relative humidity. They had unrestricted access to water and were fed a specific rodent diet that ICDDR, B had created. The mice were kept in these circumstances for a week before the experimental procedures started in order to guarantee acclimation and reduce stress. Every experiment followed the Federation of European Laboratory Animal Science Associations' (FELASA) rules.[7] The Animal Ethics Committee at the State University of Bangladesh granted consent prior to any animal research, with reference number 2024-03-29/SUB/I-ERC/005.

Acute toxicity test. Acute oral toxicity testing of *P. aculeata* extract was conducted in mice (4-5 weeks old) using a range of doses (500-2000 mg/kg). Following OECD protocols, mice were fasted and closely monitored after receiving a single oral dose. No mortality, behavioral changes, or physiological abnormalities were observed. Based on this safety data, researchers selected dosages of 200 and 400mg/kg for further *in vivo* investigations.[8]

DPPH free radical scavenging assay. The antioxidant potential of the plant extracts was assessed using the DPPH free radical scavenging test. The assay involved mixing a DPPH methanol solution (20 µg/ml, 3 ml) with varying concentrations of the plant extract solution (500-0.977 µg/ml, 2 ml). The fading of the purple DPPH solution caused by the plant extract was compared to a reference compound, butylated hudroxy toluene (BHT), to assess the extract's ability to neutralize free radicals.[9]

% Inhibition of free radical DPPH = (1-Absorbance of sample / Absorbance of the control reaction) × 100

Total phenolic content. The Folin-Ciocalteu Reagent (FCR) method was used to quantify the total phenolic content of the extracts, following Harbertson's protocol.[10] Gallic acid served as the reference standard for the generation of a standard curve. The total phenolic content was expressed in milligrams of gallic acid equivalent (GAE) per gram of extract based on the standard curve.

Brine shrimp lethality bioassay. A brine shrimp hatching solution was prepared by dissolving 38 g of NaCl in 1000 mL of water and adjusting the pH to 8.0. Vincristine sulfate (VS) served as the

reference standard, with test concentrations varying between 400 and 0.78125 µg/mL. After counting the nauplii, 5 mL of the prepared saltwater solution was used to expose them to the test samples, and cytotoxicity was assessed based on their survival rate.[11]

***In vitro* thrombolytic assay.** The process adhered to the Bhowmick *et al.* technique.[12] Healthy volunteers' venous blood samples were put in Eppendorf tubes that had been previously weighed, and they were incubated for forty-five minutes at 37°C to cause clot formation. The serum was separated, and the clot weight was calculated. Following that, the clots were exposed to 100 µl of distilled water, streptokinase (as a positive control), or plant extract. Every tube was incubated at 37°C for 90 minutes. Clot lysis was assessed by calculating the percentage weight difference before and after lysis.

% Clot lysis = (Weight of the clot after lysis / Weight of the clot before lysis) × 100

Heat-induced haemolysis. Two sets of tubes with isotonic buffer (2.0 mg/ml plant extract) and red blood cells were incubated at 54°C or on ice. A control with no extract was included. After centrifugation, hemoglobin release in the supernatant was determined at 540 nm.[12]

% inhibition of hemolysis =

{1- (OD2-OD1) / (OD3-OD1)} × 100

Where, OD1 = test sample unheated; OD2 = test sample heated; and OD3 = control sample heated.

Hypotonic solution-induced haemolysis. RBC suspensions in hypotonic solution were treated with plant extracts (2.0 mg/mL), acetyl salicylic acid (0.1 mg/ml, positive control), or left untreated (negtive control). After 10 minutes of incubation and centrifugation, hemoglobin release was measured at 540 nm.[12]

% inhibition of hemolysis =

{(OD1-OD2) / OD1}× 100

Where, OD1 represents the optical density of the hypotonic-buffered saline solution alone (control), and OD2 signifies the optical density of the test sample in the hypotonic solution.

Acetic acid triggered writhing. Plant extract (200 & 400 mg/kg), diclofenac-Na (10 mg/kg) and control (1% Tween 80) were administered to four groups of six mice each. Mice were given an injection of 0.6% acetic acid solution (10 ml/kg) thirty minutes after treatment, and their writhing reactions were monitored for 30 minutes.[13]

Castor oil-induced diarrhea test. Four sets of six mice each were given access to water and fasted for a full day. Vehicle (1% Tween-80) was given to Group 1, loperamide (2 mg/kg) was given to Group 2, and plant extracts (200 and 400 mg/kg) were given to Groups 3 and 4. All mice received castor oil an hour later, and the weight, severity, and commencement of diarrhea were tracked.[14]

Oral glucose tolerance test. Four groups of fasted mice were created: control (1% Tween-80), glibenclamide (2 mg/kg), and extract fractions (200 and 400 mg/kg). After glucose administration, blood sugar levels were measured at 0, 30, 60, 90, and 180 minutes to evaluate the extract's effect on blood sugar control.[15]

RESULTS AND DISCUSSION

Isolated phytochemicals from *P. aculeata*. Chemical analysis of the PAH from *P. aculeata* identified four compounds using various chromatographic and spectroscopic techniques (Figure 1). These compounds were confirmed to be Oleic acid (**1**)[16], methyl palmitate (**2**), 7,9-Pentadecanediol (**3**)[17], and methyl 5-eciosenoate (**4**)[18]by comparing their ^{1}H NMR spectra with data from published scientific literature.

The ^{1}H NMR (400 MHz, $CDCl_3$) of Compound **1** exhibited signals indicative of oleic acid. It displayed triplet proton signals at δ_H 2.34 (J=7.6 Hz) which indicated the proton bonded to the α-CH_2 proton of the carboxyl group as well as at 2.01 which indicated protons bonded to methylene adjacent to the C=C group. Another strong triplet peak at δ_H 0.87 (J=7.0 Hz) indicated a terminal methyl group. At δH 1.25, a broad singlet was found for a long chain of methylene Protons. Two multiplets at δ_H 5.34 proved the presence of unsaturated proton.

A sharp singlet at δH 3.66 indicated a proton of carboxymethylene in the ^{1}H NMR (400 MHz, $CDCl_3$) of Compound **2**. A multiplet was found at δ_H 1.62 proved the presence of β proton of carboxymethyl group and another multiplet at δ_H 1.28 showed a signal for the long aliphatic chain. At δ_H 0.88 (*J*=2.4 Hz), a triplet indicated a terminal proton. Another triplet at δ_H 2.34 (*J*=2.4 Hz) provided a signal for protons adjacent to the α carbon of the $COOCH_3$ group.

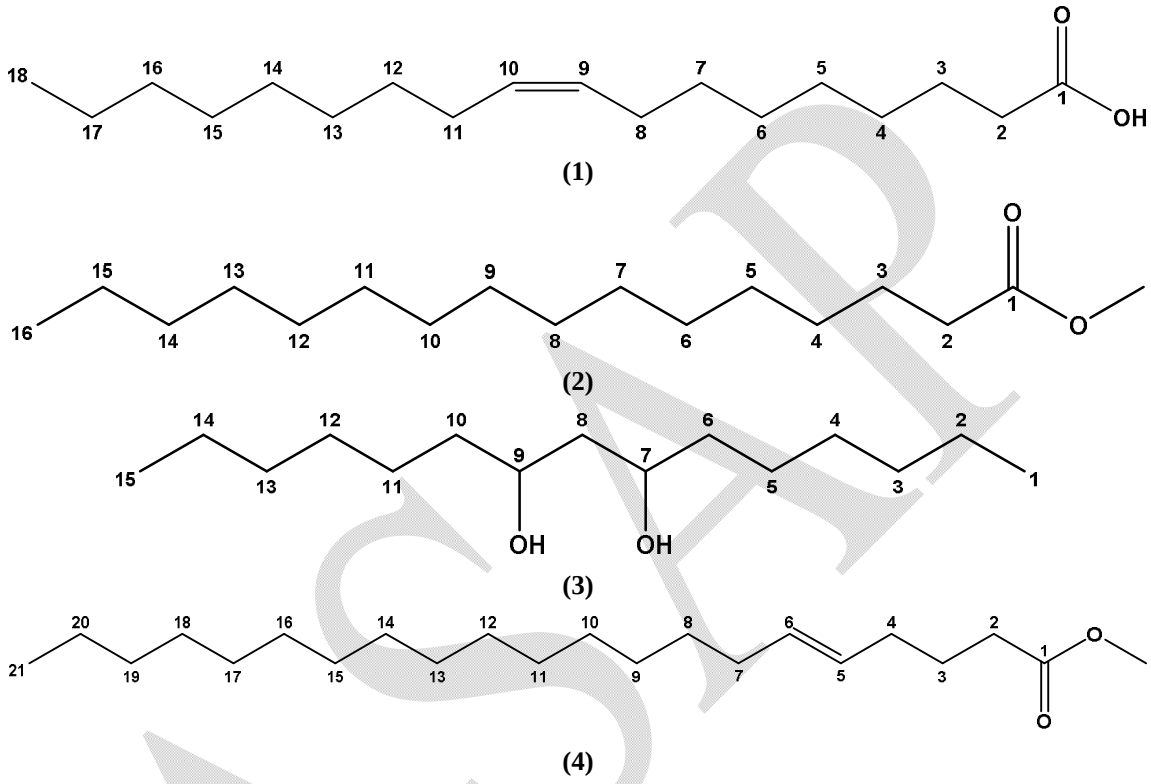

Figure 1. Structures of the isolated phytochemicals from *P. aculeata*: Oleic acid (**1**), Methyl Palmitate (**2**), 7,9-Pentadecanediol (**3**), Methyl 5-Eciosenoate (**4**).

The ^{1}HNMR (400 MHz, $CDCl_3$) of Compound **3** showed a multiplet peak at δ_H 0.87 which indicated a terminal methyl proton. A peak at δ_H 1.25 proved the presence of carbon chain methylene protons and a multiplet peak at δ_H 1.63 showed α $–CH_2$- proton adjacent to the C-OH group. A triplet peak at δ_H 2.34 proved the presence of methylene proton. Two doublet proton signals of hydroxylated carbon were found at δ_H 3.64 and δ_H 4.01 positions.

A singlet of methoxy protons was found at δ_H 3.77, and a triplet of $-CH_2$ protons was observed at δ_H 2.24 in the ^{1}H NMR (400 MHz, $CDCl_3$) of Compound **4**. The identification of methyl esters was confirmed by these two different peaks. Other peaks were found at δ_H 0.85 owing to terminal methyl protons, δH 1.25 due to carbon chain methylene protons. Another peak at δ_H 2.09 was due to $–CH_2$ protons adjacent to the alkene group and at δ_H 5.25 due to olefinic proton.

Effect of *P. aculeata* extracts on DPPH free radical scavenging activity. The PAA fraction demonstrated the most potent activity, with an IC_{50} value of 12.65 µg/ml. Interestingly, PAEA exhibited a similarly strong effect, also reaching an IC_{50} of 12.65 µg/ml. PAH showed moderate activity (IC_{50}=38.16 µg/ml), while the PAD fraction displayed the weakest effect (IC_{50}=313.325 µg/ml), compared to the standard BHT (IC_{50}=10.1 µg/ml) . The IC_{50} values of different fractions of *P. aculeata* are shown in figure 2.

Total phenolic content. The total phenolic content of *P. aculeata* extracts varied significantly. The PAEA fraction had the highest content, reaching 47.62 mg of gallic acid equivalent (GAE) per gram of extract followed by PAA (47.54 mg of GAE/g). In comparison, PAD and PAH fractions contained progressively lower phenolic content at 32.86 mg GAE/g and 21.66 mg GAE/g, respectively, as presented in Table 1.

Effect of *P. aculeata* extracts on brine shrimp lethality bioassay. PAD exhibited the strongest toxicity with an LC_{50} of 0.258 µg/ml, indicating that a very low concentration was needed to kill half of the brine shrimp, in contrast to the standard VS with an LC_{50} of 4.817 µg/ml. PAA showed moderate toxicity (LC_{50}=0.427µg/ml), while the PAEA fraction had a slightly lower effect (LC_{50}=0.82 µg/ml). The PAH fraction displayed the weakest toxicity (LC_{50}=1.765 µg/ml). The LC_{50} values of different fractions of *P. aculeata* are shown in figure 3.

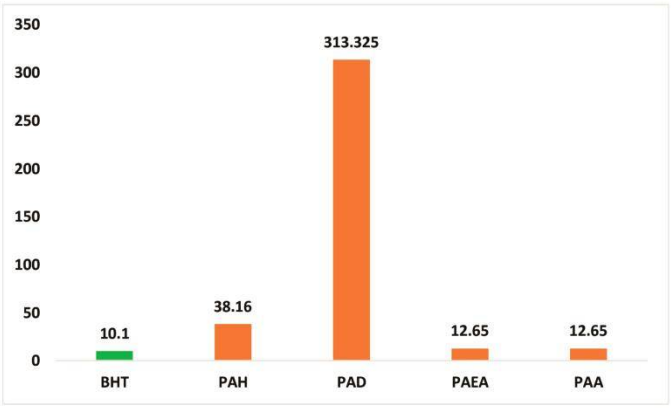

Figure 2. Comparison among IC_{50} values of different fractions of *P. aculeata* with the standard (BHT).

Table 1. Total phenolic content (mg of GAE/gm Extract) of different fractions of *P. aculeata.*

Plant Part	Sample Code	Absorbance	Total phenolic content (mg of GAE/ gm of extract)
Stem and Leaves	PAH	0.645	21.66
	PAD	0.942	32.86
	PAEA	1.333	47.62
	PAA	0.271	47.54

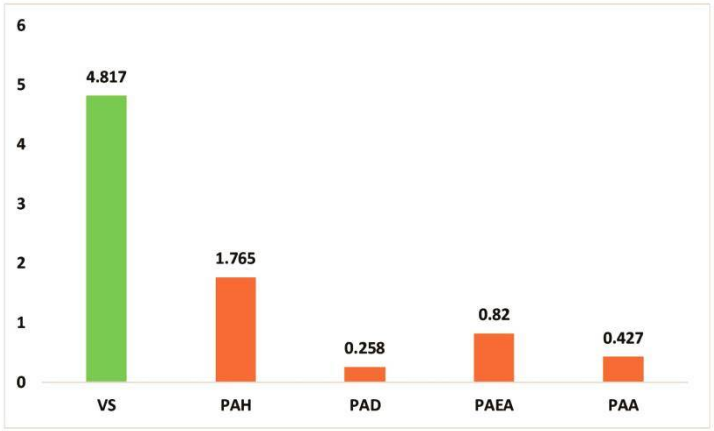

Figure 3. Comparison among LC_{50} values of different fractions of *P. aculeata* with the standard (VS).

Effect of *P. aculeata* extracts on thrombolytic assay. The findings, as detailed in Table 2, revealed that among the tested extracts, PAEA fraction of *P. aculeata* demonstrated the strongest clot lysis activity (45.99%), when compared to streptokinase, a positive control (65.09%). PAA and PAD fractions exhibited moderate activity (around 23%), while the PAH fractoin showed the weakest effect (13.61%). These findings suggest PAEA as the most promising fraction for further investigation of its clot-dissolving properties.

Table 2. Effects of different fractions of *P. aculeata* crude extract on thrombolytic and membrane stabilizing assay.

Sample	% clot lysis	% inhibition of heat-induced hemolysis	% inhibition of hypotonic solution-induced hemolysis
PAH	13.61%	83.38%	28.08%
PAD	22.28%	88.43%	22.51%
PAEA	45.99%	95.70%	14.37%
PAA	23.74%	86.97%	49.60%
SK	65.09%	-	-
AcSA	-	88.56%	51.10%

Effect of *P. aculeata* extracts on membrane stabilizing assay. The PAEA fraction displayed the strongest inhibitory activity against heat induced hemolysis, reaching 95.70%. The other fractions also showed promising results, with PAD, PAA, and PAH achieving inhibition percentages of 88.43%, 86.97%, and 83.38%, respectively, in descending order (Table 2). PAA emerged as the most effective inhibitor of hypotonic solution-induced hemolysis, achieving a remarkable 49.60% inhibition, when compared to the standard the effect of acetylsalicylate (AcSA), a common pain reliever (51.10%). The PAH fraction also displayed moderate inhibitory activity at 28.08%. In contrast, PAEA, which exhibited strong activity in the other test, surprisingly showed the weakest effect in this experiment (14.37%).

Effect of *P. aculeata* extracts on peripheral analgesic activity. In the peripheral analgesic study, the crude MeOH extract (PA) significantly inhibited writhing at both doses (200 & 400 mg/kg) comparing to the control. The inhibition is dose-dependent, meaning that the higher the dose of PA, the greater the inhibition of writhing. The highest inhibition of writhing is seen at the highest dose of PA (400 mg/kg) which is comparable to the standard (77.65%).

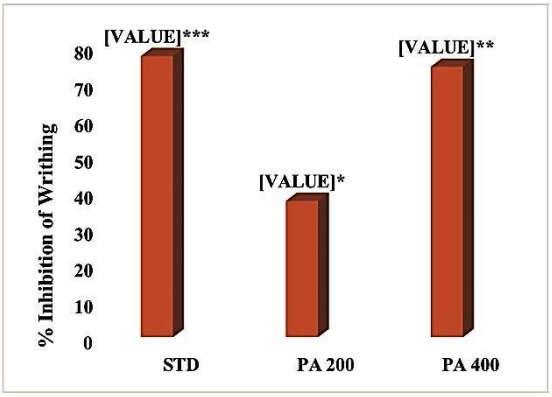

Figure 4. Comparative study of peripheral analgesic activity exhibited by diclofenac and methanolic extract of *P. aculeata*. (*p<0.05, **p<0.01, ***p<0.001).

Effect of *P. aculeata* extracts on antidiarrheal activity. When compared to the control group, PA substantially reduced the mean number of defecations, and the treatment group reduced 84.62% of diarrhea caused by castor oil. The percentage of inhibition of diarhhoea are same for both the doses (58.97%) and are statistically significant ($p<0.01$).

Effect of *P. aculeata* extracts on hypoglycemic activity. Plasma glucose levels decreased in a dose-dependent manner when PA was administered in comparison to the control group. A significant decrease in plasma glucose was observed at all tested doses (200 mg/kg and 400 mg/kg) of PA ($p<0.05$).

The most pronounced hypoglycemic effect was elicited at the highest dose of PA (400 mg/kg) which is 58.99%.

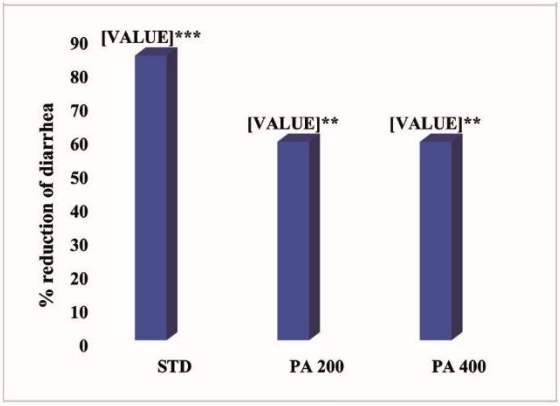

Figure 5. Comparative study of anti-diarrheal activity exhibited by loperamide and methanolic extract of *P. aculeata*. (*p<0.05, **p<0.01, ***p<0.001).

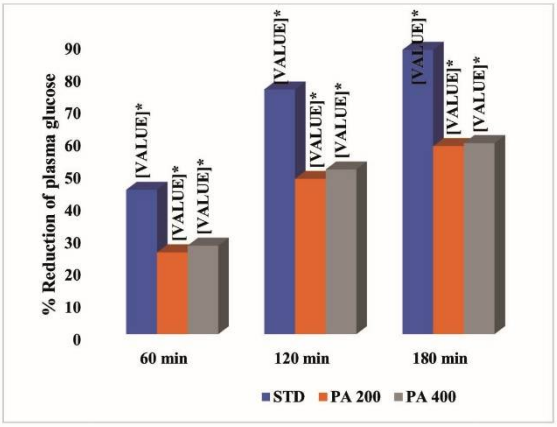

Figure 6. Comparative study of hypoglycemic activity exhibited by Glibenclamide and methanolic extract of *P. aculeata*. (*p<0.05, **p<0.01, ***p<0.001).

Unlike primary metabolites essential for basic life functions, secondary metabolites serve specialized roles. These compounds are produced by organisms for specific ecological purposes. They can act as defensive tools against predators, attract pollinators, aid in competition with other organisms, or help the organism adapt to challenging environmental conditions.[19] Isolating secondary metabolites from plants is crucial to identify, characterize, and study their properties, bioactivities, and potential applications.

The goal of the current study was to investigate the phytochemical components and pharmacological potential of *P. aculeata*. Four phytochemicals were isolated and characterized as a result of the chemical analysis: methyl palmitate, 7,9-pentadecanediol, oleic acid, and methyl 5-eicosenoate.

The observed potent antioxidant activity of the PAEA and PAA fractions, as evidenced by the DPPH assay, is noteworthy (IC_{50}=12.65 µg/ml). This activity correlates positively with the high phenolic content of these fractions (47.62 and 47.54 mg GAE/g, respectively). Phenolic compounds are well-known for their radical scavenging properties, and the results obtained in this study support this notion.[20,21] The strong antioxidant potential of *P. aculeata* extracts advocates their possibilities as a source of natural antioxidants for various applications.

The brine shrimp lethality assay revealed a significant cytotoxic effect of the PAD fraction (LC_{50}=0.258 µg/ml). While this finding indicates potential toxicity, further studies are warranted to evaluate the selective toxicity of this fraction against specific cell lines and to determine its safety profile. The moderate cytotoxicity exhibited by the PAEA fraction (0.82 µg/ml) also necessitates careful evaluation before considering its therapeutic applications.

Natural sources of thrombolytic agents are essential for developing safer, cost-effective alternatives to synthetic drugs, potentially with fewer side effects and sustainable, renewable therapeutic options. This study found that the PAEA of the plant exhibited the highest thrombolytic activity (45.99%), compared to the standard, which had a clot lysis rate of 65.09%, making it an excellent candidate for researching natural thrombolytic drugs.

Corticosteroids (steroids) and non-steroidal anti-inflammatory drugs (NSAIDs) achieve their anti-inflammatory effects by stabilizing cell membranes, but they can have significant side effects.[22,23] Therefore, exploring natural products capable of stabilizing cell membranes could be a preferable alternative for anti-inflammatory treatments. In this

study, all the fractions demonstrated notable inhibitory effects on hemolysis in heat-induced hemolysis. The highest inhibition was observed in PEAE and PAA achieving 95.70% and 86.97% inhibition in heat-induced hemolysis and 49.60% (PAA) inhibition in a hypotonic solution. In comparison, the standard AcSA showed slightly higher inhibitory percentages, with 88.56% in hypotonic and 51.10% in heat-induced hemolysis. These findings suggest that the plant is highly promising for further investigation.

The experiment also revealed that the plant extract exhibited a significant analgesic effect in the acetic acid-induced writhing assay ($p<0.05$ for 200 mg/kg dose and $p<0.01$ for 400 mg/kg dose), highlighting its potential for peripheral pain inhibition. The writhing response is commonly seen as a reflexive indicator of pain. Intraperitoneal administration of acetic acid triggers the production of prostaglandins and sympathomimetic mediators, including PGE2 and PGF2, leading to an increase in prostanoids and lipoxygenase-derived compounds in the peritoneal fluid of mice. This process activates pain-related signaling to the central nervous system, stimulating the release of mediators like prostaglandins, which heighten nociceptor sensitivity. Consequently, a decrease in the number of writhing episodes suggests analgesic activity by reducing prostanoid and lipoxygenase product levels.

The lack of synchronization between the motility of intestinal smooth muscles and the absorption patterns in the gastrointestinal tract can lead to diarrhea.[24] The antidiarrheal effect of the PA (58.97%, $p<0.01$) might be due to several mechanisms, such as reducing the secretion of prostaglandins[25], modulating the production of COX-1, COX-2, and lipoxygenase (LOX), which can inhibit the synthesis of prostaglandins and autacoids[26], or limiting intestinal motility and hydro-electrolytic secretions[27]. Further research is necessary to fully understand the underlying mechanism of this effect.

When compared to the control group, the administration of PA resulted in a dose-dependent decrease in plasma glucose levels. Both tested doses of 200 mg/kg and 400 mg/kg resulted in significant drops in plasma glucose ($p<0.05$). The highest dose of 400 mg/kg produced the most noticeable hypoglycemic impact, lowering blood sugar levels by 58.99%. These results demonstrate PA's therapeutic potential for controlling blood glucose levels and call for more research into its long-term effects and mechanisms of action.

It is crucial to remember that this work is only an initial exploration of the pharmacological potential of *P. aculeata*. To determine and characterize the bioactive compounds responsible for the observed effects, elucidate their mechanisms of action, and evaluate their safety and effectiveness in human and animal models, more investigation is needed.

CONCLUSION

The results of this study on the pharmacological potential and phytochemical components of *P. aculeata* were encouraging. The plant's chemical diversity and therapeutic potential are highlighted by the detection of strong antioxidant activity in the PAEA and PAA fractions as well as the isolation and characterisation of four compounds. The PAD fraction's notable cytotoxic activity calls for more research to ascertain its safety profile and selectivity. Additionally, the investigation found that certain *P. aculeata* extracts and fractions had noteworthy thrombolytic, anti-inflammatory, analgesic, antidiarrheal, and hypoglycemic qualities. These findings suggest that this plant could be a valuable natural chemical source for the identification and creation of novel medicinal medicines.

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