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Study of Pharmacological Profile of Chloroform Leaf Extract of *Ludwigia perennis* - A Wetland Plant

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The main objective of this study was to find out various therapeutic properties of chloroform leaf extract of the wetland plant Ludwigia *perennis*.
Place and Duration of Study: Department of Biotechnology, Sree Narayana Guru College, Coimbatore, Tamilnadu, Scigen Research and Innovation in Thanjavur, Tamil Nadu; and the Sri Lakshmi Narayana Institute of Medical Sciences in Osudu, Pondicherry between April 2023 and August 2023.
Methodology: Chloroform leaf extract of *Ludwigia perennis* was employed for evaluating the antioxidant, antidiabetic, anti-nutritional, anticancer, anti-inflammatory, and antipyretic properties, respectively.

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Results: The DPPH assay found that the IC_{50} for the leaf extract was 48.78µg/mL, while the IC_{50} for the standard was 49.74µg/mL. The IC_{50} for the phosphomolybdenum test was 32.31µg/mL for the leaf extract and 33.73µg/mL for the standard. The IC_{50} values for the hydroxyl radical scavenging experiment were 55.6µg/mL for the standard and 47.54µg/mL for the leaf extract. The IC_{50} of the standard in the α-amylase inhibitory assay is 394.48µg/mL, and the extract's IC_{50} is 330.38µg/mL, respectively. The IC_{50} of the standard in the α-glucosidase inhibitory assay was 394.06µg/mL, and the extract's IC_{50} was 335.45µg/mL, respectively. Antinutrient content such as alkaloids was 21.4%, oxalate was 6.6%, phytate was 4.18%, and saponin was 30%, respectively. The IC_{50} of HeLa cells treated with leaf extract was 180µg/mL. After 4hours of treatment with chloroform leaf extract, the inhibition was 51.75% when 50mg/kg was used and 58.74% when 250mg/kg was used. Here, the inhibitory percentage of indomethacin is 59.45%. This means that the leaf extract has anti-inflammatory activity. Chloroform leaf extracts produced significant antipyretic effects in 250 and 500mg/kg doses.
Conclusion: Therefore, the chloroform leaf extract of *Ludwigia perennis* has shown to have anti-oxidant, anti-diabetic, anti-nutrient, anti-cancer, anti-inflammatory, and anti-pyretic activities. These evidences suggest the plant is a potential source for developing new drugs.

Keywords: Antioxidant; anti-diabetic; anti-nutritional; anticancer; anti-inflammatory; antipyretic.

1. INTRODUCTION

Natural materials have traditionally been used by humans for basic purposes, especially in the field of healthcare. Phytochemicals present in plants have both physiological and therapeutic value in the human body [1-3]. Herbal medicines are becoming increasingly common since they often have fewer or no side effects [4]. The usage of herbal medication has protracted history; however, alternative and complementary treatment plans that use trendy technology can fill the space in long-standing practises [5-7]. The majority of nations have employed medicinal herbs for therapy since ancient times. Therefore, we should continue looking for novel plant-based therapies that make use of nature's extensive body of information. [8-9].

Ludwigia perennis, the common name of the plant, is water primrose. This plant belongs to the family Ongraceae. These are mainly found in wet areas and swamps. The range of this plant is Tropical Asia, East and Southeast Asia, Australia, and New Caledonia. Phytochemicals are found in plants and serve a number of functions, including preventing and treating illnesses. Some examples include alkaloids, flavonoids, tannins, steroids, terpenoids, carbohydrates, saponins, phenols, reducing sugars, and cardiac glycosides. [10-15]. The aim of the research was to investigate any possible biological activity of the unstudied chloroform extract of *Ludwigia perennis* leaf. To achieve this, studies were conducted on the chloroform extract of the leaves to determine its anti-oxidant, diabetic, nutritional, anti-cancer, anti-inflammatory, and anti-fever properties.

2. EXPERIMENTAL DETAILS

2.1 Collection and Identification of the Plant Material

Ludwigia perennis was verified at the Durva Herbal Centre in Pammal, Chennai after a sample was taken from a wetland region in the hamlet of Nandipulam in the Thrissur district of Kerala, India.

2.2 Preparation and Extraction of the Plant Material

The leaves of *Ludwigia perennis* were cut into smaller pieces, properly washed, and then air dried. The leaves were preserved in airtight plastic containers after being ground into a coarse powder using an antique grinding mill. The ground material was extracted with chloroform for a duration of 72 hours at room temperature using Soxhelet apparatus. Using Whatsman No.1 filter paper, the extract was filtered and stored for eventual use.

2.3 Animal Preparation

The study included Wistar Albino rats (180-200 g) of both sexes. The animals were donated by Sri Lakshmi Narayana Institute of Medical Sciences in Pondicherry. The animals were housed in polypropylene cages with rice husk bedding, and after being randomly divided into two groups, either a control or a treatment was given to them. In accordance with industry standards, the animals were provided with a photoperiod (12:12 h dark:light cycle), a constant

temperature of 27℃, and a relative humidity range of 30 to 70%. Each cage held six animals at a time. Each rat was provided with unlimited access to clean water and M/S. Hindustan Lever Ltd.'s standard fare of commercial pelleted rat chaw.

2.4 Antioxidant Activity

The chloroform leaf extracts of *Ludwigia perennis* were tested for their capacity to scavenge free radicals using standard protocols with modifications wherever applicable.

2.4.1 DPPH

The 1,1-diphenyl-2-picrylkydrazil (DPPH) technique, a reliable free radical test, was used to study antioxidant activity. [16]. Using the serial dilution method, leaf extracts were extracted with chloroform and diluted in methanol to concentrations of 400, 200, 100, 50, 25, 12.5 and 6.25 µg/mL.[17].

2.4.2 Phosphomolybdenum Assay

Antioxidant activity was determined using a conventional approach including the phosphomolybdenum method [18].

2.4.3 Hydroxyl radical scavenging assay

The deoxyribose technique was used to test the ability of a sample to scavenge hydroxyl radicals. [19].

2.5 *In vitro* Antidiabetic Activity

2.5.1.α- Amylase activity

In vitro anti-diabetic efficacy was assessed using alpha-amylase activity [20-21]. Various sample concentrations (100-500 µg/ml) were combined with a low dose of - amylase (0.5 µg/ml). Starch at a concentration of 1% was then added to 100 mL of phosphate water (pH 6.9). The reaction was terminated by adding 2 ml of the 3-dinitrosalicylic acid reagent after 5 minutes at 37℃. The next step is to add 10 cc of extremely cold distilled water to the process. The spectrophotometer reading for α- amylase concentration was taken at 540 nm.

2.5.2.α – Glucosidase activity

The *in vitro* antidiabetic effectiveness [22] was determined using alpha-glucosidase assay. In 1 ml of 0.2 M Tris buffer (pH 8), starch doses of 100–500 mg/ml were added. The inhibitory activity of the glucosidase enzyme was then assessed. After assessment, the tubes were placed in an incubator for 10 minutes at 37℃. Then, 1 ml of the α-glucosidase enzyme and 2 ml of 6N HCl were added to the test tubes to halt the process. The resulting solution was read at 540 nm.

The data were transformed into an inhibition percentage using the formula;

$$\% \text{ inhibitory activity} = (Ac-As)/Ac \times 100$$

Where As is the sample's absorbance and Ac is the absorbance of the control.

2.6 Determination of Antinutrient Composition

2.6.1 Alkaloid content

The total alkaloid content was determined according to standard procedure [23]. To 200mL of 10% acetic acid in ethanol, 5gm of the pulverised material were first added. The jar containing the mixture was then sealed and set out for 4 hours at room temperature. The concentrated filtrate obtained during the filtering process was made up to five times the volume of initial quantity. The concentrated filtrate was then treated with drop-wise flows of concentrated ammonium hydroxide (NH4OH) to produce a foggy fume precipitate. Using diluted ammonium hydroxide and filter paper, the solution was cleaned once it had settled. The remnant was dried and weighed.

2.6.2 Oxalate content

This protocol was executed as per standard procedure [24]. 75 mL of 3M H_2SO_4 (sulfuric acid) was mixed with 1g of the powdered material and macerated in a conical flask. After stirring, the compound was filtered. The collected filtrate was then heated to 70℃ in a 25 mL flask. The end point hue remained a very hazy light pink for 15 seconds after the heated aliquot was carefully titrated against 0.05 M KMnO4 (potassium permanganate).

2.6.3 Phytate content

This method was used to evaluate the amount of phytic acid in the sample [25].2% hydrochloric acid (HCl) was used to macerate 2 g of the powdered material for three hours. To determine the concentration of ammonium thiocyanate in

the combination, 25 mL of the filtered mixture was combined with 53.5 mL of distilled water, 5 mL of the indicator solution, and 5 mL of the filtrate. The combination was titrated against a standard solution of iron (III) chloride (0.00195 g iron per mL) after the endpoint color had been maintained for roughly five minutes.

2.6.4 Saponin content

This approach was used for the analysis of the total saponin content present in the sample [26].40 mL of 20% ethanol and 1 g of powdered material were combined, homogenized, and then heated to 55°C for 4 hours. A vacuum pump coupled to a filtration machine was used to filter the mixture. 20 mL of 20% ethanol were used to remove the residue once again. The filtrate was passed through a separating funnel after being heated to 90℃ in a water bath until its mL volume reached 40. The remainder of the diethyl ether, 20 mL, was then added, and everything was thoroughly mixed. The bottom part of the ether was left behind after the upper layer was removed. The reintroduced sub-fraction was forcedly blended once again after receiving butan-1-ol. A 5% sodium chloride (NaCl) aqueous solution was then added in a volume of 5 mL. After collection, butan-1-ol from the top was evaporated in an oven to a consistent weight.

2.7 Anticancer Activity

2.7.1 Cell culture maintenance

We obtained Cervical cancer HeLa cell lines from the NCCS Cell Repository in Pune, India. Dulbecco's Modified Eagle medium (DMEM) with 10% foetal bovine serum (FBS) was used to sustain the cell line. Penicillin (100 U/ml) and streptomycin (100 µg/ml) were added to the solution to stop bacterial growth. The cell line medium was maintained at 37°C in a humidified 5% CO2 the atmosphere.

2.7.2 MTT assay

The chloroform leaf extracts of *Ludwigia perennis* were tested for cytotoxicity on HeLa cells [27].

2.7.3 Acridine orange/ethidium bromide (AO/EB) dual staining technique for measuring apoptotic induction

A fluorescence microscopic examination of apoptotic cell death was performed [28].

2.8. Anti-inflammatory Activity

2.8.1 Carrageenan-induced rat paw edema model

Ludwigia perennis leaf extract was tested for its anti-inflammatory effects using a rat model of carrageenan-induced paw edoema. [29].Two doses of *Ludwigia perennis* leaf extract (50 and 250 mg/kg) were utilised in the study, with the standard anti-inflammatory medicine indomethacin (10 mg/kg) serving as a control. Four groups of six Wistar albino rats each were created by randomly selecting 24 healthy Wistar albino rats. Group II acted as the reference control and got oral indomethacin (10 mg/kg), whereas Group I functioned as the vehicle control and was given 0.1% carboxymethylcellulose solution. The leaf extract of *Ludwigia perennis* was given orally to Groups III and IV at 50 and 250 mg/kg, respectively. Both the control and experimental groups had 0.1 ml of 1% (w/v) carrageenan injected into the plantar area of their left paw 30 minutes later. The right paw served as a benchmark for the uninflamed paw for comparison. Three groups of rats (controls, rats treated with Indomethacin, and rats treated with *Ludwigia perennis* extract) had the thickness of their left paws measured with a digital calliper every hour following carrageenan administration. Paw thickness was assessed after 0, 30, 60, 120, and 240 seconds. It was observed that the paw volume had decreased. Additionally determined was the paw thickness inhibition percentage with time.

% Inhibition = 100 (Vc-Vt/Vc), where, (Vc-Control mean paw thickness, Vt – Test mean paw thickness)

2.9 Antipyretic Activity

2.9.1 Brewers's yeast induced pyrexia

Ludwigia perennis leaf extract was evaluated for its antipyretic properties in a rat model of fever caused by brewer's yeast [30]. The investigation was conducted using 180–200-gm Wistar albino rats. Using digital telethermometers, we recorded each rat's initial rectal temperature. A suspension of Brewer's yeast in distilled water at a concentration of 15% (w/v) at a dosage of 10 millilitres per kilogramme of body weight was injected subcutaneously to induce the fever. Only animals whose body temperatures increased by at least 0.6℃ (or 1 F) in the 18 hours after Brewer's yeast injection were chosen for the

research. Four groups of six rats each were created at random. Group I was given normal saline through stomach intubation tube at a rate of 1 ml/kg. Group II received their customary dosage of 100 mg/kg of paracetamol by oral administration. Three groups received 250 mg/kg and four groups received 500 mg/kg of *Ludwigia perennis* by oral administration. At 0, 1, 2, and 4 hours post-treatment, rectal temperatures were measured and recorded.

2.10.2 Statistical analysis

The mean ± SEM of the data were displayed. Dunnett's 't' test was used to examine the data after a one-way ANOVA was performed. Significant data are those with P <0.05.

3. RESULTS

3.1 Antioxidant Activity

3.1.1 DPPH

The DPPH radical scavenging capacity of *Ludwigia perennis* chloroform leaf extract was shown to increase with concentration (Fig. 1). Fig. 1 displays that the DPPH radical scavenging activity of *Ludwigia perennis* was statistically equivalent to that of the reference chemical, ascorbic acid, across the full range of concentrations studied. The ability to scavenge DPPH radicals also varied greatly across the various extract concentrations tested. Fig. 1 demonstrates that the concentration of the extract directly correlates to its capacity to neutralise DPPH radicals. Additionally, whereas the sample's IC_{50} value was discovered to be 48.78 µg/mL, the reference's IC_{50} value was 49.74 µg/mL. (Table1).

Table 1. Inhibitory % of leaf extract on DPPH Assay

Concentration (µg/ml)	Inhibitory % of DPPH	
	Sample	Standard
6.25	44	46.3
12.5	50.3	51.3
25	56	57.8
50	61.3	65.6
100	68.7	71.3
200	74	77.4
400	80	83.5
IC_{50}(µg/mL)	48.78	49.74

3.1.2 Phosphomolybdenum assay

As shown in Fig. 2, *Ludwigia perennis* and the standard substance, ascorbic acid, have comparable total phosphomolybdenum activity. Total phosphomolybdenum test results (Fig. 2) favoured the extract's highest concentration. The extract has an IC_{50} of 32.31 µg/mL, whereas the standard was 33.73 µg/mL. (Table 2).

Table 2. Inhibitory % of leaf extract on total phosphomolybdenum assay

Concentration (µg/ml)	Inhibitory % of Total Phosphomolybdenum assay	
	Sample	Standard
6.25	46	46
12.5	54.7	54.7
25	64	64
50	74	74
100	81.7	81.7
200	84.3	84.5
400	89	90
IC_{50}(µg/mL)	32.31	33.73

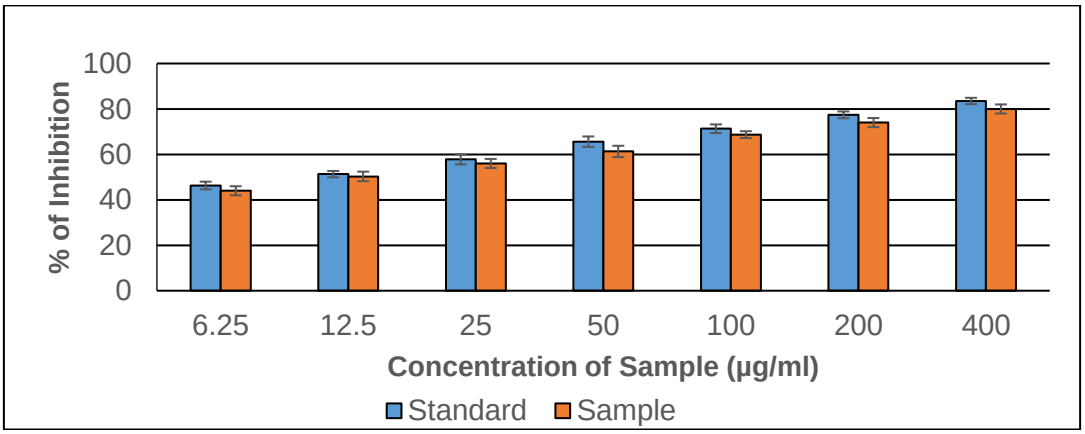

Fig. 1. Antioxidant activity of chloroform leaf extract in DPPH

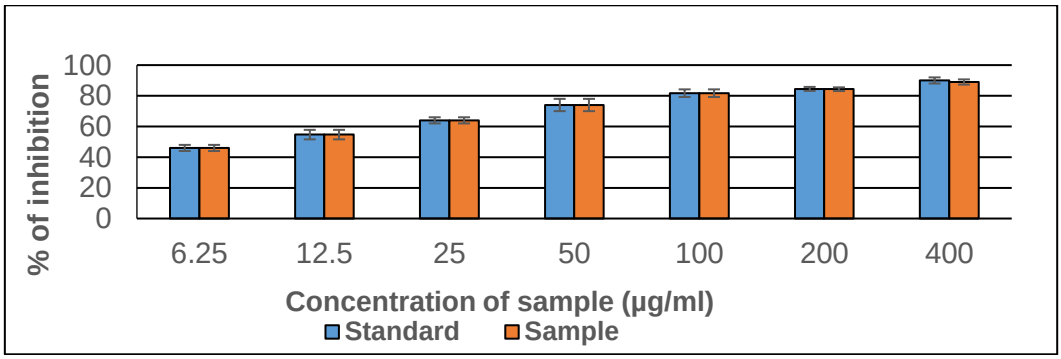

Fig. 2. Antioxidant activity of chloroform leaf extract in Phophomolybdenum assay

3.1.3 Hydroxyl radical scavenging assay

The *Ludwigia perennis* chloroform leaf extract demonstrated strong hydroxyl radical scavenging action at all doses and the activity was directly proportional to the concentration (Fig. 3). Compared to the reference value of 55.6 µg/mL, the extract's IC_{50} was found to be substantially lower at 47.54 µg/mL. (Table 3).

Table 3. Inhibitory % of leaf extract on hydroxyl scavenging assay

Concentration (µg/ml)	Inhibitory % of Hydroxy Radical Scavenging assay	
	Sample	Standard
6.25	42	53.33
12.5	52	57
25	58	63
50	64.7	72.67
100	70.7	83
200	78.7	92.33
400	87.3	96
IC_{50}(µg/mL)	47.54	55.6

3.2 *In vitro* Antidiabetic Activity

3.2.1 α Amylase inhibitory activity

Fig. 4 shows that when the concentration of the chloroform extract of *Ludwigia perennis* leaves was increased, so too was the percentage of amylase activity that was inhibited. The chloroform leaf extract demonstrated 36.3, 41.7, 52.5, 63.8, 71.7, and 77.5 percent inhibition at 100, 200, 300, 400, 500, and 600 µg/mL, respectively, with an IC_{50} of 330.38 µg/mL.. Comparatively, the common medication acarbose showed inhibitions of 48.8%, 55.2%, 60%, 66.1%, 81.5%, and 91.2% at doses of 100, 200, 300, 400, 500 and 600 µg/mL, respectively, with an IC_{50} of 394.48 µg/mL (Table 4).

3.2.2 α – Glucosidase activity

Table 5 provides evidence of the chloroform leaf extract's *invitro* α-glucosidase inhibitory action. "The highest concentration of the sample (600 µg/mL) demonstrated the greatest percentage inhibition of α -glucosidase (100%) when tested at concentrations between 100 and 600 µg/mL.: 14.7%, 25%, 41.3%, 50.9%, 61.7%, and 76.7%, when compared to conventional acarbose, which has inhibitory percentages of 31.7%, 40.2%, 46.7%, 54.8%, 75.3%, and 88.2%. Acarbose standard and chloroform leaf extract both have IC_{50} values of 335.45 µg/mL and 394.06 µg/mL, respectively (Table 5).

Table 4. α-amylase inhibition by *Ludwigia perennis* chloroform leaf extract

Concentration (µg/mL)	Inhibitory % of α- amylase inhibitory activity	
	Sample	Standard
100	36.3	48.8
200	41.7	55.2
300	52.5	60
400	63.8	66.1
500	71.7	81.5
600	77.5	91.2
IC_{50} (µg/mL)	330.38	394.48

Table 5. α-glucosidase inhibition by *Ludwigia perennis* chloroform leaf extract

Concentration (µg/mL)	Inhibitory % of α-glucosidase inhibitory activity	
	Sample	Standard
100	14.7	31.7
200	25	40.2
300	41.3	46.7
400	50.9	54.8
500	61.7	75.3
600	76.7	88.2
IC_{50} (µg/mL)	335.45	394.06

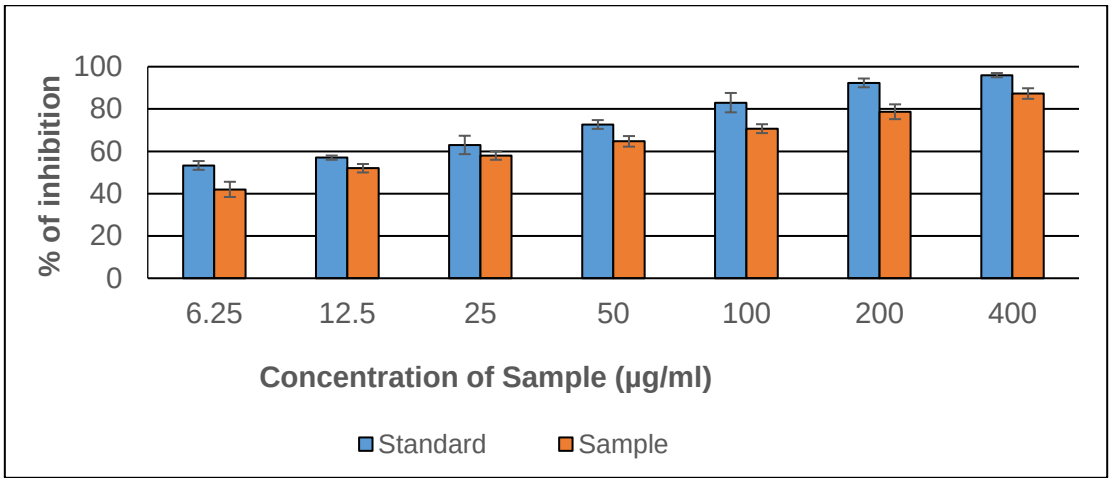

Fig. 3. Antioxidant activity of chloroform leaf extract in hydroxyl radical scavenging assay

3.3 Anti-nutrient Composition

Fig. 6 depicts *Ludwigia perennis* anti-nutrient make up. The findings showed that leaf contained anti-nutrients such as alkaloid (21.4%), oxalate (6.6%), phytic acid (4.18%), saponin (30%).

3.4 Anticancer Studies

3.4.1 Cytotoxic activity of *Ludwigia perennis* chloroform leaf extract on human cervical cancer cell lines

Ludwigia perennis chloroform leaf extract was subjected to anti-cancer studies using HeLa cell line. At concentrations between 50 and 300 µg/ml, the cytotoxic effects of chloroform-dissolved leaf extracts from the plant *Ludwigia perennis* were evaluated. With an increase in concentration, a steady decline in the percentage of viable cells was observed. Leaf extract from Chloroform showed an IC_{50} of 180 µg/ml.

3.4.2 Acridine orange/ Ethidium bromide staining

Fluorescence microscopy was used to examine Hela cells that had been treated with leaves (180 µg/ml) for 24 hours and labelled with the dual dye AO/EB. The absence of apoptosis was shown by the green fluorescence of the control cells. Early apoptotic cell death, as shown by yellow fluorescence and condensed or shattered nuclei, and late apoptotic cell death, as indicated by orange fluorescence, were seen in the leaf-treated cells.

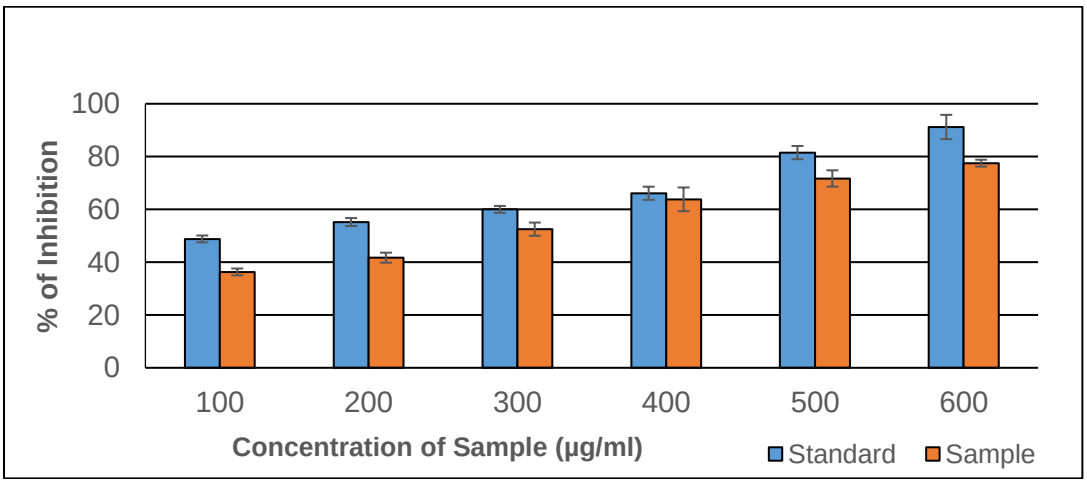

Fig. 4. Effect of chloroform leaf extract on alpha amylase inhibition activity

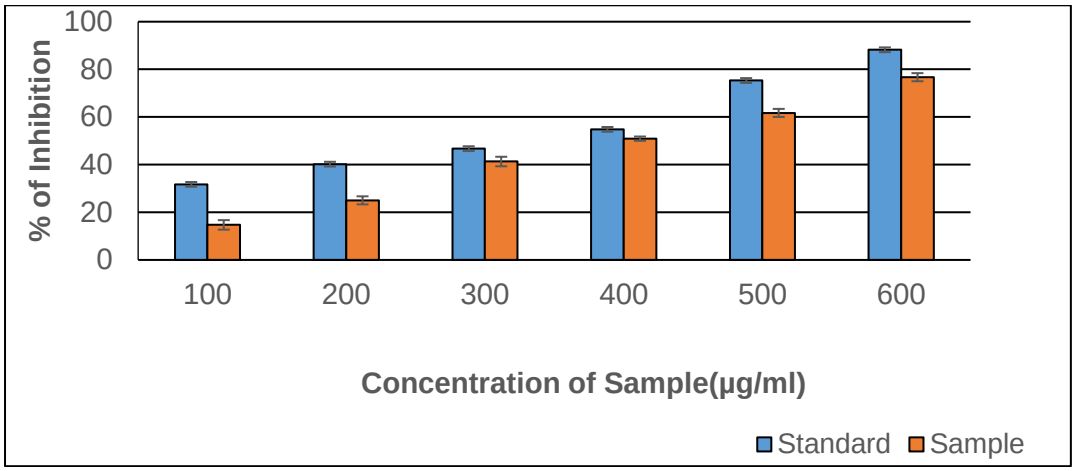

Fig. 5. Effect of chloroform leaf extract on alpha glucosidase inhibition activity

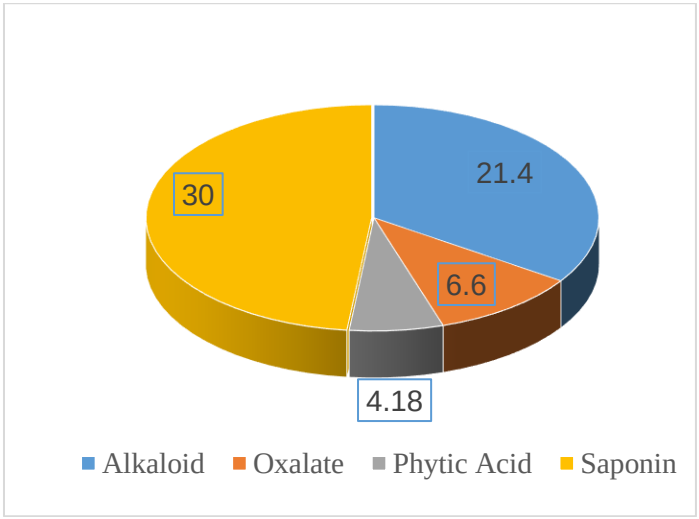

Fig. 6. Antinutritional composition of *Ludwigia perennis* chloroform leaf extract

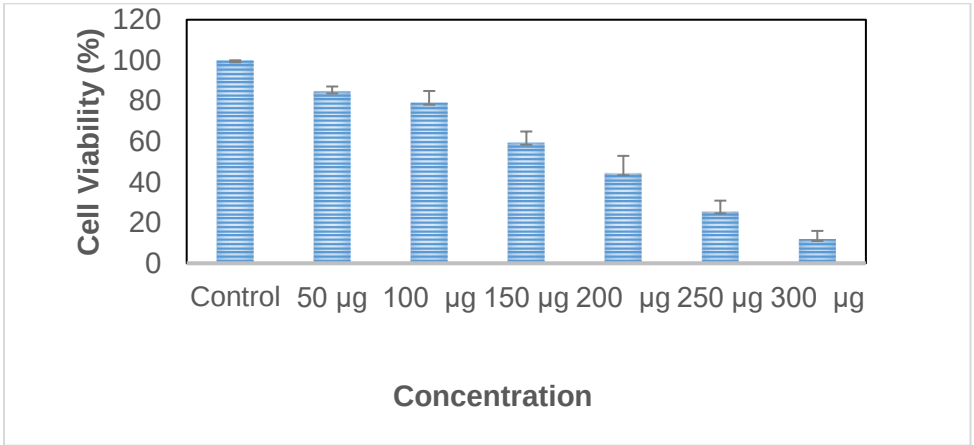

Fig. 7. Cytotoxicity of *Ludwigia perennis* leaf extracts in HeLa cells treated with 50, 100,150,200,250, and 300 µg/mL of by MTT assay

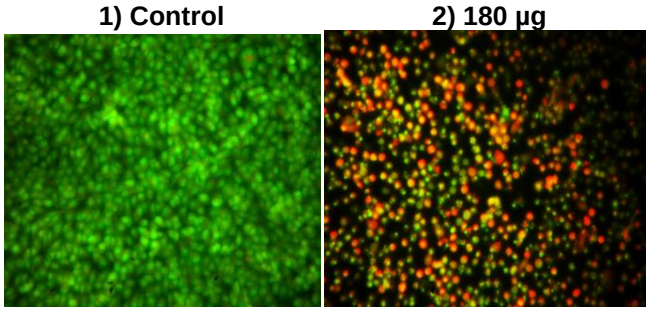

Fig. 8. Effect leaf extract in HeLa cell-lines inducing apoptosis

3.5 Anti-inflammatory Activity

3.5.1 Carrageenan induced paw edema in rats

Chloroform leaf extract concentrations of 50 mg/kg and 250 mg/kg significantly reduced paw volume. Paw volume was significantly decreased between hours 2 and 4 after application of chloroform leaf extract at 50 and 250 mg/kg (P<0.01 and P<0.001, respectively). Paw size was considerably (P<0.001) reduced at the two and four hour intervals when indomethacin (10 mg/kg) was compared to vehicle control. At 2 hours, it was discovered that chloroform leaf extract at 50 mg/kg and 250 mg/kg inhibited changes in paw volume by 34.58% and 47.24%, respectively. However, the maximal percentage inhibition was discovered to be at 4h (51.75% and 58.74% for 50mg/kg and 250mg/kg of chloroform leaf extract, respectively). When compared to a carrageenan control animal, Indomethacin (10 mg/kg) was shown to have a percentage inhibition at 2nd and 4th hours of 55.61% and 59.44%, respectively.

3.6 Anti-Pyretic Studies

3.6.1 Brewers's yeast induced pyrexia

Chloroform leaf extracts showed significant antipyretic effectiveness (*P*< 0.01). The high rectal temperature dramatically lowered four hours after being treated with either 250 or 500 mg/kg of chloroform extract. Initial and final rectal temperatures in the chloroform extract (250 and 500 mg/kg) and paracetamol (150mg/kg) groups were 103.9±21.76 and 98.95±1.20, 103.44±1.33 and 98.33±1.50, and 102.96±1.60 and 98.35±1.88 F, respectively. The antipyretic effects of both paracetamol and the chloroform extract persisted throughout the 4-hour test period.

Table 6. Anti-inflammatory activity (% of inhibition) at different time intervals

Drug Treatment	Paw Thickness (mm)			
	30min	60min	120min	240min
Control 0.1% CMC (1ml/kg)	-	-	-	-
Indomethacin (10mg/kg)	40.60	41.60	55.61	59.44
Ludwigia perennis leaf extract 50mg/kg	23.54	31.29	34.58	51.75
Ludwigia perennis leaf extract 250mg/kg	34.34	40.46	47.24	58.74

Table 7. Effects of chloroform leaf extract of *Ludwigia perennis* on Brewer's yeast-induced pyrexia in rats

Groups	Drug Treatment	Rectal Temperature in Fahrenheit				
		Basal	0hr	1hr	2hr	4hr
I	Vehicle Control Saline (1ml/kg)	98.34±1.22	103.75±1.45	103.89±1.05	103.79±1.66	102.60±1.54
II	Reference Control Paracetamol (100mg/kg)	98.57±1.65	102.96±1.60	100.08±1.12*	98.95±1.20**	98.35±1.88**
III	*Ludwigia perennis* (250mg/kg)	98.44±1.32	103.92±1.76	102.75±1.08	100.62±1.87*	98.95±1.20*
IV	*Ludwigia perennis* (500mg/kg)	98.54±1.40	103.44±1.33	101.20±1.47*	99.62±1.87**	98.33±1.50**

*Values are in mean ± SEM; (n = 6) *P < 0.05, **P < 0.01,***P < 0.001, Vs Vehicle Control*

4. DISCUSSION

The present study intended to assess the pharmacological potential of *Ludwigia perennis* chloroform leaf extracts. Antioxidants prevent cell and tissue damage in addition to acting as scavengers. Cells can protect themselves from excessive free radicals by using preventative procedures, repair mechanisms, physical barriers, and antioxidants [31]. Antioxidant activity has been reported for the first time in the plant *Ludwigia perennis*. Postprandial glucose elevation is caused by the conversion of dietary starch and oligosaccharides to glucose by the enzymes α-amylase and α-glucosidase. [32]. The chloroform leaf extract of *Ludwigia perennis* has anti-diabetic properties. Phytic acid, tannins, steroids, alkaloids, saponins, flavonoids, and cyanogens are few of the anti-nutrients that can be found in plants, even though they do provide nutrition. These anti-nutrients prevent the body from utilising proteins and minerals like iron and zinc or interfere with their assimilation [33]. Phytate, oxalate, alkaloids, and saponin are the anti-nutrients reported in this plant's leaves. According to reports, the bioactive chemicals derived from plants target a variety of cancer-causing pathways, such as NF-B, apoptosis, and CDK inhibitors [34]. Chloroform leaf extract of *Ludwigia perennis* was treated with HeLa cell lines (for cervical cancer), and it shows anticancer activity. As a result, in this scenario, it is best to use all-natural ingredients that also have anti-inflammatory and antioxidant qualities. There are several plant extracts with anti-inflammatory properties in addition to antioxidant properties [35]. Numerous studies indicate that phytochemicals increase cell lifetime, prevent ageing, and reduce propensity to certain inflammatory illnesses [36]. Carrageenan-induced paw edema was used in a rat in vivo experiment to measure the anti-inflammatory effects. Rats with yeast-induced hyperpyrexia were used to examine the extract's antipyretic effects. The rat models used in this study provided clear evidence of the sample's anti-inflammatory and fever-reducing properties. Altogether, this study explains *Ludwigia perennis* chloroform leaf extract's therapeutic properties because it has antioxidant, antidiabetic, antinutritional, anticancer, anti-inflammatory, and antipyretic properties.

5. CONCLUSION

According to the current study, *Ludwigia perennis* chloroform leaf extract contains medicinally useful components which can reduce or cure complicated ailments. The creation of new bioactive chemicals for treating various diseases is anticipated to be aided by further study of the active components of plants.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The experimental procedures, protocols, and use of animals in this study were evaluated by the institution's animal ethics committee (938/Po/Pe/S/06/CPCSEA) to ensure they satisfied ethical requirements. (Proposal Number: SLIMS/06/IAEC/2022–23).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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