Research Article

Evaluation of Effect of Cytotoxic and Antioxidant Activity of *Paederia foetida* Linn. Leaf Extract

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Received: 12-01-2024; **Revised:** 25-02-2024; **Accepted:** 04-03-2024; **Published on:** 15-03-2024.

ABSTRACT

This plant's main chemical components include stigmasterol, sitosterol, iridoid glycosides, alkaloids, volatile oil, carbs, protein, and amino acids. Phytochemical studies have revealed the presence of paederolone, paederone, -sitosterol, paederoside, asperuloside, and their related glucosides in *Paederia foetida*. Previous studies have discovered 77 components, including a range of terpenoids and steroids, in the volatile oils of *P. foetida's* leaves, stems, and flowers—some of which are present in high amounts.

Distillation produces a volatile oil that smells strongly like freshly crushed leaves. Two alkaloids were identified: a- and b-Paederine. The leaves are used to extract an indole. Vit C and beta-carotene content are high in the leaves. The plant produces a variety of chemicals, including beta-sitosterol, Friedelan-3-1, and epifriedelinol. The leaf contains a variety of compounds, including iridoid glycosides, paederoside, asperuloside, and scandoside; sitosterol, campesterol, stigmasterol, palmitic acid, ursolic acid, and methyl mercaptan; and palmitic acid. Methyl mercaptan is the source of the plant's foul smell.

The last ten years have seen a rise in the popularity of the indigenous or traditional medical system. Many people now turn to traditional healers, who use medicinal plants—among which is *Paederia foetida*—to meet their fundamental medical needs. Its many conventional uses are supported by the thorough material in this review. *Paederia foetida* has antibacterial, antioxidant, anti-inflammatory, hepatoprotective, cardio protective, anti-diarrheal, anti-ulcer, and anthelmintic properties, among other properties.

Keywords: ABTS, ALD50, OCED, HPTLC, ICDDR, EMSA, Nrf2.

INTRODUCTION

An important family of angiosperms found mostly in Asia and North America is the genus Paederia of the Rubiaceae family. There are 33 recognized species of plants in the genus Paederia, according to The Plant List (The Plant List: Paederia genus). The deciduous climbing plant *Paederia foetida* Linn. (also known as Paederia scandans) is the lectotype species of the Paederia genus. It has a firm lignified stem and may grow up to 5.5 meters in height. When the leaves or stems of the plant are crushed or bruised, they release a pungent, sulfurous stench[1]. The Latin word foetida, which refers to the species, implies "stinky" or "foul-smelling." The plant grows as a ruderal on steep, wooded slopes, sandy or rocky seashores, thickets and woodlands, forest boundaries, secondary evergreen to deciduous forest, clearings in primary forests, and montane vegetation up to 3000 m.

For the treatment of arthritis, vesical calculi, inflammation, asthma, diarrhea, dysentery, piles, diabetes, seminal weakness, and a host of other conditions, P. foetida has a long history of use in Chinese, Ayurvedic, and other traditional medical systems. Its strong nutraceutical properties also make the plant a vegetable[2].

Figure 1: *Paederia foetida*

A few of assessments have been conducted about the plant's pharmacological, botanical compounds, and pharmacognostical characteristics. But a thorough analysis that includes all current data is necessary. That's why a thorough synopsis of P. foetida's phytochemistry and pharmacology was created for this review[3,4]. Detailed phytoconstituents categorization and a methodical examination of biological activity documented in the literature up until November 2022 are included. A wide range of biological and pharmacological activities, such as analgesic, anti-inflammatory, anti-arthritic, antimicrobial,

hepatoprotective, anti-diabetic, antioxidant, gastrointestinal, antihyperuricemic, anthelmintic, cytotoxic, renoprotective, cardiotonic, wound healing, sedative, anxiolytic, anticonvulsant, etc., are displayed by the extracts, essential oils, and compounds isolated from P. foetida. There were 217 phytoconstituents in all, including terpenoids, phytosterols, glycosides, anthraquinones, and phenolic derivatives[4, 5].

Figure 2: *Paederia foetida* Leaves

Because they are utilized for food, fuel, fodder, and other things, plants play a vital role in civilization. Herbal plants are a significant part of society's primary healthcare. The biggest obstacle to the acceptance of herbal medications is the lack of proof and quality control. It is therefore vitally important to try to standardize the plant material that will be utilized as medication[2]. *Paederia foetida*, known as "skunkvine" in English but locally called Gandhavadulia. Rubiaceae is the family to which it belongs[3]. Asian plants of the temperate and tropical varieties *Paederia foetida* are found in Southeast Asia, Japan, and India. It smells bad and has a nasty flavor. Though it can thrive at high tree levels in many different types of habitats, such as xeric sand hill communities and mesic hammocks, P. foetida is more commonly found in sunny flood plains and bottomlands. Growing in disturbed areas and tree gaps, it is also a common sight[4, 5]. There are many different ailments that the plant can be used to treat, including rheumatoid arthritis, constipation, hepatic disorders, diabetes, wounds, itches, coughs, stomachaches, dysentery, asthma, diarrhea, pain, teeth aches, cancer, flatulence, body aches, typhoid, pneumonia, and bone fractures[5]. Sustaining the integrity of aromatic and medicinal plants in their natural habitat requires continued use and proper knowledge of these species. Plants such as *Paederia foetida* Linn require special care in terms of proper propagation, upkeep, and chemical characterization due to their many uses[6].

Botanical description

Paederia foetida is a perennial plant that is long and thin. Its 1.5–7m long, scented branches twine together. Young stems are rosy brown or purple, and they might be densely bushy or almost smooth[7,8]. The ancient stems have a shade that ranges from yellow-brown to gray, and they are glossy and smooth. The leaf is simple, completely egg-shaped, round, oval, and measures approximately 2–21 cm by 0.7-9 cm. The base of the leaf is heart-shaped, somewhat distorted, or occasionally hastened, whilst the top is sharply tapering. The color range of the pale to dazzling yellow earthy color is bald to dramatically furry. The average length of the petiole is 0.5–6(–9) cm. Stipules are in 1.5-5mm x 2-3mm diameters and may be modified, expanded, or placed in an interpetiolar or three-sided construction. Usually, it's hairy, naked, or entire[9]. An extremely large terminal or axillary cymose panicle makes up the inflorescence. It grows from a widely spread paniculate that is over one meter long to a smaller one that is usually 10 centimeters long. Typically seen in tightly looping cymes with a peduncle ranging in length from 2 to 30 mm, the bracts are either leaf-like or small and straight, bearing few too many flowers[10].

Figure 3: Photograph of *Paederia foetida*

Scientific classification[10]:

Domain: Eukaryota

Kingdom: Plantae

Phylum: Spermatophyta

Subphylum: Angiospermae

Class: Dicotyledonae

Order: Gentianales

Family: Rubiacea

Genus: Paederia

Species: *Paederia foetida*

MATERIALS AND METHODS

Preparation of samples:

Trees grown in private gardens in Gelugor, Penang, Malaysia, provided plant material for the collection. Samples were cleaned with water as soon as they arrived at the lab to get rid of any dirt. The plants were deprived of their leaves and twigs of *P. foetida* and *S. aqueum*. 500 g of plant material were split up into fresh and dried samples. The dried samples were allowed to air dry for 14

days at 30 °C room temperature or until a consistent weight was reached. After being air dried for 24 hours at 30 °C, fresh samples were immediately submerged in the extraction solvents[11, 12].

Extraction Procedure:

In a soxhlet extractor, the powdered plant parts (22 gm) were extracted in stages using 250 ml of distilled methanol (40–60°C), ethanol, and chloroform at a high temperature. Upon extraction, all extracts were stored at 4°C in a refrigerator for later use, along with the appropriate labeling to facilitate identification[12].

Preparation of extracts:

- A 1:10 methanol to water ratio was used to extract all materials, both dried and fresh.
- After that, the solvent was extracted in vacuum by filtering the aqueous methanol solution using Whatman No. 4 filter paper.
- To create solutions at various concentrations (0.005 - 0.1 mg mL-1), the crude extract was put into a 100 ml volumetric flask and ethanol was added up to the mark.
- At a temperature of -20 ºC, the extracts were kept.
- The ABTS free radical scavenging and antioxidant activity towards β-carotene oxidation were tested on these crude leaf extracts from P. foetida and S. aqueum, while the total phenolic content was analyzed using all of the crude leaf and twig extracts[12, 13].

Collection, Identification and Processing of Plant Samples:

- The National Herbarium of Bangladesh, Mirpur-1, Dhaka, assisted in the taxonomy identification of *Paederia foetida* leaves that were gathered in June 2014 from the University of Dhaka's Botanical Garden, Curzon Hall.
- Leaf dried in the sun for seven days.
- After being dried, the leaves were crushed into a coarse powder using a high capacity grinding machine. They were then sealed in an airtight container and maintained in a cold, dry, and dark area for the duration of the inquiry. The leaf powder had the required marks for identification[13, 14].

Determination of acute toxicity (ALD50):

The research investigation assessed the acute toxicity of P. foetida methanol extract in albino mice housed in normal circumstances. Before the experiment, the animals were starved for the whole night. For toxicity investigations, the acute oral toxicity-acute toxic class technique (OCED Guideline no. 423, Annexure – 2d) established by CPCSEA, Government of India, was used. Following the oral administration of the 2000 mg/kg b.w test sample, the mortality was noted. During the seven days of monitoring, common adverse effects of treatment groups of animals were depression, weight loss, and moderate diarrhea[15].

HPTLC examination of P. foetida methanol extract:

Using a high-performance thin layer chromatography (HPTLC) technology, the methanol extract of P. foetida was assessed for β-sitosterol at 540 nm using qualitative estimate. Using a mobile phase consisting of toluene, ethyl acetate, and formic acid in an 8:2:0.05 v/v/v ratio, the commercially available β-sitosterol (Sigma-Aldrich, India) and methanol leaf extract of P. foetida were run concurrently. As a visualizing reagent, anisaldehyde sulphuric acid was used[16, 17].

Pharmacognostical Evaluation:

Macroscopic study:

The fresh plant material was subjected to morphological analyses, including measurements of color, size, odor, taste, surface characteristics, and breakage. Evans WC[15] and Indonesian Herb Pharmacopoeia[14] provided the keywords and descriptions that were used to study them. Photographs were shot in the original setting and the organoleptic features were documented and examined[14, 15].

Microscopic study:

Microscopic analysis was conducted on transverse slices of fresh leaves, stems, and roots. By hand, a transverse section was created for this purpose. Using a sharp razor, numerous small parts were cut from the leaf, stem, and root that were placed between the pith. Chloral hydrate solution was used to clean the acquired portions.

Chloral hydrate suspension was macerated with a tiny amount of various powder materials. A single drop of the solution was placed on a slide, warmed by a spirit lamp, and inspected under a microscope. Various tissues were examined under a microscope and captured on camera[16, 17].

Physicochemical Evaluation:

In accordance with official procedures and WHO recommendations for the quality control of medicinal plant material, physicochemical assessment of samples was conducted to determine loss on drying, moisture content, total ash, water soluble, and acid insoluble ash value. The drug's powdered extractive values (alcohol, water, and ether soluble) were ascertained using the procedures outlined in Indonesian Herb Pharmacopoeia[14]. The fluorescence characteristics of the powdered and extract material were seen and evaluated under both normal and ultraviolet light, both with and without chemical treatment[19].

Moisture content:

Up to 2 grams of the powdered material that had air dried was put in a watch glass, baked at 1050 degrees for a while, and allowed to dry until a steady weight was achieved. The weight differential between the powdered material's pre-

and post-oven drying states indicated its moisture content[14, 15].

Total Ash value:

The bottom of the constant weights crucible had up to 2 g of powdered material distributed in a thin, equal layer. Gradually raising the temperature to 400°C allowed it to ignite and become white, signifying that it is carbon-free. Following cooling, the crucible was weighed. Until the constant weights were reached, the process was repeated. Using the air-dried powdered sample as a guide, the total Ash value was computed[16].

Water-soluble ash value:

Five minutes were spent boiling the entire amount of ash in 25 milliliters of chloroform water. After being gathered on ash-free filter paper, the insoluble material was cleaned with hot water. Before being torched for 15 minutes at a temperature not to exceed 400oC, the insoluble ash was put into a pre-weighed silica crucible, cooled, and then weighed again until the weight remained constant. With reference to the air-dried medications, the percentage of water soluble ash was computed[15-16].

Acid-insoluble ash value:

For five minutes, 25 milliliters of diluted hydrochloric acid (2N) were heated with the resulting total ash. After gathering the insoluble ash on an ash-free filter and giving it a hot water wash, it was put into a silica crucible that had been previously weighed, lit, cooled, and weighed again until a consistent weight was reached. Using the powder sample that had been air-dried as a reference, the percentage of acid insoluble ash was determined. Acid-insoluble ash value: 25 ml of diluted hydrochloric acid (2N) was heated with the collected total ash for five minutes. The insoluble ash was gathered onto an ash-free filter, cleaned with hot water, and then placed into a silica crucible that had been previously weighed. It was then fired, cooled, and weighed again until a consistent weight was reached. Using the air-dried powder sample as a reference, the percentage of acid insoluble ash was computed[18, 19].

Ethanol-soluble extractive value:

Accurately weighed up to 10 g of coarsely powdered, air-dried material was macerated for 24 hours with 100 ml of 90% ethanol. The following phase was shaking it often for six hours before leaving it alone for eighteen. A dry filter was used to quickly filter the mixture. Up to 25.0 ml of the filtrate were evaporated until completely dry in a dish with a tarred bottom, dried at 105°C in the oven, and then weighed. Using the air-dried sample as a reference, the extractable matter value was determined[20].

Water-soluble extractive value:

Accurately weighed material that had been air-dried and coarsely powdered up to 10 g was macerated for 24 hours, shaking regularly for the first 6 hours, and then left to stand for 18 hours in a glass-stopper conical flask with 100 ml of chloroform water. In a flat-bottomed plate covered with tar, 25 milliliters of the filtrate were quickly filtered while being cautiously dried at 1050 degrees Celsius and weighed. Calculations were made using the air-dried medication to determine the percentage of the water-soluble extractive value[19-20].

Ether soluble extractive value:

We used ether to determine the extractable stuff in the ether by following the same approach as the water extractive value. With the medication air-dried, the ether extractive value was computed[11].

Fluorescence character:

As per the method described by Kokhasi19, a fluorescence research was conducted on powdered leaves. Both visible and ultraviolet (366 nm) light were used to view a tiny amount of the powder, either with or without chemicals. The substances included ammonium hydroxide, 50% sodium hydroxide, 50% nitric acid, 50% sulfuric acid, and 50% methanol[20, 21].

Phytochemical Screening:

Using 25 g of powdered material, a preliminary phytochemical screening was performed by extracting it successively for 30 minutes in a reflux device using 250 ml of ethanol20. A rotary evaporator was used to filter and concentrate the extraction. Using the ethanolic extract and established methods outlined by the Indonesian Herb Pharmacopoeia[14] and Harborne[21], many phytoconstituents including alkaloids, glycosides, steroids, phenolic compounds, tannins, flavonoids, and carotenoids were qualitatively analyzed[18-20].

Chromatographic Profile:

Using 10 g of powdered material, the chromatographic profile was created by successively extracting it in a Soxhlet device using 150 ml of hexane, dichloromethane (DCM), and 70% ethanol, respectively[22]. To concentrate the extracts, a rotary evaporator was employed. RF values were ascertained by performing a typical method of thin layer chromatography profile of n-hexane, DCM, and ethanolic extracts. The chromatographic profile was observed using three extracts that were produced on a silica gel 60 F254 TLC plate. The plates were created with a variety of solvent systems, including glacial water, n-hexane, ethyl acetate, and chloroform-ethyl acetate (6:4), as well as ethyl acetate-formic acid-acetic acid (5:0.5:0.5:1). Upon spraying with 10% sulfuric acid and heating at 105°C for 5–10 minutes, the dry plates were examined under UV and visible light. It was then decided what the RF values were[22].

Total Flavonoid Content:

A modified version of the aluminum chloride colorimetry technique was used to ascertain the total flavonoid concentration in the extracts. The calibration curve was created by diluting the extract sample with methanol up to 100µg/ml and diluting the quercetin standard in methanol

(0–100 µg/ml). 0.1 ml of a 10% (w/v) aluminum chloride solution and 0.1 ml of a 0.1 mm sodium acetate solution were combined with the diluted quercetin extract (2.0 ml). A half-hour at room temperature was spent incubating the combination. Next, a UV-Vis spectrophotometer was used to detect the mixture's maximum absorbance at 415 nm. Using the equation of the standard curve, the total flavonoid content was calculated and represented as mg of quercetin/g of extract[18, 19].

Experimental Animal:

The experiment involved the collection of Swiss albino mice from ICDDR, B, Dhaka, which were 4-5 weeks old, of either sex, and weighed between 10-24 gm. Standard environmental conditions were provided for the animals, including full access to food and water and a temperature of 27.0 ± 1.0 °C, relative humidity of 55–65%, and a 12-hour light/12-hour dark cycle. Prior to the studies, the animals were given a week to become used to being in a laboratory. The institutional animal ethics committee authorized all procedures pertaining to using animals in research[16, 17].

Gastrointestinal Motility Determination:

- The experiment was conducted on 48 Swiss Albino mice, weighing between 10 and 20 g, who were carefully chosen and kept in a suitable environment for 10 days. Six mice each group comprised the eight groups of animals on test day.
- They were weighed, given no food, but were allowed to drink water at will.
- Three hours following their deprivation of food, the animals in group 1 were given 5 ml/kg body weight of 0.9% NaCl (normal saline) orally by gavages as a control group, whereas group 2 received 5 mg/kg body weight of Butapen (hyocine butyl bromide) as the standard group.
- As indicated in the table, the remaining six groups were administered their corresponding dosages. Following ninety minutes, each animal received an oral gavage with 0.3 milliliters of an aqueous solution containing five percent charcoal in normal saline.
- After sixty minutes (150 minutes), they were able to freely obtain meals.
- The animals were watched for a maximum of 300 minutes, at 5-minute intervals, until the charcoal-containing feces were completely removed.
- When the black dots were clearly apparent, charcoal was noticed on the feces under normal light. If not, a microscope was used to aid identify them.
- The duration of the charcoal's elimination was the basis for the results[16].

Anti-pyretic Activity:

Before the experiment began, 48 Albino Swiss mice of both sexes (10–20 grams) were split into 8 groups at random and allowed to fast for the whole night with unrestricted access to water. Each mouse's normal body temperature was recorded and monitored rectally at pre-arranged intervals. The procedure outlined was followed to create a fever. A thermometer probe, lubricated, was inserted 3–4 cm deep into the rectum and secured to the tail using adhesive tape. Using a digital thermometer, the temperature was recorded. Upon taking the animals' baseline rectal temperature, 10 ml/kg of 20% w/v brewer's yeast in NSS was subcutaneously injected into the mice's dorsum. The mice were subsequently put back inside their cages[17, 18]. After receiving a brewer's yeast injection, the animals were once more sedated for the purpose of taking their rectal temperatures, as previously mentioned.

In this investigation, mice exhibiting a minimum 10°C rise in body temperature were employed. Four groups of animals received oral administration of the extracts at levels of 200 and 400 mg/kg body weight. Oral paracetamol (50 mg/kg body weight) was given to the standard group, while a 0.9% NaCl solution (1 ml/kg body weight) was administered to the control group. During the four hours following the extract/drug delivery, rectal temperature was recorded every hour. Normal (normothermic) rats' rectal temperatures were likewise taken every hour for seven hours. By applying Makonnan's formula[19], the findings are reported as a percentage of the pre-drug temperature that was observed for the identical animals.

Neuropharmacological Study:

Two types of experiments—an open field test and a swimming test—are conducted to examine the neuropharmacological effects or side effects of drugs[11, 12].

Open Field Test (OFT):

An open field test was used to observe the behavioral reactions of mice put in a novel and bright arena, in accordance with earlier research that was slightly modified20. Brightly lit locations tend to repel rodents. Additionally, a variety of locomotor activity and exploratory behaviors generated by anxiety are evaluated in this experiment. Eight groups of five mice each were created from the animals. The initial group was the control and received 10ml/kg of 1% Tween 80 orally. Group 2 received conventional treatment of 2 mg of Clonazepam per kg of body weight. Groups 3, 4 were given a methanol extract of P. foetida leaves at 200 and 400 mg/kg of body weight, whereas groups 5, 6 were given an ethanol extract at 200 and 400 mg/kg of body weight[20, 21]. 200 and 400 mg/kg of body weight of P. foetida leaf extracts were given to Groups 7 and 8. The hardboard used for the open field equipment is 60 cm by 60 cm with 40 cm walls. Thirty-six squares are created on the floor by blue lines. Squares measuring 10 cm by 10 cm, alternately painted in black and white, with a prominently indicated Central Square (10 cm

by 10 cm) in the center. After the experimental crude extracts were administered orally for 0, 30, 60, 90, and 120 minutes, the number of squares visited by the animals was measured for two minutes at each location[20].

Forced Swimming Test (FST):

Swimming tests were conducted with minor modifications, according to Porsolt 21. The eight groups of animals—five mice each—were randomly selected. A standard of 2 mg of Clonazepam per kg of body weight was administered to Group 2, whereas a control group of 1 received 10 ml/kg of 1% Tween 80. Two hundred and four hundred mg/kg of body weight of P. foetida leaf methanol extracts were given to groups three and four. A 200 and 400 mg/kg body weight ethanol extract of P. foetida leaf was given to groups 5 and 6. P. foetida chloroform extracts were administered at 200 and 400 mg/kg of body weight to groups 7 and 8. In an open acquire water tank device of 29 cm by 19 cm by 20 cm and holding 9 cm of water at 25 ± 1°C, mice were individually subjected to the forced swim test. The four minutes of immobility were assessed according to the guidelines provided. When a mouse stopped struggling and stayed in the water without moving, it was considered immobile and could only move in order to maintain its head above the water. The length of the immobility was noted. The FST's reduced immobility period was used to gauge the antidepressant's effectiveness[21].

Antioxidant activity:

Coupled oxidation of β-carotene and linoleic acid:

The tests for bleaching β-carotene were carried out with minor alterations from the earlier descriptions[21, 22]. Tween® 40 (20 ml, Sigma Chemical Co.), linoleic acid (1.0 g, Sigma Chemical Co.), and β-carotene (60 mg, Sigma Chemical Co.) were combined and dissolved in 20 ml of Merck's chloroform. Using a rotary evaporator, the chloroform was eliminated at 40 °C. The mixture was added right away to 25 milliliters of oxygenated distilled water after it evaporated to create an emulsion. Test tubes containing extracts (1.0 ml) were filled with the emulsion (25 ml), and the mixture was then carefully stirred. Five milliliters of 95% ethanol were pipetted into one milliliter of the mixture at 0°C. Using a Hitachi U-2000 Spectrophotometer, the absorbance of the samples at 450 nm was measured in triplicate every 20 minutes for a total of 160 minutes. Using DL-α-tocopherol (Sigma Chemical Co.) and quercetin (Sigma Chemical Co.) as standards, the aforesaid process was repeated. With the same sample concentration, a blank solution devoid of β-carotene was made. Using the following formula, the total antioxidant activity was determined: AA is equal to [1 - (As 0 - As 160)/(Ac 160 - Ac 160)]. X 100, where as 0 represents the sample's absorbance at 0 minutes, As 160 represents the sample's absorbance at 160 minutes, Ac 0 represents the control sample's absorbance at 0 minutes, and Ac 160 represents the control sample's absorbance at 160 minutes[20, 21].

Quantitative determination of total phenolic content:

By applying a modified Folin Ciocalteu technique with ferulic acid (Sigma Chemical Co.) as a standard, the total phenolic content of the crude methanol extract was ascertained25. The tube was vortexed, 20% aqueous sodium carbonate solution (1.2 ml) was added, and the mixture was then incubated for 40 minutes. The methanolic extract solution (1.0 mg mL-1, 10 ml) was mixed with 0.25 ml of Fluka's folin-Ciocalteu reagent. The absorbance was measured at 725 nm using a Hitachi U-2000 Spectrophotometer, and a blue hue emerged. There were three duplicates of each measurement, and the findings were reported as milligrams of ferulic acid per gram of material[17, 18].

Analysis of Intracellular Tyrosinase Activity:

Equal numbers of cells were seeded and exposed to PFE at different concentrations (50, 100, and 200 µg/ml) in the melanin contents test. Following centrifugation to remove the media, the cell pellets were treated in lysis buffer (radio-immuno precipitation assay) containing a 1% protease/phosphatase inhibitor cocktail for 10 minutes in order to test for tyrosinase activity. To lyse the cells, they were scraped, collected in an e-tube and vortexed three times every ten minutes. Using 0.1M sodium phosphate buffer to dilute 80 µL of L-DOPA (2 mg/ml) and 20 µL of each measured protein sample, the mixture was put and incubated at 37◦C for two hours after centrifugation at 15,000 rpm for 30 minutes at −8◦C. A single measurement of the absorbance at 490 nm was made for each concentration following a two-hour reaction to determine the optical density (O.D) value[19-21].

Analysis of Cell Melanin Content:

After a day, it was verified that all of the cells were fully adhered to the 6-well plate that they had been distributed onto at a concentration of 5.0 × 104 cells/well. Afterwards, each well received a duplicate pretreatment of PFE diluted with different quantities, followed by a two-hour incubator. After treating the cells with α-MSH (200 nm), the cells were incubated for an additional 72 hours at 37 ºC. Following the completion of the cell culture, the medium was withdrawn, the color of the cell pellet was noted, and the cells were twice washed with phosphate buffered saline (PBS) in order to analyze the melanin content of the cell. Following the direct addition of 1 ml of 1N NaOH containing 10% DMSO to each well, the cells were lysed at 80 °C for 1 hour at 300 rpm, and the absorbance at 405 nm was determined[22].

Cytotoxic Activity:

Paederoside, an iridoid glycoside isolated from P. scandens, demonstrated significant antitumor effectiveness by preventing tumor promoters from prematurely activating the Epstein-Barr virus. In the test, paederoside's claimed inhibition was higher (89.5%) than genipin's, suggesting that paederoside might be a good option for cancer treatment avoidance [45]. This finding

suggests that paederoside could be used as a preventative measure against cancer. Regarding its antitumor mechanism of action in vivo, more research is necessary. Recent research by Yu et al. from P. scandens produced four ursane-type triterpenoids: 3β, 13β-dihydroxy-urs-11-en-28-oic acid, 2α, 3β, 13β-trihydroxyurs-11-en-28-oic acid, 2α-hydroxyl ursolic acid, and ursolic acid. With IC50 values ranging from 1.9 to 65.3 µmol/L, these triterpenoids showed strong cytotoxic effects in HepG2, HL-60, and Mata cell lines [15]. Unfortunately, P. foetida extract was not able to block NF-κB/DNA interactions, according to the findings of the electrophoretic mobility shaft assay (EMSA) on many human cell lines (erythroleukemia K562, B-lymphoid Raji, T-lymphoid jurkat, and erythroleukemia HEL cell lines). P. foetida may not be an anti-tumor source, according to this[18-22].

RESULTS

Test evaluating Gastrointestinal Motility (GI):-

Table 1 illustrates the results of the test for gastrointestinal motility. In the current study, 100 and 200 mg/kg body weight of methanol, ethanol, and chloroform extract were administered.

In contrast to the effect produced by normal saline, the results showed that the doses of ethanol extract (100 and 200 mg/kg b.w.) and higher the dose of chloroform extract (200 mg/kg b.w.) showed maximum charcoal defection time[15-17].

Table1: Evaluating GI motility using the charcoal defection time (min)

Group	Doses (mg/kg)	Charcoal defection Time (min)
Control (0.9%Nacl)	5ml/kg	90.2±3.21
STD (Butapan)	5mg/kg	130 ±4.44
Methanol extract	100	96.8±7.61
	200	102.2±7.007
Ethanol extract	100	120.6± 14.90
	200	133.4± 8.50
Chloroform extract	100	93.8±7.58
	200	113.8± 3.50

(*p<0.05; **p<0.01; ***p<0.001 significant when compared with the comparable value of the control group. Values are expressed as mean ± S.D. (n=6).)

Antioxidant activity:

Coupled oxidation of β-carotene and linoleic acid:

The tests for bleaching β-carotene were carried out with minor alterations from the earlier descriptions[21, 22]. Tween® 40 (20 ml, Sigma Chemical Co.), linoleic acid (1.0 g, Sigma Chemical Co.), and β-carotene (60 mg, Sigma Chemical Co.) were combined and dissolved in 20 ml of Merck's chloroform. Using a rotary evaporator, the chloroform was eliminated at 40 °C. The mixture was added right away to 25 milliliters of oxygenated distilled water after it evaporated to create an emulsion. Test tubes containing extracts (1.0 ml) were filled with the emulsion (25 ml), and the mixture was then carefully stirred. Five milliliters of 95% ethanol were pipetted into one milliliter of the mixture at 0°C. Using a Hitachi U-2000 Spectrophotometer, the absorbance of the samples at 450 nm was measured in triplicate every 20 minutes for a total of 160 minutes. Using DL-α-tocopherol (Sigma Chemical Co.) and quercetin (Sigma Chemical Co.) as standards, the aforesaid process was repeated. With the same sample concentration, a blank solution devoid of β-carotene was made. Using the following formula, the total antioxidant activity was determined: AA is equal to [1 - (As 0 - As 160)/(Ac 160 - Ac 160)]. X 100, where as 0 represents the sample's absorbance at 0 minutes, As 160 represents the sample's absorbance at 160 minutes, Ac 0 represents the control sample's absorbance at 0 minutes, and Ac 160 represents the control sample's absorbance at 160 minutes[18-22].

DISCUSSION

The results of this investigation unequivocally showed that S. aqueum and P. foetida both exhibit strong antioxidant activity. Compared to dried samples, fresh samples showed stronger antioxidant activity and higher phenolic levels. Between P. foetida leaves and twigs, there is not much of a variation in total phenolic content. An excellent correlation (R2 = 0.9878) was found between the β-carotene oxidation and ABTS techniques. According to the current study, P. foetida and S. aqueum may be important sources of naturally occurring antioxidant chemicals with strong potential health benefits[15, 16,17].

The current in vivo experiment provided the evidence needed to support P. foetida's use in the management of peptic ulcers. The present study further solidifies the results of our earlier in vitro research. The cholinergic action of Nrf2 mediated antioxidant and anti-secretary properties may mediate P. foetida's ulcer-preventive function. The methanol extract's β-sitosterol's anti-inflammatory and anti-gastro ulcerative action may play a crucial part in P. foetida's ulcer-preventive activities. None the less, research is being done to determine how the extract's primary active ingredients contribute to its ulcer-preventive properties[11-15].

An estimated 30% of adult Western populations report having suffered from abdominal pain and cramps on a regular basis. The anti-cholinergic medication hyoscine butyl bromide has a strong affinity for muscarinic receptors on the smooth muscle cells of the gastrointestinal tract, according to pharmacological research. The smooth-muscle relaxing/spasmolytic effect is a result of its anti-cholinergic. When compared to the effect generated by normal saline (control), the drug significantly decreased (*p<0.05; **P<0.01; ***P<0.01) intestinal motility due to its outstanding anti-motility effect on ethanol extract 100& 200 mg/kg b.w. and

chloroform extract 200 mg/kg b.w. The reason for its application in the management of cramp-related abdominal discomfort is its blockade of muscarinic receptors in the GI tract. But hyoscine butyl bromide is still accessible at the site of action in the colon and continues to have a local spasmolytic effect due to its strong tissue affinity for muscarinic receptors[19-21].

We have listed the current conventional medical applications as well as the results of phytochemical, pharmacological, and clinical research on the well-researched Paederia species P. foetida and P. scandens. Numerous scholarly citations demonstrate that contemporary pharmacological research has assessed the traditional medicinal applications of these two Paederia species. More information regarding the pharmacology and mechanism of action of these herbs is required to gain a better understanding of the theoretical underpinnings of Traditional Chinese Medicine as well as the relationship between traditional uses and contemporary pharmacology of these two Paederia species, despite the fact that a growing number of studies have been conducted on the subject[17, 18, 20].

Source of Support: The author(s) received no financial support for the research, authorship, and/or publication of this article

Conflict of Interest: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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