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Investigation of Antioxidant, Analgesic, Antimicrobial, and Anthelmintic Activity of the Aerial parts of *Paederia foetida* (Family: Rubiaceaea)

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ABSTRACT

Paederia foetida Linn. (Family: Rubiaceae) has been traditionally used in Bangladesh as medicinal plant for the treatment and prevention of different diseases. The aim of the present study is to investigate the antioxidant, analgesic, antimicrobial, and anthelmintic activities of the aerial parts of *P. foetida*. Preliminary phytochemical analysis of ethanol extract of the aerial parts of *P. foetida* Linn. exhibited the presence of carbohydrate, glycosides, tannins, alkaloids, acidic compounds, steroids, terpenoids, and flavonoids. In the TLC-based qualitative antioxidant assay using DPPH, *P. foetida* extract showed free radical scavenging properties indicated by the presence of yellowish white spot on a purple background on the TLC plate. In the quantitative antioxidant assay using DPPH, IC_{50} value was approximately 75.52 µg/mL, where IC_{50} value of the ascorbic acid was approximately 15.78 µg/mL. *P. foetida* extract displayed hydroxyl radical scavenging activity (SC_{50} = 196.03 mg/L) which was comparable to the standard ascorbic acid (SC_{50} = 80.83 mg/L). The total phenolic content (TPC) and total tannin content (TTC) were found to be ~55.36 mg and ~36 mg GAE/gm of the dried plant extract respectively, using gallic acid calibration curve. Total flavonoid content was determined ~60 mg QE/gm of dried plant extract using quercetin calibration curve. The extract showed antibacterial activity against *S. aureus, V. cholerae,* and *P. aeruginosa* species at the dose of 250 and 500 µg/disc in comparison with standard drug ciprofloxacin (30 µg/disc) and flucloxacillin (30 µg/disc). The extract showed significant ($p<0.01$) acute peripheral analgesic activity at the doses of 250 mg/kg (34.66% writhing inhibition) and 500 mg/kg (65.91% writhing inhibition) determined by acetic acid induced writhing reflex in mice as compared to control Diclofenac sodium (79.55%). In the anthelmintic test, the extract showed dose dependent decrease in paralysis and death time of the parasites (Trematode and Nematode). The phytochemicals present in this plant may be responsible for the aforementioned pharmacological effects.

Keywords: *Paederia foetida* Linn, phytochemical study, antioxidant activity, antimicrobial activity, analgesic activity, anthelmintic activity.

1. INTRODUCTION

Plants produce a rich and diverse array of natural products. Plants made many chemical compounds for their important biological functions, including defense against insects, fungi, and herbivorous mammals. These chemicals are also exploited as pharmaceutical drugs, agrochemicals, within the food and drink industry, and for a wide variety of other industrial biotechnology applications.

Paederia foetida Linn. (Family: Rubiaceae) is commonly known as gandhabhaduli, gandhal, and gandhaprasarini. It is found in the Himalayas up to an altitude of 1800 meters above sea level. As its common name suggests, *P. foetida* is a bad-odoring climber. It produces slender stems that may reach 30 feet (9 m) long. Leaves are evergreen, opposite and heart-shaped. Sulfur compounds in the leaves and stems give the herb its smelly odor.[1] Flowers are short-lived and fruits are round, about the size of a large pea. Fruits contain 1 or 2

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wingless seeds that are up to 5.5 mm long.[1]

Leaves are rich in carotene and vitamin C; and also contain high protein content, consisting of arginine, histidine, lysine, tyrosine, tryptophan, phenylalanine, cystine, methionine and valine. Aerial parts also contain a crystalline keto alcohol paederolone, a keto compound, paederone, β- and δ-sitosterols and two volatile alkaloids, paederine and paederenine. Leaves contain a volatile oil of an offensive odor due to the presence of methyl mercaptan hentriacontane, hentriacontanol, methyl mercaptan, ceryl alcohol, palmitic acid, sitosterol, stigmasterol, campesterol, ursolic acid and iridoid glycosides - paederoside, paederosidic acid, scandoside, asperuloside and deacetyl asperuloside have also been isolated from leaves and stems.[2, 3]

P. foetida is useful in relieving a number of ailments like rheumatism, paralysis, abscesses, gout, diarrhea, dysentery, infertility, colic and flatulence. The entire plant is tonic, diuretic, astringent, and aphrodisiac. The leaves are tonic, styptic, vulnerary and astringent; used as a remedy in diarrhea, dysentery, herpes infection and to relive distension due to flatulence. The fruits of the plant are used to relieve tooth pain and for whitening blackened teeth. Juice of the root is used as an emetic, useful in piles and beneficial for relieving spleen inflammation as well as pain in the chest and liver.[4] This study was conducted on the aerial parts of *P. foetida* to explore the antioxidant, analgesic, antimicrobial, and anthelmintic activities.

2. MATERIALS AND METHODS

2.1 Plant identification and collection

The aerial part of *P. foetida* was collected from the Khulna region (Azad Nursery, Joragate, Khulna). The time of collection was July 20, 2016 at the daytime. During collection, any type of adulteration was strictly prohibited. The plant was identified by the experts of Bangladesh National Herbarium, Mirpur, Dhaka, where a voucher specimen was submitted (voucher specimen no. 44944 DACB**)** for future reference.

2.2 Preparation of the plant extract

The aerial part of plant was dried in shade to ensure the active constituents free from light decomposition. After complete drying, the sample was cut into small pieces and then slashed to coarse powder with the help of mechanical grinder and the powder was stored in a suitable container. About 150 gm of powder was extracted by maceration over 15 days with 800 mL 96% ethanol accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material. Then it was filtered through filter paper. Then concentrated filtrate was evaporated using rotary evaporator at 25^0C. Thus, the extract obtained 3.49gm was used for experimental purposes.

2.3 Drugs

The standard drugs Diclofenac sodium and Albendazole powder were collected from Beximco Pharmaceuticals Ltd. Dhaka, Bangladesh. Marketed Ciprofloxacin and Flucloxacillin (ACI Pharmaceuticals Ltd.) prepared disc were used as standards for antibacterial assay.

2.4 Experimental animals

Swiss-Albino mice of either sex (20-25 gm body weight) were collected from animal resources branch of the International Center for Diarrhoeal Disease Research, Bangladesh (ICDDR, B) and were used for the experiments. The animals were kept in the standard polypropylene cages and provided with standard diets (ICDDR, B formulated) and tap water. The animals were allowed to acclimate for 7 days before starting the experiments in the animal house of the Pharmacy Discipline, Khulna University, Bangladesh under standard Laboratory conditions (relative humidity 55-60%, room temperature 25 ± 2^0 C and 12 hours light: dark cycle).

2.5 Microorganisms

Both Gram-positive and Gram-negative bacterial strains were collected from the Microbiology Lab. of

Pharmacy Discipline, Khulna University, Khulna, Bangladesh.

2.6 Phytochemical tests

The crude extract was subjected to preliminary phytochemical screening for the detection of major functional groups according to Evans *et al*.[5] Then, the extract was used for pharmacological screening.

2.7 Determination of Antioxidant Activity by DPPH scavenging assay

2.7.1 Qualitative Analysis

Antioxidant properties of *P. foetida* was evaluated by DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical scavenging assay. DPPH based qualitative analysis was performed according to the method followed by Sadhu *et al*.[6] Commercially prepared TLC plate was used. The sample and ascorbic acid were spotted. Here, ascorbic acid was used as standard. The chromatogram was developed by ascending technique using three types of solvent systems i.e. non polar (*n*-hexane: Ethyl acetate = 2:1), medium polar ($CHCl_3$: CH_3OH = 5:1) and polar ($CHCl_3$: CH_3OH: H_2O = 40:10:1). The solvent system was allowed to move up to a previously marked line. The plates were then dried at ambient temperature. The plates were viewed under UV detector both in short (254 nm) and long (360 nm) wavelengths. DPPH forms deep pink color when it dissolved in 0.02 % ethanol solution. When it was sprayed on the chromatogram of the extract, it formed pale yellow or yellow color which indicates the presence of antioxidants.

2.7.2 Quantitative Analysis

DPPH based quantitative analysis of seeds of *P. foetida* was performed according to the method followed by Lu, Shipton *et al*.[7] Ten (10) concentrations (1, 2, 4, 8, 16, 32, 64, 128, 256, and 512 µg/mL) of plant extract and ascorbic acid were prepared in DMSO by dilution technique and DPPH was dissolved in methanol to make 0.007886% (w/v) solution. 10 µL of different concentrations of plant extracts and ascorbic acid were taken in each well of the micro plate and 190 µL DPPH in methanol was added to each well and 190 µL of DPPH was applied as blank. After mixing for 5 minutes in the micro plate the sample in the plate was kept standing at room temperature in light for 30 minutes, absorbance was measured at 517 nm using micro plate reader.

2.8 Determination of Antioxidant Activity by Hydroxyl Radical Scavenging Assay

The hydroxyl radical scavenging activity can be measured by studying the competition between deoxyribose and the plant extracts for hydroxyl radicals generated with Fe^{3+} /ascorbate/EDTA/H_2O_2 system.[8,9] Hydroxyl radical scavenging activity of the examined compounds was measured based on the method of Halliwell, Gutteridge *et al*.[10] with a slight modification according to Jiang, Gutteridge *et al*.[11] 0.5 mL 2-deoxy 2-ribose solution (2.8 mM) was mixed with 12.5 µL of different concentrations of sample extracts or standard. Then 1 mL of 200 µM $FeCl_3$, 1 mL of 1.04 mM EDTA, 0.5 mL of 1 mM H_2O_2 and 0.5 mL of 1 Mm of ascorbic acid were added to prepare the reaction mixture. Then the test tubes were incubated for 1 hour at 37^0 C. After incubation, 3.75 mL of 2.8% TCA (Trichloroacetic acid) and 3.75 mL of 1% TBA (Thiobarbituric acid) were added and kept at 100^0 C for 20 minutes. The absorbance was measured at 530 nm. Blank was prepared simultaneously containing all the reagents except the extract and the standard.

2.9 Determination of Total Phenolic Content (TPC)

Folin-Ciocalteau Colometry method is based on Folin-Ciocalteau (FC) reagent, a mixture of phosphomolybdate and phosphotungstate. It detects all the potentially oxidizable phenolic groups in extracts. The method is based on the reaction of FC Reagent with different types of phenols, which involves the oxidation of phenolic compounds by FC reagent in alkaline medium, producing a blue colored complex. The produced complex was then measured using UV spectrophotometer at 765 nm. Thus, the method

provides a very useful index of phenolic content. In such case, Gallic acid is usually used as reference standard and the result is usually expressed as gallic acid equivalence.[12] Total phenolic content was determined by modified Folin-Ciocalteu method.[13] 0.005 g of plant extract was mixed with 5 mL of methanol and 0.01 gm gallic acid was dissolved in 10 mL methanol. A standard solution of 8 concentration of the gallic acid was prepared by serial dilution method. 0.5 mL standard solution of each concentration (0.15, 0.1, 0.08, 0.06, 0.04, 0.02 mg/mL) and 0.5 mL sample extract were taken separately into different test tubes. Then 5 mL of dilute FC reagent (1/10) was added to every test tube. Then 4 mL solution of 7% Na_2CO_3 was added to it and vortex for 15 seconds. Then they were kept at 40^0C temperature. Blank was prepared by following all the above steps except the addition of gallic acid and sample. After 30 minutes, the UV absorbance was measured at 765 nm.

2.10 Determination of Total Tannin Content (TTC)

Folin-Ciocalteau Colometry method is based on Folin-Ciocalteau (FC) reagent, a mixture of phosphomolybdate and phosphotungstate. It detects the number of potentially oxidizable phenolic groups in extracts. The method is based on the reaction of FC Reagent with different types of phenols, which involves the oxidation of phenolic compounds by FC reagent in alkaline medium, producing a blue colored complex. The produced complex is then measured using UV spectrophotometer at 725 nm. Thus, the method provides a very useful index for tannin content. In such case, Gallic acid is usually used as standard and the result is usually expressed as gallic acid equivalence.[14] Total tannin content was determined by modified Folin-Ciocalteu method.[15] 0.005 gm extract of *Paederia foetida* was mixed with 5 mL of ethanol and 0.01 gm gallic acid was dissolved in 10 mL ethanol. At first, 0.1 mL standard solution of each concentration (0.5, 0.4, 0.3, 0.2, 0.1 mg/mL) and 0.1 mL extract sample were taken separately in different test tubes. 7.5 mL of distilled water was added to the each test tube. Then 0.5 mL of FC reagent was added to the test tube. 1 mL of 35% Na_2CO_3 was added to the test tube and the solution was diluted Q.S. to 10 mL with distilled water. Then all test tubes were vortexed for 15 second and kept at room temperature. Blank was also prepared by following all the above steps except the addition of gallic acid and sample. After 30 minutes the absorbance of the solution was measured at 725 nm.

2.11 Determination of Total Flavonoid Content (TFC)

The total flavonoid content determined by aluminum chloride method may represent the real content of total flavonoids. The principle of aluminum chloride colorimetric method is that aluminum chloride forms acid stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonols.[16] In addition, aluminum chloride forms acid labile complexes with the orthodihydroxyl groups in the A- or B-ring of flavonoids. The produced complexes are then measured at 510 nm. Thus, the method provides a very useful index for flavonoid content.[17] For total flavonoid content determination, quercetin is usually used as reference standard and the result is usually expressed as quercetin equivalence. Total flavonoid content was determined by Aluminum trichloride colorimetric method proposed by Zhishen, Mengcheng *et al*.[18] In this method 0.005 gm of *Paederia foetida* extract was weighed and mixed with 5 mL methanol. It was sonicated for 20 min. 0.01 gm Quercetin in methanol Q.S. to 10 mL. At first, 1 mL standard solution of each concentration (1, 0.75, 0.5, 0.25, 0 mg/mL) and 1 mL sample extract was taken separately into different test tubes. Then 4 mL of distilled water was added and 0.3 mL of 5% w/v $NaNO_2$ was added to every test tube and kept for 5 minutes. Then 0.3 mL of 10% w/v $AlCl_3$ solution and 2 mL of 1 M NaOH was

also added to every test tube and added distilled water Q.S. to 10 mL of the solution. Then they were kept for 15 minutes at room.[19] After 15 minutes, the UV absorbance was measured at 510 nm against blank for each concentration, the absorbance was taken for two times and the mean was used for accuracy.

2.12 Antibacterial activity

Antibacterial activity of the ethanol extract of *P. foetida* was determined using disc diffusion method followed by Valgas *et al*. 2007 & Ahmed *et al.* 2003.[20, 21] Nutrient agar and nutrient broth media was prepared by adding water to a dehydrated product that contains all the ingredients (peptone, beef extract, yeast extract, sodium chloride, and agar). Practically all media are available commercially in powdered form. Sample impregnated discs (5 mm filter paper discs were dissolved in sample solution at the concentration of 250 and 500 µg/10µL of 50% ethanol), standard antibiotic discs (Ciprofloxacin & Flucloxacillin) and negative control discs (50% ethanol) were placed gently on the seeded agar plates with the help of sterile forceps to assure complete contact with medium surface. The plates were then inverted and kept in refrigeration for about 2 hours at 4°C to allow the material to diffuse into a considerable area of the medium. Finally, the plates were incubated at 37°C for 16–18 hours. After proper incubation, the antibacterial activity of the test agent was determined by measuring the diameter of zone of inhibition in terms of millimeter with a slide calipers.

2.13 Determination of analgesic activity

The analgesic activity of the extract of *P. foetida* was studied using acetic acid induced writhing model in mice. Experimental animals were randomly selected and divided into four groups, denoted as Control group, Positive control group and Test group I and Test group II, consisting of five (05) mice in each group. Control group received orally 1% Tween-80 at the dose of 10 mg/kg body weight and Positive control group received orally diclofenac sodium at the dose of 25 mg/kg body weight. Test group I and Test group II were treated with test sample orally at the doses of 250 and 500 gm/kg body weight. A thirty minutes interval was given to ensure proper absorption of the administered substances. Then the writhing inducing chemical, acetic acid solution (0.7%) was administered intra-peritoneally to each of the animals of a group. Five (05) minutes were given for absorption of acetic acid and number writhing was counted for 15 minutes. The animals do not always perform full writhing. The incomplete writhing was taken as half-writhing and so two half-writhing were taken as one full writhing. This is why total writhing was halved to convert all writhing to full writhing or real writhing according to Whittle, *et al.* 1964 and Ahmed *et al.* 2004.[22, 23]

2.14 Anthelmintic activity test

Anthelmintic activity of the plant extract was determined based on Hossain *et al*.[24] Live parasites (Trematode & nematode) were collected from freshly slaughtered cattle at local abattoirs. After cleaning, parasites were stored in 0.9% phosphate-buffered saline (PBS) of pH 7.4 prepared with 8.01 gm NaCl, 0.20 gm KCl, 1.78 gm Na_2HPO_4 and 0.27 gm KH_2PO_4 in 1 litre of distilled water at 37±1°C. Extract at the concentrations of 25 and 50 mg/mL and reference standard albendazole at the concentrations of 15 mg/mL of 10 mL in PBS were prepared and transferred to Petri dishes. Control group was treated with 0.1% tween-80 in PBS. Six parasites were placed in each Petri dish and observed. The time of paralysis was recorded when no movement was observed unless shaken vigorously. The death time was recorded after evaluating that the parasites did not move when shaken vigorously, dipped in warm water (50°C) or subjected to external stimuli. Anthelmintic activity is expressed as the time required for paralysis and death of parasites as compared to control.

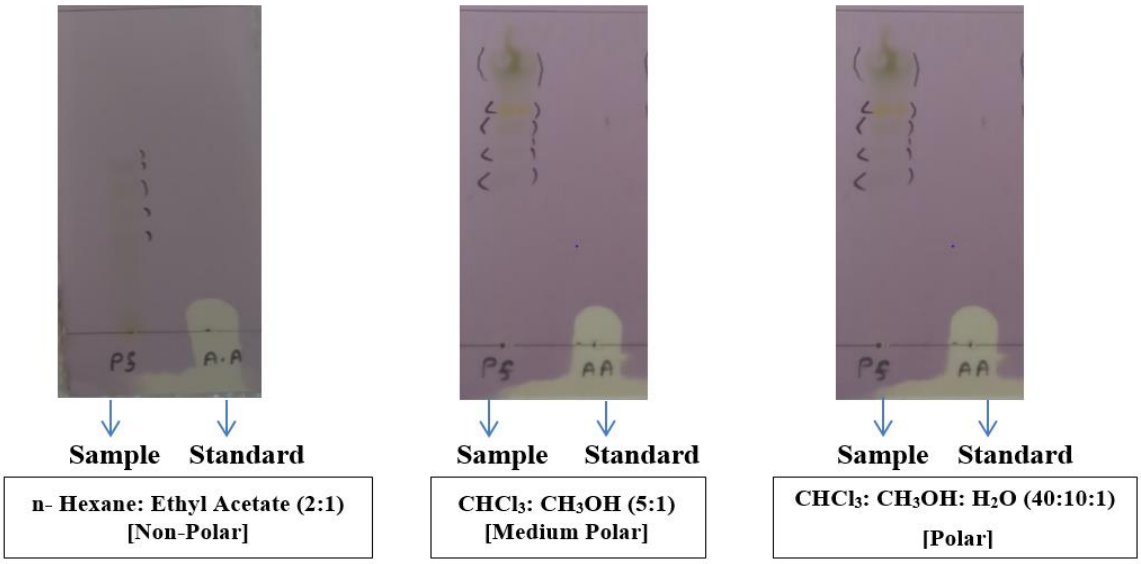

Figure 1: Comparison of TLC plate for *P. foetida* with Standard (Ascorbic acid) after applying 0.02% DPPH solution of ethanol.

3. RESULTS

3.1 Phytochemical tests

The preliminary phytochemical screening of *P. foetida* extract exhibited the presence of carbohydrate, glycosides, tannins, alkaloids, acidic compounds, steroid, terpenoids and flavonoids (Table 1).

3.2 Antioxidant Activity test by DPPH scavenging assay

In the TLC-based qualitative antioxidant assay using DPPH, *P. foetida* extract showed free radical scavenging properties indicated by the presence of yellow spot on a purple background of the TLC plate (Fig. 1). This free radical scavenging activity is comparable to that of L-ascorbic acid, a well-known antioxidant. In the quantitative antioxidant assay, *P. foetida* revealed free radical scavenging activity in the DPPH assay with approximate IC_{50} value 75.52 µg/mL, whereas the IC_{50} of the standard ascorbic acid was 15.78 µg /mL (Table 2 & 3, Figure 2).

Table 1. Presence and absence of different phytochemical groups

Constituents	Result
Reducing sugar	-
Tannins	+
Flavonoids	+
Saponin	-
Carbohydrate	+
Steroids	+
Alkaloids	+
Glycoside	+
Proteins	-
Terpenoids	+
Acidic compounds	+

Table 2. DPPH Scavenging Assay of Ascorbic acid.

Conc.(µg/mL)	Log conc.	Abs.1	Abs. 2	Abs. 3	Average	%Inhibition	IC_{50} (µg/mL)
Control	0	0.808	0.814	0.805	0.809		
1	0	0.759	0.763	0.768	0.763	5.67	
2	0.3	0.699	0.703	0.707	0.703	13.10	
4	0.6	0.638	0.621	0.649	0.636	21.38	
8	0.9	0.541	0.556	0.521	0.539	33.37	
16	1.2	0.398	0.377	0.392	0.389	51.92	**15.78**
32	1.51	0.267	0.260	0.240	0.256	68.36	
64	1.81	0.145	0.147	0.152	0.148	81.71	
128	2.11	0.079	0.083	0.069	0.077	90.48	
256	2.41	0.038	0.035	0.043	0.039	95.18	
512	2.71	0.029	0.026	0.030	0.028	96.54	

Table 3. DPPH Scavenging Assay of *P. foetida.*

Conc.(µg/mL)	Log conc.	Abs. 1	Abs. 2	Abs. 3	Average	%Inhibition	IC_{50} (µg/mL)
Control(Blank)	0	0.808	0.814	0.805	0.809	0.0	
1	0	0.760	0.760	0.757	0.759	6.18	
2	0.3	0.719	0.721	0.715	0.718	11.25	
4	0.6	0.657	0.663	0.649	0.656	18.91	
8	0.9	0.603	0.601	0.605	0.603	25.46	
16	1.2	0.552	0.548	0.560	0.554	31.52	
32	1.51	0.494	0.498	0.488	0.493	39.06	**75.52**
64	1.81	0.435	0.431	0.439	0.435	46.23	
128	2.11	0.362	0.365	0.357	0.361	55.38	
256	2.41	0.288	0.283	0.293	0.288	64.40	
512	2.71	0.204	0.203	0.205	0.204	74.78	

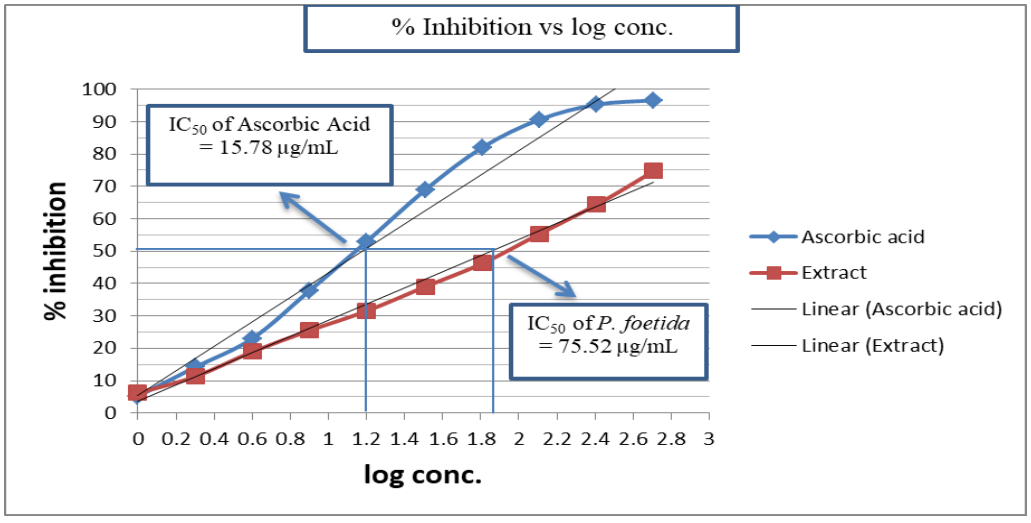

Figure 2: % of inhibition vs. log concentration graph for standard (ascorbic acid) vs. *P. foetida* in DPPH scavenging Assay.

3.3 Antioxidant Activity test by Hydroxyl Radical Scavenging Assay

In the assay, *P. foetida* extract possesses hydroxyl radical scavenging activity (SC_{50} = 196.03 mg/L) which is comparable to that of ascorbic acid (SC_{50} = 80.830 mg/L) (Table 4 & 5, Figure 3).

Table 4: Hydroxyl Radical Scavenging Assay of Ascorbic acid.

Conc. of Ascorbic acid (mg/L)	Log conc.	Abs.1	Abs. 2	Abs. 3	Average	% Scavenged	SC_{50} (mg/L)
Control (Blank)	--	0.707	0.698	0.701	0.702		
6.25	0.796	0.640	0.643	0.640	0.641	8.69	
12.5	1.097	0.590	0.593	0.587	0.590	15.95	
25	1.401	0.503	0.509	0.511	0.508	27.64	
50	1.701	0.419	0.423	0.415	0.419	40.31	80.83
100	2	0.331	0.339	0.335	0.335	52.28	
200	2.301	0.240	0.238	0.240	0.239	65.95	
400	2.602	0.142	0.147	0.145	0.145	79.34	
800	2.903	0.058	0.049	0.054	0.053	92.45	

Table 5: Hydroxyl Radical Scavenging Assay of *P. foetida*.

Conc. (mg/L)	Log conc.	Abs.1	Abs. 2	Abs. 3	Average	% Scavenged	SC_{50} (mg/L)
Control (Blank)	--	0.707	0.698	0.701	0.702		
6.25	0.796	0.654	0.661	0.659	0.658	6.27	
12.5	1.097	0.610	0.605	0.612	0.609	13.25	
25	1.401	0.548	0.551	0.554	0.551	21.65	
50	1.701	0.493	0.499	0.501	0.498	29.06	196.03
100	2	0.446	0.453	0.451	0.450	35.90	
200	2.301	0.383	0.377	0.380	0.380	45.87	
400	2.602	0.288	0.295	0.292	0.292	58.40	
800	2.903	0.146	0.151	0.153	0.150	78.63	

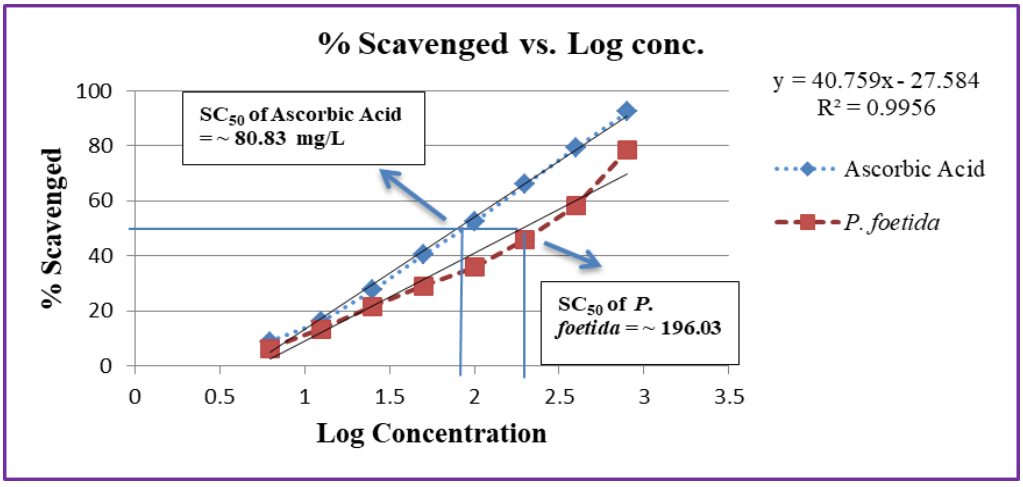

Figure 3: Comparison of % Scavenged versus log conc. for ascorbic acid and *P. foetida*.

3.4 Determination of Total Phenolic Content (TPC)

In the present study, Gallic acid calibration curve (Figure 4) was drawn by plotting absorbance versus concentration. Absorbance of both the sample and the standard was taken at 765 nm. The obtained equation, y = 7.2658x+0.0758; R^2= 0.9784 from gallic acid calibration curve was used to get Gallic Acid Equivalent (GAE) and GAE was subsequently applied to determine total phenolic content of *P. foetida* extract. The total phenolic content of *P. foetida* extract was ~55.36 mg GAE/gm of dry extract. (Table 6, 7 & 8).

Table 6. UV Absorbance of gallic acid (standard) at 765 nm.

Concentration (mg/mL)	Absorbance 1	Absorbance 2	Average
0.15	1.159	1.160	1.1595
0.10	0.875	0.871	0.873
0.08	0.623	0.619	0.621
0.06	0.443	0.441	0.442
0.04	0.371	0.373	0.372
0.02	0.259	0.255	0.257

Table 7. UV absorbance of ethanol extract of *P. foetida* at 765 nm.

No.	1st reading	2nd reading	Average
1	0.481	0.475	0.478

Table 8. Determination of phenolic content in *P. foetida* extract.

Extract name	Average absorbance at 765 nm	GAE for dried extract	Total phenolic content for dried extract (mg GAE/g)
P. foetida	0.478	0.05355	55.36

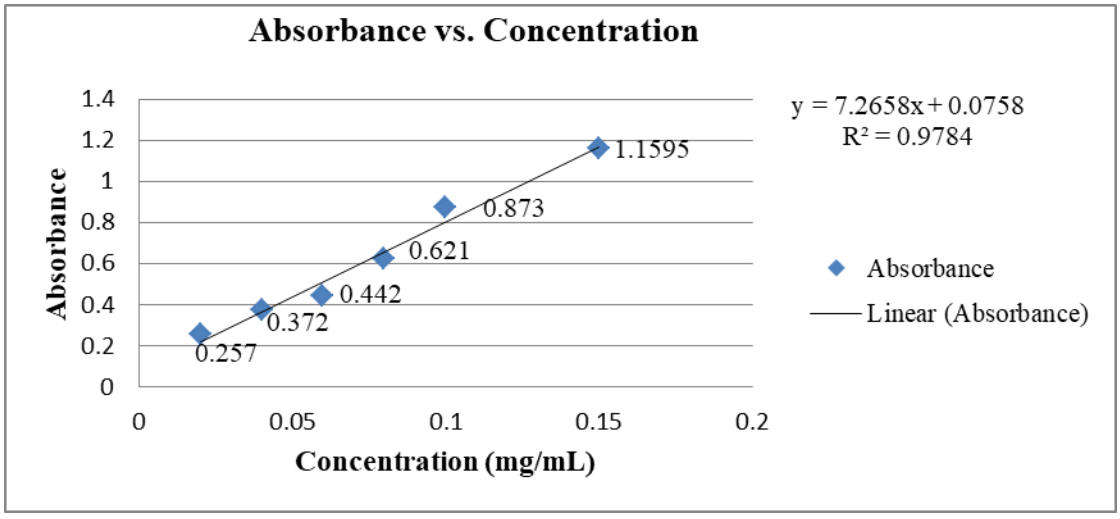

Figure 4: Total phenolic content determination of *P. foetida* extract with the help of gallic acid standard calibration curve.

3.5 Determination of Total Tannin Content (TTC)

Total tannin content is ~36 mg QE/g for the *P. foetida* extract. It was expressed using a linear calibration curve equation of Gallic acid. Gallic acid calibration curve (Figure 5) was drawn by plotting absorbance versus concentration. The obtained equation, y = 1.3765x-0.0103; with coefficient of determination, R^2 = 0.9804 was used to calculate Gallic Acid Equivalent (GAE) and then GAE was used to determine total phenolic content of *P. foetida* extract. Where y is absorbance at 725 nm and x is total tannin content in the *P. foetida* extract. (Table 9, 10 & 11).

Table 9. UV Absorbance of gallic acid (standard) at 725 nm.

Concentration (mg/mL)	Absorbance 1	Absorbance 2	Average
0.1	0.108	0.107	0.1075
0.2	0.265	0.267	0.266
0.3	0.420	0.422	0.421
0.4	0.579	0.580	0.5795
0.5	0.640	0.638	0.639

Table 10. UV absorbance of sample at 725 nm.

Sample	1st reading	2nd reading	Average
Paederia foetida extract	0.040	0.038	0.039

Table 11. Determination of tannin content of *P. foetida* extract.

Extract	Average absorbance at 725 nm	GAE of dried extract	Total tannin content of dried extract (mg GAE/g)
P. foetida	0.039	0.0358	~ 36

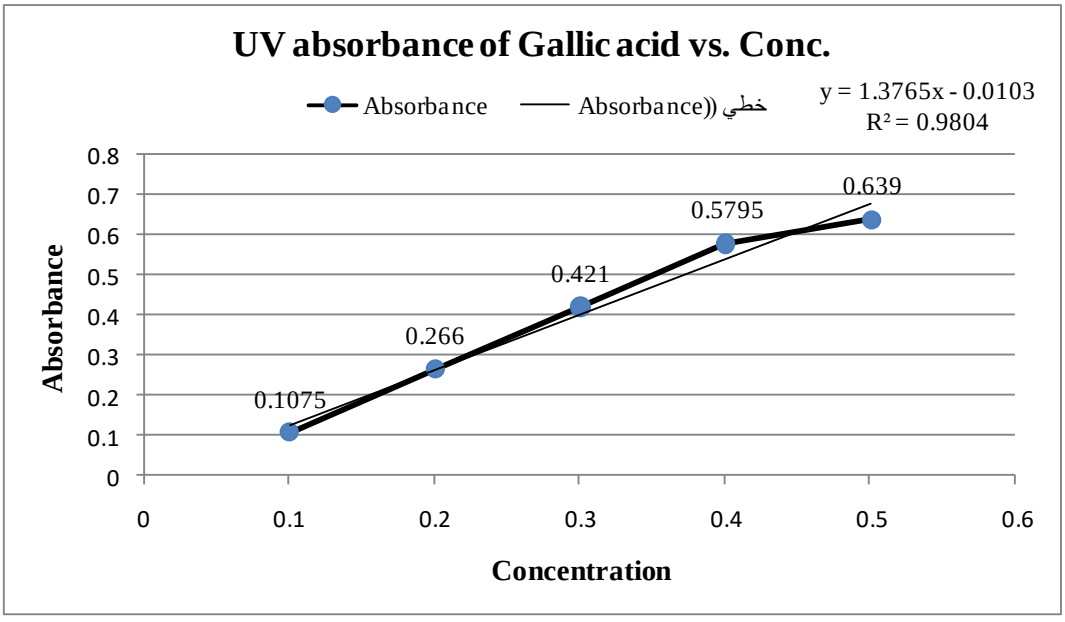

Figure 5: Total tannin content determination of *P. foetida* extract with the help of gallic acid standard calibration curve.

3.6 Determination of Total Flavonoid Content (TFC)

By using a linear calibration curve equation of Quercetin, y = 0.515x-0.0011 with coefficient of determination, R^2 = 0.9957, Where y is absorbance at 510 nm and x is total flavonoid content in the *Paederia foetida* extract (Figure 6). Total flavonoid content is ~ 60 mg QE/g for the *Paederia foetida* extract. (Table 12, 13 & 14).

Table 12. UV Absorbance of Quercetin (standard) at 510 nm.

Concentration (mg/mL)	Absorbance 1	Absorbance 2	Average
0	0.012	0.013	0.0125
0.25	0.1136	0.1137	0.1135
0.5	0.243	0.245	0.244
0.75	0.399	0.398	0.3985
1.0	0.514	0.513	0.51375

Table 13.UV absorbance of sample at 510 nm.

Name of sample	1st reading	2nd reading	Average
Paederia foetida extract	0.032	0.028	0.030

Table 14. Determination of flavonoid content of *P. foetida* extract.

Name of sample	Average absorbance at 510 nm	Quercetin equivalence mg/mL for extract	Total flavonoid Content for extract (mg QE/g)
P. foetida extract	0.030	0.060	~ 60

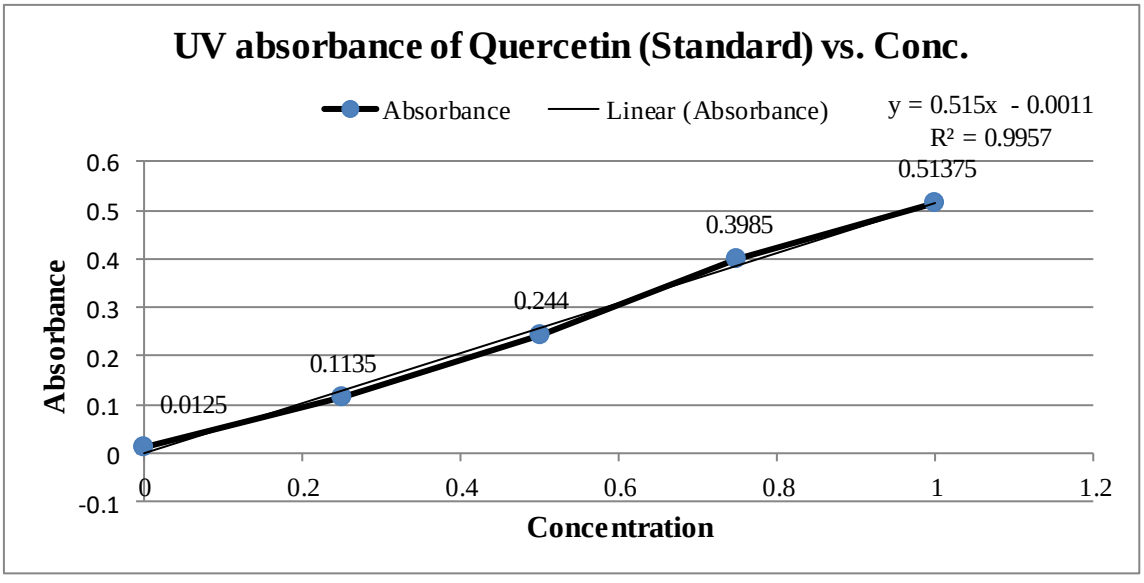

Figure 6: Total flavonoid content determination of *P. foetida* extract with the help of Quercetin standard calibration curve.

3.7 Antibacterial activity test

The antibacterial activity was assessed against nine pathogenic bacterial strains (Both gram positive and gram negative) at the doses of 250 and 500 µg/disc, and the results were compared with the activity of the positive controls, ciprofloxacin (30 µg/disc) and flucloxacillin (30 µg/disc). At 250 µg/disc, the extract showed no activity against *Pseudomonas aeruginosa* but showed activity at the dose of 500µg/disc where the zone of inhibition was 13 mm. At the doses of 250 and 500 µg/disc, the extract showed antibacterial activity against *Vibrio cholera* (12 & 16 mm), and *Staphylococcus aureus* (12 & 17.5 mm) as compared with Flucloxacillin (30 µg/disc) and Ciprofloxacin (30 µg/disc) (Table 15).

Table 15. *In vitro* antibacterial activity of crude extract of *P. foetida* using Ciprofloxacin and Flucloxacillin antibiotic disc as positive control.

Bacterial strains	Type of bacteria	Diameter of zone of inhibition (mm)				
		Blank	Extract (250 µg/disc)	Extract (500 µg/disc)	Flucloxacillin (30 µg/disc)	Ciprofloxacin (30 µg/disc)
Staphylococcus pyogenes	Gram(+)	0	0	0	36	36
Vibrio cholerae	Gram (-)	0	12	16	0	30
Shigella dysenteriae	Gram (-)	0	0	0	0	25
Proteus Species	Gram (-)	0	0	0	18	26
Salmonella enteritidis	Gram (-)	0	0	0	12	23
Pseudomonas aeruginosa	Gram (-)	0	0	13	30	30
Staphylococcus aureus	Gram(+)	0	12	17.5	35	35
Salmonella typhi	Gram (-)	0	0	0	0	35
Escherichia coli	Gram (-)	0	0	0	36	22

Here, Aver.: Average; Gram (-): Gram Negative Bacteria; Gram (+): Gram Positive Bacteria; (0): No inhibition

3.8 Analgesic activity test

The results of the test showed that the ethanol extract of *P. foetida* at the dose of 250 mg/kg and 500 mg/kg body weight exhibited significant inhibition of writhing reflex by 34.66% and 65.91% respectively, while the standard drug's, Diclofenac Sodium, inhibition was found to be 79.55% at a dose of 25 mg/kg body weight**.** So it can be said that the analgesic activity of the ethanol extract of *P. foetida* was significant in comparison with control, at the doses of 250 and 500 mg/kg body weight showed significant decrease in acetic acid induced writhing reflex of mice (Table 16).

Table 16.Statistical evaluation of Analgesic activity of *P. foetida*

Animal group	Mean of writhing	%Writhing	SD	SE	% Inhibition of writhing	T-test (value of p)
Negative control	35.2	100	1.4	0.7	0	
Positive control Diclofenac Sodium (25 mg/kg)	7.2	20.45	1.30	0.58	79.55	26.94 P<0.01

Animal group	Mean of writhing	%Writhing	SD	SE	% Inhibition of writhing	T-test (value of p)
Extract (250 mg/kg)	23	65.34	1.58	0.71	34.66	10.96 P<0.01
Extract (500 mg/kg)	12	34.09	1.00	0.45	65.91	23.93 P<0.01

SD = Standard deviation, SE = Standard error.

3.9 Anthelmintic activity test

From the observations, the higher concentration (50 mg/mL) of extract produced paralytic effect earlier and the time to death was shorter. Evaluation of anthelmintic activity was compared with reference standard Albendazole (Table 17).

Table 17. Anthelmintic activity of the ethanol extract of *P. foetida*

Treatments Concentration (mg/mL)	Worm no.	Time taken for paralysis (min.)	Mean time of paralysis (min.) ±S.E.	Time taken for death (min.)	Mean time of death in (min.) ±S.E.
Control 0.1 % Tween-80 in PBS	C_1	---	---	---	---
	C_2	---			
	C_3	---			
	C_4	---			
	C5	---			
	C6	---			
Standard Albendazole 15 mg/mL	S_1	7.1	7.5 ± 0.1770	15.2	16.23 ± 0.4738
	S_2	7.8		14.5	
	S_3	7.3		16.6	
	S_4	7.1		17.4	
	S5	8.2		14.8	
	S6	7.5		16.5	
Extract 25 mg/mL	T_1	34.23	34.12 ± 0.4442	45.24	43.45 ± 0.6181
	T_2	32.46		43.36	
	T_3	35.27		44.45	
	T_4	33.54		42.16	
	T5	34.26		44.26	
	T6	32.56		41.23	
Extract 50 mg/mL	T_1	23.43	24.15 ± 0.5584	30.24	32.19 ± 0.7611
	T_2	22.38		29.45	
	T_3	25.26		33.26	
	T_4	22.08		31.17	
	T5	24.09		32.23	
	T6	25.23		34.39	

DISCUSSION

Preliminary phytochemical screening of *P. foetida* extract revealed the presence of flavonoids, tannins, carbohydrates, alkaloids, glycosides, steroids, terpenoids and acidic compounds. But in previous research (Upadhyaya, 2013), the ethanolic extract of leaves of *P. foetida* showed the presence of saponins, tannins, phenols, flavonoids, terpenoids, cardiac glycosides, alkaloids and reducing sugars. However, steroids and anthraquinones were not detected.[25] The variation in results in these two researches may be due to the experimental variations or to the variation in the chemical composition among regions; because in these two researches, the plant was collected from different regions in different countries with different climate conditions. Among these constituents; phenolic compounds, flavonoids, tannins and alkaloids are the most valuable for therapeutic activity.[26]

Phytochemical screening of *P. foetida* extract revealed the presence of polyphenolic compounds like flavonoids, tannins, terpenoids and previous research showed that the leaves are rich in carotene and vitamin C which are known as antioxidant compounds. These antioxidant compounds in *P. foetida* extract can scavenge free radical like DPPH. So, this plant may be a good source of antioxidant compounds.

Important biomolecules can be modified by the introduction of oxygen atoms by means of non-oxidative hydroxyl radicals.[27] In addition, hydroxyl radicals can reduce disulfide bonds in proteins, specifically fibrinogen, resulting in their unfolding and scrambled refolding into abnormal spatial configurations. Consequences of this reaction are observed in many diseases such as atherosclerosis, cancer and neurological disorders, and can be prevented by the action of non-reducing substances.[27]

Phenolic compounds protect different organs of the body from free radical damage and associated diseases by scavenging them and also maintain normal human health. Free radicals are often generated as byproducts of biological reactions or from exogenous factors.[27] The result of the present study showed that the extract of *P. foetida* contained moderate amount of phenolic compounds.

Tannins are widely distributed in plants. They have been reported to possess anticarcinogenic and antimutagenic potentials as well as antimicrobial properties.[28] The result of the present study showed that the extract of *P. foetida* contain moderate amount of tannin compounds exhibiting antioxidant activity.

Flavonols that have ortho or para hydroxyl group in the 2- phenyl ring are known to have strong antioxidant properties, while free hydroxyl at the 5, 7 positions proved to have a pro-oxidant effect.[27] The results of the present study showed that the extract of *P. foetida* which contain moderate amount of flavonoid compounds exhibited good antioxidant activity. So, *P. foetida* extract may be used in further studies to unravel novel treatment strategies for disorders associated with free radicals induced tissue damage.

The phytochemicals; flavonoids, polyphenols, tannins, quinones, terpenoids, essential oils and alkaloids show antibacterial activity. Flavonoids and tannins may protect plants from invasion of pathogenic microorganism due to their antimicrobial and antifungal properties. Polyphenols and tannins exert antimicrobial activity through enzyme inhibition, membrane disruption and metal ion complexation. Alkaloids exert antimicrobial activity through intercalating into cell wall and DNA of parasites.[27,28] *P. foetida* was effective against both gram positive and gram-negative bacteria.

Phytochemical groups present in *P. foetida* like polyphenols, flavonoids, tannins and alkaloids may be responsible for this antibacterial activity. Further investigation may be conducted using pure compounds of this extract to determine the responsible compounds for this pharmacological effect.

According to Ravishankar *et al.* 1994, butanol fraction of a methanol extract of leaves of *P. foetida* produced a significant anti-inflammatory response.[30] The mechanism of induction of inflammation and pain is quite

similar and related to each other. Both are induced due to the synthesis of prostaglandins. From this experiment, it can be concluded that the extract showed dose dependent analgesic activity in acetic acid induced writhing method. Phytoconstituents like terpenoids, gums, flavonoids and tannins are responsible for analgesic activity.[28]

P. foetida Linn. is used traditionally to treat intestinal worm infections, and showed significant anthelmintic activity. Anthelmintic effects of plants are normally due to the presence of secondary metabolites such as alkaloids, terpenoids or polyphenols such as proanthocyanidins.[28] The experimental evidence obtained from this laboratory model could provide a rationale for the traditional use of this plant as anthelmintic. The plant may be further explored for its phytochemical profile to recognize the active constituent that is responsible for anthelmintic activity.

CONCLUSION

According to the above discussion, *P. foetida* Linn contains important chemical constituents for which it is known as a medicinal plant. Identification of the nature of the compounds present in extracts is essential to evaluate the biological activity of the extract. It is already reported that the polyphenolic compounds, as phenolic acids, flavonoids and tannins are commonly found in different plants and exert multiple biological response, including antioxidant, antimicrobial, anthelmintic, antidiarrhoeal activity. Phytoconstituents like terpenoids, carbohydrates, flavonoids and tannins are responsible for analgesic activity.[27,28] So, different phytochemical groups present in *P. foetida* are responsible for antioxidant and pharmacological activity.

Phytochemical analysis of *P. foetida* showed the presence of flavonoids, tannins, carbohydrates, alkaloids, glycosides, steroids, terpenoids and acidic compounds which have potential role in its antioxidant, antibacterial, analgesic and anthelmintic activities. These preliminary studies do not describe the mechanism of action of these different pharmacological effects. But it could provide a rationale for the traditional uses of this plant and suggest more investigations to identify the mechanism of action and to isolate the bioactive compounds that are responsible for each pharmacological activity.

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نشاط مضادات الأكسدة والمسكنات ومضادات الميكروبات والديدان من الأجزاء الهوائية لبايديريا فويتيدا (العائلة: الروبيكيا)

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ملخص

العائلة (لين) *Paederia foetida* (Rubiaceae) يستخدم تقليديا في بنغلاديش كنبات طبي لعلاج والوقاية من الأمراض المختلفة. الهدف من هذه الدراسة هو التحقيق في أنشطة مضادات الأكسدة والمسكنات ومضادات الميكروبات والديدان المضادة للأنسولين في الأجزاء الهوائية لـ .P. foetida تحليل كيميائي نباتي أولي لمستخلص الإيثانول للأجزاء الهوائية من .P. foetida Linn عرض وجود الكربوهيدرات، الجليكوسيدات، العفص، قلويدات، المركبات الحمضية، الستيرويدات، terpenoids، والفلافونويدات. في مقايسة مضادات الأكسدة النوعية المستندة إلى TLC باستخدام DPPH، أظهر مستخلص P. foetida خواص تنظيف جذرية حرة مشار إليها في وجود بقعة بيضاء صفراء على خلفية أرجوانية على لوحة .TLC في مقايسة مضادات الأكسدة الكمية باستخدام DPPH، كانت قيمة IC50 حوالي 75.52 ميكروغرام/مل، حيث كانت قيمة IC50 من حمض الأسكوربيك حوالي 15.78 ميكروغرام/مل P. foetida .استخراج عرض هيدروكسيل جذري الكسح النشاط(SC50 = 196.03) ملغم/ لتر (والتي كانت مماثلة لحمض الاسكوربيك القياسية SC50 = 80.83) ملغ/ لتر. تم العثور على إجمالي محتوى الفينول (TPC) ومحتوى التانين الكلي (TTC) ليكون 55.36 ~ ملغ و 36 ~ ملغ / GAE جم من مستخلصات النباتات المجففة على التوالي، باستخدام منحنى حمض الغاليس المعايرة. تم تحديد المحتوى الكلي للفلافونويد 60 ~ ملليجرام كمي / جم من مستخلصات النباتات المجففة باستخدام منحنى معايرة كيرسيتين . أظهر المستخلص نشاطًا مضادًا للبكتيريا ضد أنواع *S. aureus* و *V. cholerae* و *P. aeruginosa* بجرعة 250 و 500 ميكروغرام/ قرص مقارنة مع سيبروفلوكساسين دواء قياسي (30) ميكروغرام / قرص (والفلوكسلاسيلين 30) ميكروغرام / قرص. أظهر المستخلص نشاط مسكن هامشي هام ($p < 0.01$) بجرعات 250 ملغم / كغم) تثبيط تثبيط 34.66 ٪ (و 500ملغم / كغم) تثبيط تثبيط التثبيط بنسبة (65.91 ٪) يتحدد بواسطة رد فعل التثبيط الناجم عن حمض الأسيتيك في الفئران السيطرة ديكلوفيناك الصوديوم. (79.55 ٪) في اختبار الديدان، أظهر المستخلص انخفاضًا يعتمد على الجرعة في الشلل ووقت موت الطفيليات تريماتود والديدان الخيطية .(قد تكون المواد الكيميائية النباتية الموجودة في هذا المصنع مسؤولة عن الآثار الدوائية المذكورة أعلاه).

الكلمات الدالة: *Paederia foetida* Linn ، دراسة كيميائية نباتية، نشاط مضاد للأكسدة، نشاط مضاد للميكروبات، نشاط مسكن، نشاط مضاد للديدان.

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