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Bioactive Potential of *Parthenium hysterophorus* and Cytotoxicity Assay of Parthenin

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Abstract

Parthenium hysterophorus belongs to the family Asteriaceae. It is rich in hormones, carbohydrates, alkaloids, steroids, tannins, saponins etc. and hence can be used as traditional medicine. Solvent extracts of leaves was tested against pathogenic bacteria and fungi, by using Disk Diffusion method. Qualitative analysis of aqueous leaf extract was studied for the phytochemical screening. Phytochemical analysis shows the presence of steroids, phenol, saponins, tannin, flavonoids, terpanoids, carbohydrates etc. Fluorescence analysis of leaf powder was carried out. Parthenin compound was extracted from *Parthenium hysterophorus* and was tested for used for cytotoxicity activity using MBMD231, breast cancer cell line. Further studies were carried out on detection of anti-diabetic activity by two ways i.e. α-amylase inhibition which showed 75 ± 1.04 % activity and glucose diffusion inhibitory study showed 20% relative movement. *Parthenium* leaf extract have 74.3 ±0.3 % anti-oxidant activity using DPPH method while 0.81 ±0.04 mg of Trolox equivalent/ gram using FRAP assay. But only 18.18±0.02 % of anti-arthritic and 52.72 ± 0.018 % thrombolytic activity. Cytotoxic assay was on MDMB231 cell line showed that parthenin have very low cytotoxicity. Anti-helminthic activity was done using *Asonia putida* earthworm. No paralysis or death of earthworm was found within 24 hours by aqueous leaf extract of *P. hysterophorus*.

Keywords
Anti-arthritic and thrombolytic, Anti-diabetic, Anti-oxidant, Anti-helminthic, Cytotoxicity assay, Fluorescence analysis, Parthenin.

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1. INTRODUCTION:

Parthenium hysterophorus is an aggressive ubiquitous annual noxious weed. It is usually known as carrot grass, white top, congress grass, star weed, santa-maria feverfew, bitter weed. This harmful weed is frequently spotted on road sides, parks, drainage lines, water supply canals and mostly in fields with crops. Some allergic respiratory problems, contact dermatitis, mutagenicity in humans and livestock can caused due to regular contact with this weed [1]. This weed was introduced in India as a contagion in PL 480 Wheat (Public Law 480) passed

in 1954 to give food grains to developing countries in crisis condition [2]. *P. hysterophorus* was accidently introduced in India, 1955, in Pune with import of grains [3].

Chemical analysis of *P. hysterophorus* has indicated that all its parts contains toxins called sesquiterpene lactons (SQL). *P. hysterophorus* contains a bitter glycoside parthenin, a major sesquiterpene lactone [4]. Phytotoxic compounds or allelochemicals that are present in *P. hysterophorus* are hysterin, ambrosin, flavonoids such as quercelagetin 3,7-dimethylether, 6-hydroxyl kaempferol 3-0 arabinoglucoside, fumaric acid. P-hydroxy benzoin and vanillic acid, caffeic acid, p courmaric, anisic acid, p-anisic acid, chlorogenic acid, ferulic acid, sitosterol and some unidentified alcohols [1]. Parthenin, hymenin and ambrosin are found to cause health hazards in humans [5]. *Parthenium hysterophorus* present in different geographical regions exhibited parthenin, hymenin, coronopilin, dihydroisoparthenin, hysterin, hysterophorin and tetraneurin A as the principal constituents of their sesquiterpene lactones [6]. A novel hydroxyproline-rich glycoprotein as the major allergen in *P. hysterophorus* pollen was identified by Gupta *et al*., 1996 [7] and Das *et al*., (2007) [8].

P. hysterophorus affecting food and fodder crops, since the pollen and dust of this weed cause allergic contact dermatitis in humans [9], [10]. Dermatitis is a T cell-mediated immune injury and the disease manifests as itchy erythematous papules and papulovesicular lesions on exposed areas of the body [11]. Cytotoxicity of the sesquiterpene lactone Parthenin is related with the T-cell mediated immune injuries [12]. Skin inflammation, asthma, allergic rhinitis, hay fever, black spots, burning and blisters around eyes are symptoms cause due to continuous exposure to this plant. It also causes diarrhea, breathlessness and choking [4]. Allergic bronchitis is caused due to exposure to *P. hysterophorus* pollens [13].

With this negative and harmful side, it also have some positive and economical importance. Weeds are valuable sources as they are available free of cost, can grow without farming and without any specific conditions [14]. Instead of eradication of *Parthenium* its constituent can Be used for our purpose [14]. According to researchers this *P. hysterophorus* can be used as a green leaf manure, as a compost as well as biopesticide [15], [16]. Untreated *P. hysterophorus* weed biomass evaluated as a substrate for cellulose production [17].

The present study was carried out to determine antimicrobial, antioxidant, anti-helminthic, antidiabetic, anti-arthritic, thrombolytic and cytotoxicity activity of *P. hysterophorus* leaf extract.

2. MATERIALS AND METHODS:

The leaves of *Parthenium hysterophorus* were collected in month of January, 2019 from local area of Manjari (Pune, Maharashtra).

Fig 1: *P. hysterophorus* leaves samples

2.1 Extraction of *Parthenium hysterophorus* leaves:

Leaves of *P. hysterophorus* were collected, washed and dried in shade To remove surface moisture. The dried leaves were then ground to fine powder.

2.1. a Extraction method:

P. hysterophorus leaves extract was obtained using water and ethanol solvents. Extraction was done using Soxhlet apparatus and temperature was adjusted to 55°C.

2.2 Determination of phytochemicals from leaves extract:

The qualitative tests were performed for detection of phytochemicals of *P. hysterophorus* leaf extract. Phytochemical study of leaf extract was carried out for detection of phytoconstituents viz., alkaloids, flavonoids, phenol, steroids, tannins, carbohydrates and proteins [18].

2.2. a Qualitative test of phytochemicals:

i. Test for Alkaloids:

The leaf extract was dissolved in dilute HCl and used for Mayer's and Wagner's test to determine the alkaloid content.

i.a. Mayer's test:

Few ml of extract was treated with Mayer's reagent (1.36 gm of mercuric chloride and 5 gm of Potassium iodide in 100 ml water) by side of test tube. White or creamy precipitation was considered as positive test.

i.b. Wagner's test:

Few ml of extract was treated with Wagner's reagent (1.27 gm iodine, 2 gm potassium iodide, and 100 ml distilled water) by side of tube. Reddish brown precipitation was observed.

ii. Test for Flavonoids:

Few drops of dilute NaOH solution was added to 1 ml of extract. Intense yellow colour solution was become colorless after addition of few drops of dilute HCl which indicates positive test.

iii. Test for Phenol:

Extract was dissolved in water then treated with 10% $FeCl_3$. Dark green color formation indicated presence of phenol.

iv. Test for Terpenoids:

5 ml of extract was dissolved in 2 ml of $CHCl_3$ and 1 ml of concentrated H_2SO_4. Reddish brown colored interface was observed.

v. Test for Steroids:

In 0.5 ml of extract, 5 ml of Chloroform and equal volume of concentrated H_2SO_4 by sides of tube was added. Upper layered turned into red and H_2SO_4 layer showed yellow color with green fluorescence.

vi. Test for Resins:

Extract was treated with 3-4 ml of $CuSO_4$solution. After proper mixing for 1-2 min, green precipitation proves resins.

vii. Test for Tannins:

Extract was dissolved in 50 ml of distilled water and it was filtered. After addition of 1% $FeCl_3$to the filtrate, appearance of green, purple, blue or black color indicated positive test.

viii. Test for Quinones:

Extract was treated with KOH solution and red color was observed.

ix. Test for Carboxylic acid:

Extract was mixed with sodium bicarbonate solution and checked for effervescence.

x. Test for Oxylates:

4 ml of extract was mixed with 2 ml of acetic acid, 1 drop of $FeCl_3$ and 2 ml of concentrated H_2SO_4. Brown ring indicated positive test for Oxylates.

xi. Test for Carbohydrates:

Hot Fehling's solution was added to 4ml of extract and red brick colored precipitation indicated carbohydrate content.

xii. Test for Proteins:

Ninhydrin reagent was added to the extract. Mixture was boiled and blue color was observed [18].

2.2. b. Thin layer chromatography of *P. hysterophorus* leaves extract:

Thin layer chromatography technique (TLC) was carried out using ethanol extract of the *P. hysterophorus* leaves. The phytoconstituents were qualitatively determined using following solvent system.

Table 1: TLC solvent system and its developer for *P. hysterophorus* leaf extract.

Compound	Solvent system	Developer
Alkaloid	chloroform: methanol: acetone 8:1:1	Dragendorff reagent
Phenol	glacial acetic acid: n-butanol: water 1:4:1	$FeCl_3$ (2% in ethanol)
Flavonoid	chloroform: methanol: water 4:3:1	Long UV (365nm)

2.3 Fluroscence Analysis of *P. hysterophorus* leaves extract:

Dried leaves powder of 0.5gm was taken into clean and dry test tubes. To each tubes 5ml off different organic solvents like distilled water, acetone, methanol, ethanol, benzene, chloroform, diethyl ether, petroleum ether, gl acial acetic acid, picric acid, sulphuric acid, nitric acid, hydrochloric acid, 5% $FeCl_3$, 5% iodine, 1 N NaOH and 1 N NaOH + Methanol were added separately. Then all the tubes were shaken, and they were allowed to stand about 20-25 minutes. These solutions were observed under the different wavelengths i.e., visible light and UV light (254 and 365 nm) for their characteristic color reaction and compared with a standard color chart, colors were recorded [18].

2.4 Determination of antimicrobial activity of *P. hysterophorus* leaves extract:

Antimicrobial activity was done by disc diffusion method. Different human pathogens i. e. bacterial as

well as fungal were tested at O. D. =0.12 and 0.20 respectively at 600nm. [19].

2.5 Bioautography:

Bioautography is a technique used for testing antimicrobial activity against plant extract as well as separation of chemical compounds present in extract. Immersion bioautography i. e. agar overlay method was used for detection of antimicrobial activity. TLC plate was loaded with 5µl of *P. hysterophorus* leaf extract. The solvent system used was chloroform: ethanol (30: 10). Evaporation of solvent was done. Developed TLC plate was placed on Sterile Muller Hinton agar plate (15 ml) for detection of antimicrobial activity. Molten Muller Hinton agar 5 ml was seeded with 1 ml of test organism suspension and poured on TLC plate kept on Muller Hinton agar. After solidification of agar, petri plates were kept for pre-diffusion at 4°C for 2 hours. Plates were then incubated at room temperature for 24 hours. After incubation plates were checked for zone of inhibition using 2 mg/ml phenyl tetrazolium chloride [20].

2.6 *In vitro* anti arthritic activity of *P. hysterophorus* leaves extract:

Inhibition of protein denaturation was used for evaluation of *in vitro* anti arthritic activity using diclophenac sodium as standard. [21]. 0.45 ml of bovine serum albumin (5% w/v aqueous solution) and 0.05 ml of test solution (100 µg/ml of extract) were used for the preparation of 0.5ml test solution. For the preparation of test control solution (0.5ml) 0.45ml of bovine serum albumin (5% w/v aqueous solution) and 0.05ml distilled water were used. Standard solution (0.5ml) consists of 0.45ml of bovine serum albumin (5% w/v aqueous solution) and 0.05ml of diclophenac sodium. pH was adjusted at 6.3 using 1 N HCl for all solutions. The samples were incubated at 37°C for 20 minutes and then temperature was increased to 57°C for 3 minutes. 2.5 ml of phosphate buffer was added (after cooling previous solution). Using UV- visible spectrophotometer the absorbance was measured at 416nm. 100% protein denaturation was represented by control tube.

The percentage inhibition of protein denaturation could be calculated as:

% inhibition = [100 – (absorbance of test solution – absorbance of control)] × 100

The results were compared with diclofenac solution [21].

2.7 *In vitro* thrombolytic test of *P. hysterophorus* leaves extract:

The dried leaf powder extract was dissolved in dimethyl sulfoxide. In five different pre-weighed sterile micro centrifuge tubes, 5ml of venous blood were distributed and incubated at 37°C for 45 minutes. Serum was completely removed after clot formation. 100 µl of the extract was added separately into pre-weighed clot micro centrifuge tube. To determine the clot weight (Clot weight = weigh of clot containing tube – weight of tube), 100 µl of the extract was assed separately into pre-weighed clot micro centrifuge tube. 100 µl of distilled water was used for negative control. All the tubes were incubated at 37°C for 90 minutes and observed for lysis of clot. Released fluid was removed after incubation and weight of the tubes were determined. After clot disruption difference in weight was calculated and expressed in percentage of clot lysis.

Percentage of clot lysis was determined by differences in weights taken before and after clot lysis as follows:

% clot lysis = (weight of released clot / clot weight) × 100 [22].

2.8 *In vitro* Antioxidant activity of *P. hysterophorus* leaves extract:

Two methods were carried out for in *vitro* antioxidant activity of methanolic and ethanolic extract of *Parthenium hysterophorus*: 1. FRAP (Ferric Reducing Antioxidant Power) Assay 2. Free radical scavenging DPPH

2.8.1 FRAP (Ferric reducing antioxidant power) Assay:

In FRAP assay the reduction of Fe^{3+} tripyridyltriazine (TPTZ) complex (colorless complex) to Fe^{2+} tripyridyltriazine (TPTZ) complex (blue color complex) formed by the action of electron donating antioxidants at low pH was done by mixing 300mM acetate buffer, 10ml tripyridyltriazine (TPTZ) in 40mM HCl and 20 mM $FeCl_3.6H_2O$ (in proportion of 10:1:1) at 37°C i.e., FRAP reagent. 5µl of leaves extract sample was added to freshly prepared FRAP reagent and ferric tripyridyltriazine (Fe^{3+} TPTZ) complex was reduced to ferrous (Fe^{2+}) form giving intense blue colour complex. For reagent blank, 3.995 ml FRAP reagent and 5 µl distilled water were added and incubated for 30 minutes at 37°C. The absorbance was measured at 593nm [23].

2.8.1.a Determination of total phenolic content by Folin-Ciocalteu method:

The leaf extract sample (0.5ml of different dilution) was mixed with 2.5 ml of 0.2 N Folin-Ciocalteu reagent for 5 minutes and 2.0 ml of 75 grams/lit sodium carbonate were then added. Mixture was incubated at room temperature for 2 hours. Absorbance was checked at 760nm [24].

2.8.1.b Determination of total flavonoid content by $AlCl_3$ method:

20 µl leaf extract sample was mixed with 20 ml of 2% $AlCl_3.6H_2O$solution. Double distilled water was added in mixture and mixed completely to make the total volume 10ml. Incubated for 10 minutes at room temperature and absorbance was measured at 440nm [23].

2.8.2 Free radicle scavenging DPPH assay:

DPPH is a common abbreviation for the organic chemical compound 2,2-diphenyl-1-picrylhydrazyl. To explore the use of herbal extracts as antioxidants, the free radicle scavenging activity was measured. [25]. For 3 mM solution, DPPH was prepared in methanol. 1 ml of methanol and 1 ml extract were added into tubes and 2 ml of the DPPH solution was mixed. The tubes were placed in the dark at room temperature for 30 minutes after stirred by vortex. The absorbance was measured at 517 nm. Tube containing 2ml of the DPPH methanol solution and 2.5 ml of methanol was used as negative control.

Ascorbic acid was used as synthetic antioxidant of reference. A percentage of discoloration scavage the free radical.

The percentage of antioxidant activity was determined according to the following equation:

$$\% \text{ Antioxidant activity} = \frac{\text{Absorbance measured of control} - \text{Absorbance measured of test}}{\text{Absorbance measured of control}} \times 100$$

[26].

2.9 *In vitro* Antidiabetic activity of *P. hysterophorus* leaves extract:

Two methods were used for the determination of *in vitro* antidiabetic activity 1. Inhibition assay for α-amylase activity 2. Glucose diffusion inhibitory study.

2.9.1 Inhibition assay for α-amylase activity:

The different four concentrations i.e. 25mg/ml, 50mg/ml, 75mg/ml and 100mg/ml of *Parthenium* leaf extract were prepared by dissolving in double distilled water. 500 µl of 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M sodium chloride) having α-amylase solution (0.5mg/ml) and total of 500 µl leaf extract were incubated at room temperature for 10 minutes. 500 µl of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M sodium chloride) was added after pre incubation to each tube at 5s intervals. The mixture was incubated at room temperature for 10 minutes. The reaction was stopped by adding 1 ml of DNSA reagent. Test tubes were then incubated for 5 minutes in boiling water bath and then kept at room temperature to be cooled. By adding 10ml of distilled water the reaction mixture was again diluted and absorbance was measured at 540nm. Glycomate GP 0.5 tablet was used as standard

% inhibition was calculated by given formula

$$\% \text{ inhibition} = \frac{A_{540}\ \text{Control} - A_{540}\ \text{Leaf Extract}}{A_{540}\ \text{Control}} \times 100$$

([27], [28])

2.9.2 Glucose diffusion inhibitory study:

Maceration method was used for preparation of extract. In a dialysis membrane (HiMedia Laboratories, India) 1 ml of leaf extract and 1 ml of glucose solution (0.22mM in 0.15 M sodium chloride) were placed. Dialysis bag was then tied tightly at both ends using dental floss. The dialysis bag was kept in the beaker containing 10 ml of distilled water and 40ml of 0.15 M sodium chloride. 1 ml of 0.15 M sodium chloride having 22mM glucose and 1 ml of distilled water was kept as control. The beakers were then kept on shaker at room temperature. Movement of the glucose into the outer solution was observed and recorded at every half hour for total 6 times (in 3 hours). ([27], [28])

2.10 *In vitro* anti-helminthic activity of *P. hysterophorus* leaves extract:

An adult Indian earthworm *Asonia putida* was used for anthelmintic activity of ethanol and aqueous extract of *P. hysterophorus* leaves. Earthworms, each of average length of 6 cm, were placed in petri-plates containing 2 ml of each extract solution. By tapping the end of each worm with the index finger and applying a bit of pressure, the worms that were observed for the sign of paralysis, less movement and death. As a control earthworm were kept in saline. Dead earthworms were non-motile while live showed motility [29].

2.11 Extraction and fractionation of Parthenin from *P. hysterophorus* leaves powder:

10 gm of leaves powder was macerated with 60 ml of methanol in duration of 24 hours. The obtained

extract was concentrated until about half of the original volume. An equal volume of water was added. Mixture was filtered. After filtration, the obtained solution was extracted three times with 20 ml of chloroform. The chloroform extract was evaporated. Yellowish oily substance was obtained. The concentrated chloroform fraction was applied on a silica gel column and eluted with a dichloromethane: methanol (3:1) mixture. Fraction of 3 ml were collected and all were analyzed by TLC using chloroform: acetone (3:1) as mobile phase. The spots were detected with UV at 254nm and after spraying the vallinin reagent. The fraction containing parthenin, recognized by the violet zone with R_f value of about 0.6. The compound was further used for cytotoxicity assay [30].

2.12 *In vitro* Cytotoxicity assay of Parthenin compound obtained from *P. hysterophorus* leaves extract:

Annexin-V-FITC/PI. Necrotic and apoptotic cells were differentiated using the Annexin assay V-FITC kit (Sigma-Aldrich, MO, USA) as per the manufacturer's protocol. Cells from MDMB231 cell line was plated at 5×10^6 and incubated for 24 hours. Then extracted parthenin compound were added as 30 µg/ml and 50 µg/ml respectively. Plates were incubated for 24, 48 and 72 hours. The cells were centrifuged at 1000 rpm for 5 minutes and resuspended in 1× Annexin-V binding buffer. Cells were stained with 10µl of Propidium Iodide and 5µl of Annexin-V-FITC for 10 minutes in dark. Analysis was done by flow cytometry [31].

3. RESULT AND DISCUSSION:

3.1 Extraction of *Parthenium hysterophorus* leaves:

P. hysterophorus leaves were collected and shade dried. Leaf powder was used for Soxhlet extraction using water and ethanol solvent.

3.2 Phytochemical analysis:

The primary phytochemical study of *Parthenium* leaves extract for the aqueous and ethanol solvents showed the presence of alkaloids, flavonoids, phenols, terpenoids, steroids, tannin, quinone, carbohydrate and protein. *Parthenium* leaves are very rich in nutrients due to its ability to absorb all the elements from soil.

Table 2: Determination of phytochemicals of *P. hysterophorus* leaves extract

	***P. hysterophorus* leaf extract**	
	Aqueous extract	**Ethanol extract**
1. Alkaloids	+	+
a) Mayer's test	+	+
b) Wagner's test	+	+
2. Flavonoids	+	+
3. Phenols	+	+
4. Terpenoids	+	+
5. Steroid	+	+
6. Resin	+	+
7. Tannin	+	+
8. Quinone	+	+
9. Carboxylic acid	+	+
10. Oxylates	+	+
11. Carbohydrate	+	+
12. Protein	+	+

Key: + =Present

3.2.1 Thin layer chromatography of *P. hysterophorus* leaves extract: Thin layer chromatography technique was used for the quantitative determination of phytochemicals present in *Parthenium* leaves aqueous extract. R_f value of compounds were measured. Phenol had R_f value 0.83, flavonoid had R_f value 0.45 and alkaloid have R_f value of 0.9.

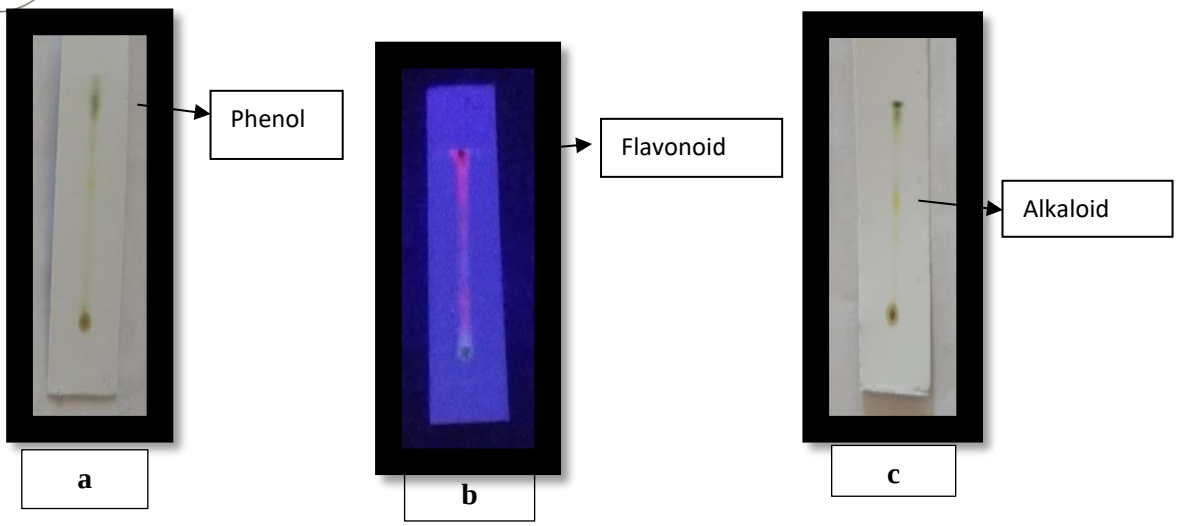

Fig 1: Thin layer chromatography of phytochemicals in the Soxhlet extract of *P. hysterophorus* leaves.

a: Thin layer chromatography of phenol using glacial acetic acid: n-butanol: water (1:4:1) solvent system and $FeCl_3$ (2% in ethanol) developer.
b: Thin layer chromatography of flavonoid using chloroform: methanol: water (4:3:1) solvent system and Long UV (365nm) developer.
c: Thin layer chromatography of alkaloid using chloroform: methanol: acetone (8:1:1) solvent system and Dragendorff reagent developer

3.3 Fluorescence analysis of *P. hysterophorus* leaf powder: The organic solvents such as acetone, methanol, 1N NaOH + methanol and chloroform showed red fluorescence under long UV i.e., under 365nm wavelength. Ethanol and diethyl ether solvents showed orange fluorescence under long UV. Other organic solvents showed black fluorescence under 365nm wavelength.

Table 3: Fluorescence analysis of *P. hysterophorus* leaf powder

Powder + Organic Solvent	Visible Rays	Short UV (254 nm)	Long UV (365 nm)
Powder + Distilled water	Pale yellow	Black	Black
Powder + Acetone	Reddish brown	Greenish black	Red
Powder + Ethanol	Greenish red	Greenish black	Orange
Powder + Methanol	Greenish red	Greenish black	Red
Powder + Glacial Acetic Acid	Brown	Black	Black
Powder + Sulfuric acid	Black	Black	Blueish grey
Powder + Nitric acid	Orange	Yellow	Black
Powder + Hydrochloric acid	Black	Black	Black
Powder + 5% FeCl3	Black	Black	Black
Powder + Picric acid	Lemon yellow	Pale yellow	Black
Powder + 5% I2	Grey	Black	Black
Powder + 1 N NaOH	Pale yellow	Greenish black	Black
Powder + 1 N NaOH and Methanol	Reddish yellow	Greenish black	Dark red
Powder + Chloroform	Wine red	Black	Dark red
Powder + Diethyl ether	Red	Black	Orange
Powder + Petroleum ether	Yellow	Yellowish green	Dark Brown

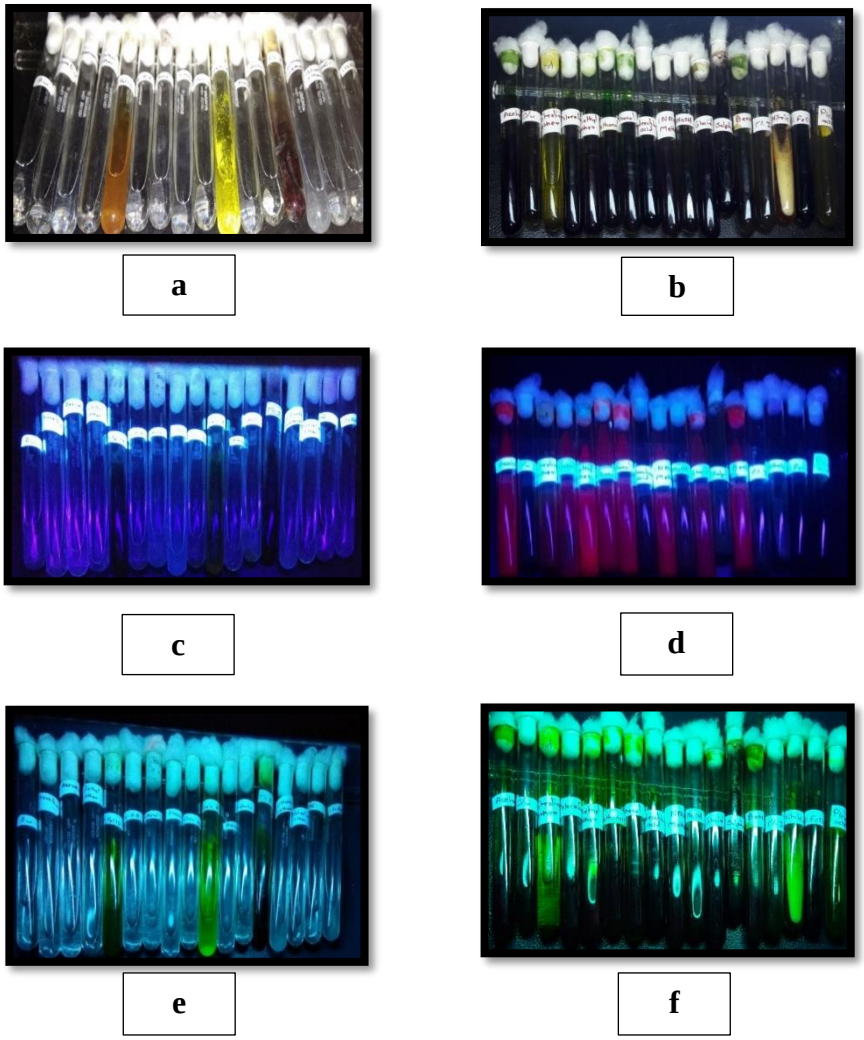

Fig 3: Fluorescence analysis of *P. hysterophorus* leaves extract observation under UV trans illuminator.

a: Solvent (Control) fluorescence under Short UV (254nm), b: Fluorescence under Short UV (254nm), c: Solvent (Control) fluorescence under long UV (365nm), d: fluorescence under long UV (365nm), e: Solvent (Control) fluorescence under visible light & f: fluorescence under visible light

3.4 Determination of antimicrobial activity of *P. hysterophorus* of leaves extract:

The aqueous and ethanolic extract of *Parthenium* leaves were tested against human pathogens i.e. bacterial as well as fungal. Those extract were effective against pathogens which were tested

Table 4: Determination of antibacterial activity

Name of pathogen	[Zone of inhibition (mm)] Mean ± SD	
	Aqueous extract	Ethanolic extract
Bacteria	**Leaves**	**Leaves**
Pseudomonas	12 ±0.15	10 ± 2.51
Klebsiella	14±0.17	15 ±1.52
Bacillus	13 ±0.10	12 ±0.36

Table 5: Determination of antifungal activity

Name of pathogen	[Zone of inhibition (mm)] Mean ± SD	
	Aqueous extract	Ethanolic extract
Fungi	**Leaves**	**Leaves**
Candida	17 ±3.51	17 ±3.05
Penicillium	14 ±2.64	15 ±0.5

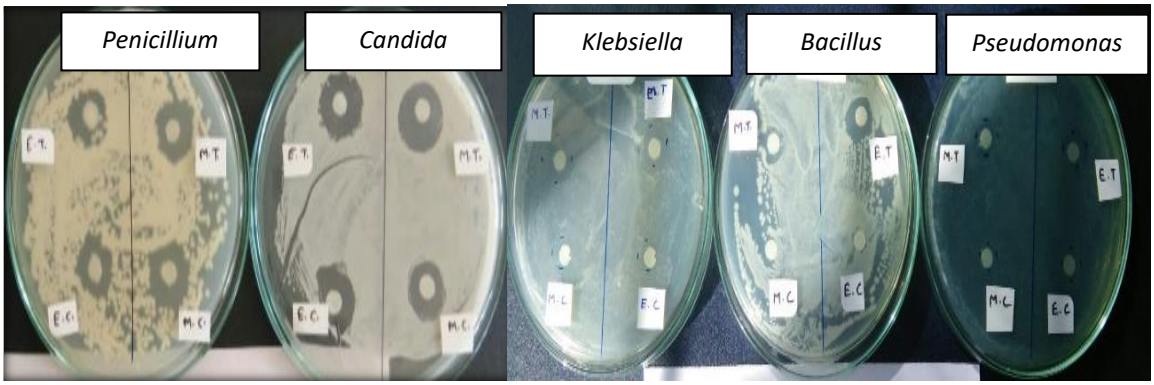

Fig 4: Antimicrobial activity of *Parthenium hysterophorus*

3.5 Bioautography: Bioautography of ethanolic extract against *Klebsiella* was done by immersion method. R*f* value of bioactive compound was found to be 0.6 and zone of inhibition was found to be 20 mm.

Fig 5: Bioautography of *Parthenium* leaves ethanolic extract against *Klebsiella*

3.6 *In vitro* anti arthritic activity of *P. hysterophorus* leaves extract result:

The aqueous and ethanol extracts were used to determine anti arthritic activity of *Parthenium* leaves. Results were compared with standard diclofenac sodium. The aqueous and ethanolic extracts showed exhibition 18.18% and 14.28% respectively where the standard showed activity 100%.

Table 6: Determination of *In vitro* anti arthritic activity:

Sr. No.	Sample used	Mean ± SD (% Activity)
1	*Parthenium* leaves aqueous extract	18.18±0.02
2	*Parthenium* leaves ethanol extract	14.28±0.12

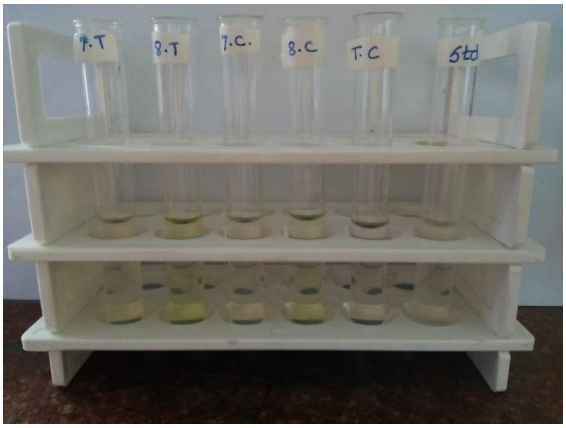

Fig 6: Anti arthritic activity of *P. hysterophorus* leaves extract

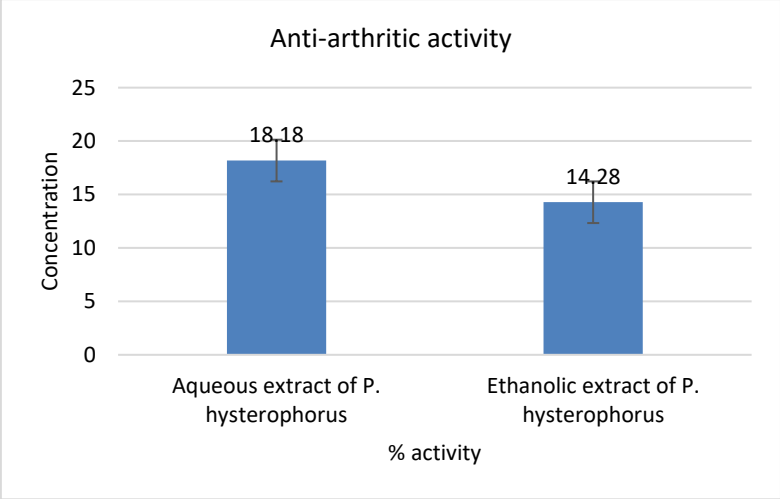

Fig 7: Bar diagram of result of anti-arthritic activity of *P. hysterophorus* leaves extract

3.7 *In vitro* thrombolytic activity of *P. hysterophorus* leaves extract result:

Parthenium leaves aqueous and ethanolic extract were used for testing thrombolytic activity. Aqueous and ethanolic extract of *P. hysterophorus* showed clot lysis 52.72% and 51.37% respectively. Distilled water was used as negative control which did not showed any activity 0% clot lysis.

Table 7: Determination of *In vitro* thrombolytic activity:

Sr. No	Sample used	Weight of the empty tube (A) gm	Weight of the tube with clot (B) gm	Weight of clot. (C) C=B – A	Weight of the tube with clot after lysis (D) gm	Weight of lysis =(B-D) (gm)	Mean ± SD (% of clot lysis)
1	*Parthenium* leaves aqueous extract	1.02	2.120	1.1	1.54	0.58	52.72 ± 0.018
2	*Parthenium* leaves ethanolic extract	1.03	2.120	1.09	1.56	0.56	51.37 ± 0.016
3	Distilled Water	1.04	2.030	0.99	2.029	0	0

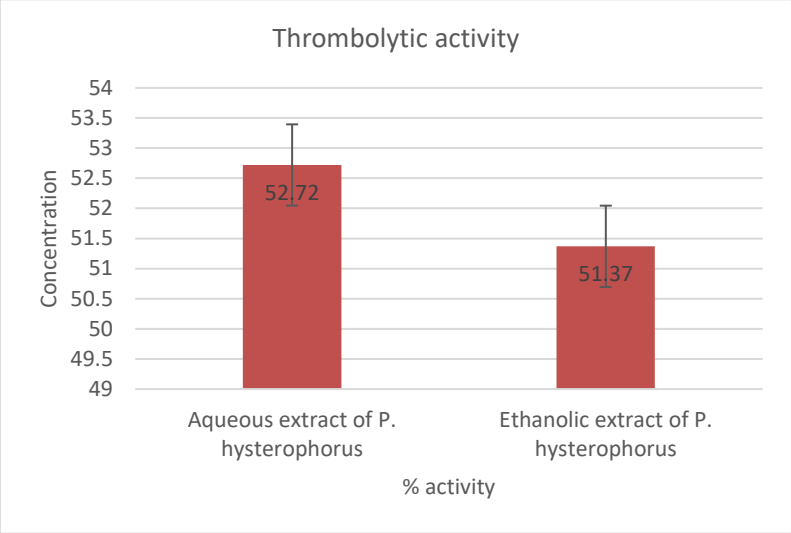

Fig 8: Bar diagram of result of *in vitro* thrombolytic activity of *P. hysterophorus* leaves extract

3.8 *In vitro* Antioxidant activity of *P. hysterophorus* leaves extract.

3.8.1 *In vitro* Antioxidant activity: FRAP Assay result

Evaluation of antioxidant activity of plant extract was determined by FRAP and DPPH assays. Total phenolic content and flavonoid content were determined. *Parthenium* hot water and hot ethanolic extracts were used for the activity. The reducing potential of an antioxidant reacting with a ferric tripyridyltriazine (Fe^{3+} - TPTZ) complex and producing a colored ferrous tripyridyltriazine (Fe^{2+} - TPTZ) was measured by FRAP assay (Rajurkar *et al.*, 2011). Absorbance was measured at 593nm at low pH, 3.6, reduction of Fe^{3+} - TPTZ complex to blur colored Fe^{2+} - TPTZ complex. Total phenolic content was measured at 760nm by using Folin-Ciocalteu method. Total flavonoid content was measured at 440 nm by using $AlCl_3$ method.

Table 8: FRAP assay result:

Sr.no	Sample used	mg of Trolox equivalent/ gram
1	*Parthenium* leaves aqueous extract	0.81 ± 0.04
2	*Parthenium* leaves ethanolic extract	0.61 ± 0.02

Fig 9: Antioxidant activity of *P. hysterophorus* using FRAP Assay.

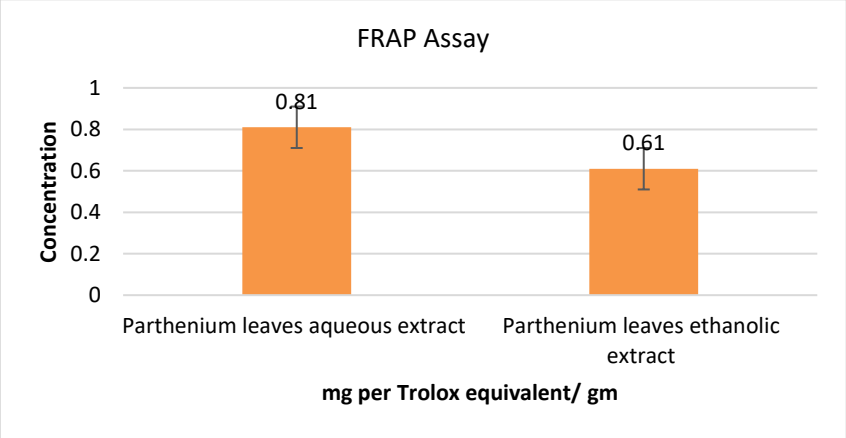

Fig 10: Bar diagram of result of *in vitro* antioxidant activity of *P. hysterophorus* leaves extract (FRAP assay)

Table no. 9 Total phenolic and flavonoid content values of *P. hysterophorus* leaves extract:

Sr. no	Sample used	Total phenolic content (mg/gm)	Total flavonoid content (mg/gm)
1	*P. hysterophorus* leaves extract	1158	464

3.8.2 *In vitro* Antioxidant activity: DPPH Assay result

DPPH assay is one of the easy methods for determination of antioxidant activity. *Parthenium* leaves aqueous extract and methanolic extracts were studied. Ascorbic acid was used as standard and gave activity 74.3%. *Parthenium* leaves aqueous extract showed 71.7% activity and methanolic extract showed similar activity as standard i.e. 74.3%.

Table 10: DPPH assay result table:

Sr. no.	Sample used	Mean ± SD (% Activity)
1	*Parthenium* leaves aqueous extract	71.7 ± 0.3
2	*Parthenium* leaves methanolic extract	74.3 ± 0.3

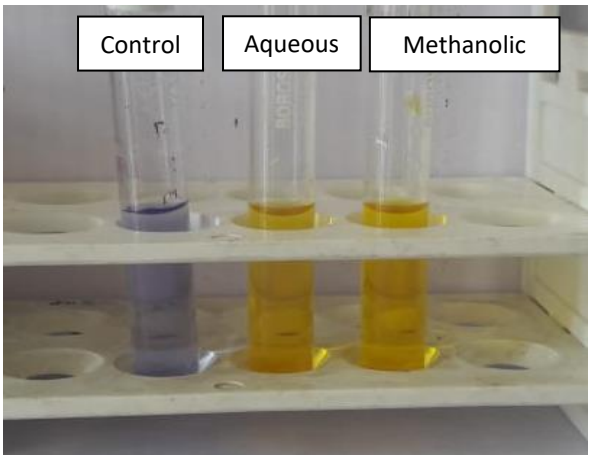

Fig 11: *In vitro* Antioxidant activity of *P. hysterophorus* leaves extract using DPPH Assay.

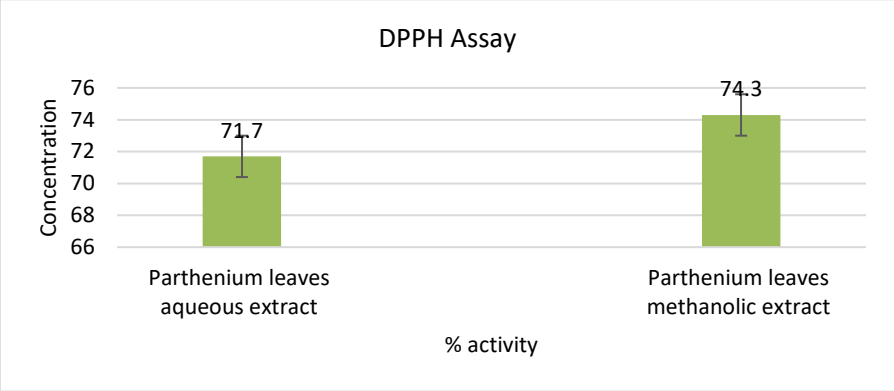

Fig 12: Bar diagram of result of *in vitro* antioxidant activity of *P. hysterophorus* leaves extract (DPPH assay)

3.9 *In vitro* Antidiabetic activity of *P. hysterophorus* leaves extract

3.9.1 Inhibition of α- amylase activity result

Antidiabetic activity was measured by α- amylase inhibition assay. Unienzyme tablet was used as source of α-amylase. 75 mg/ml concentration of *Parthenium* leaves extract showed maximum inhibition of α-amylase enzyme i.e. 75% while 100% mg/ml plant extract showed lowest inhibition of α-amylase enzyme i.e. 66%.

Fig 13: *In vitro* antidiabetic activity of *P. hysterophorus* leaves extract based on inhibition of α-amylase activity

Table 11: Inhibition of α- amylase activity result table

Sr. no	Concentration of *Parthenium* leaves extract (mg/ml)	Mean ± SD (% Activity)
1	25	70 ± 0.45
2	50	73 ± 0.65
3	75	75 ± 1.04
4	100	66 ± 1.14

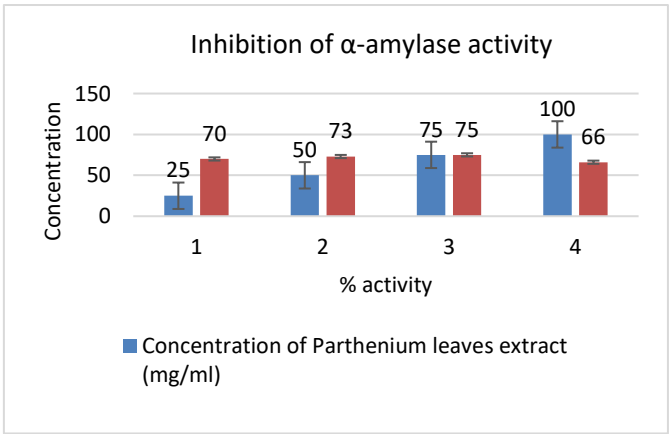

Fig 14: Bar diagram of result of *in vitro* antidiabetic activity of *P. hysterophorus* leaves extract (inhibition of α-amylase enzyme)

3.9.2 *In vitro* Antidiabetic activity result: Glucose diffusion inhibitory study: *Parthenium hysterophorus* showed least inhibitory activity in glucose diffusion till 150 minutes. At 180 minutes it showed significant inhibitory activity.

Fig 15: *In vitro* antidiabetic activity of *P. hysterophorus* leaves extract (glucose diffusion inhibitory study)

Table 12: Glucose diffusion inhibitory study result table

Sr. no.	Time (Minutes)	% Relative movement
1	30	92
2	60	96
3	90	96
4	120	96
5	150	96
6	180	20

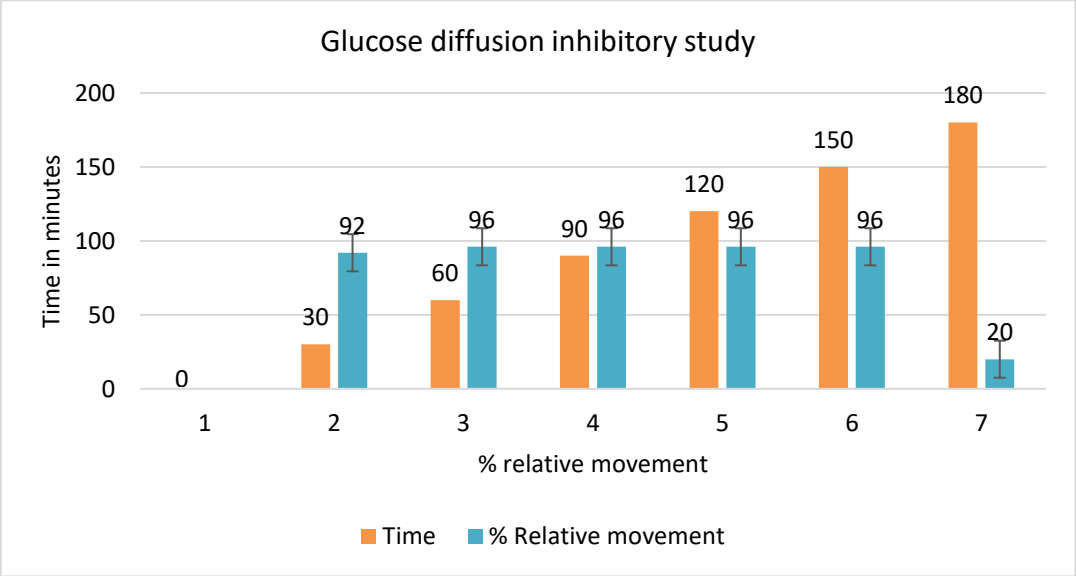

Fig 16: Bar diagram of result of *in vitro* antidiabetic activity of *P. hysterophorus* leaves extract (glucose diffusion inhibitory study)

3.10 Anti-helminthic activity of *Parthenium hysterophorus* leaves extract: An Indian earthworm *Asonia putida* was used to carry out Anti-helminthic activity. Ethanolic extract gave activity against *Asonia putida* whereas aqueous extract did not show any activity. In the presence of ethanolic extract, earthworm got paralyzed in 2 hours and after 3 hours earthworms were died. The earthworms in the presence of aqueous extract does not showed paralysis or death.

Table 13: Analysis of Anti-helminthic activity:

Sr. no.	Treatment	Paralysis time (hours)	Death time (hours)
1	Aqueous extract of *P. hysterophorus* leaf powder	-	-
2	Ethanolic extract of *P. hysterophorus* leaf powder	2 ± 0.01	3 ± 0.02
3	Saline (control)	-	-

3.11 Extraction and fractionation of Parthenin from *P. hysterophorus* leaves powder: Parthenin compound was recognized by the violet zone with R_f value of about 0.6. The compound was further used for cytotoxicity assay.

Figure 17: Extraction of parthenin by column chromatography

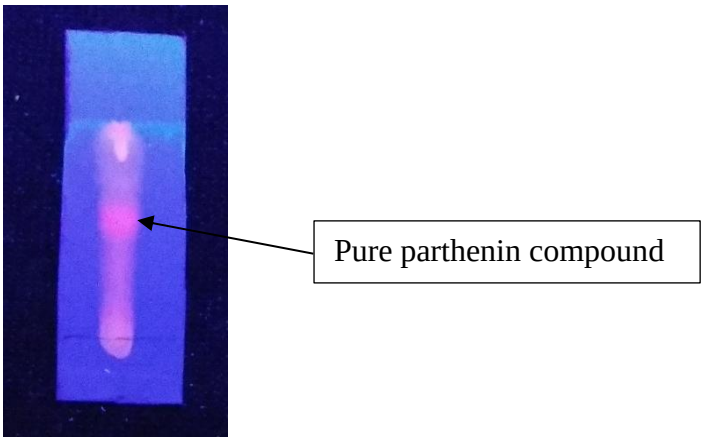

Fig 18: Thin layer chromatography of parthenin

R*f* value of compound was measured which was found to be 0.6. Solvent system used for TLC of parthenin was Chloroform: Acetone i.e. 6:2 and spots were detected with UV at 254nm.

3.12 *In vitro* cytotoxicity assay of Parthenin compound obtained from *P. hysterophorus* leaves extract results: Parthenin compound was extracted from *Parthenium hysterophorus* and was tested for

used for cytotoxicity activity using MDMB231, breast cancer cell line.

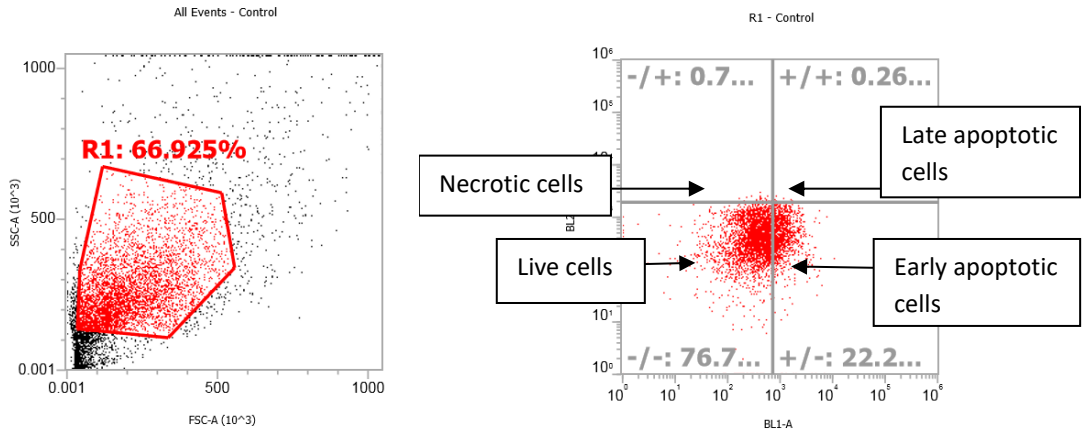

Fig 19: Cytotoxicity assay for control
Key: (+) dead cells (-) live cells

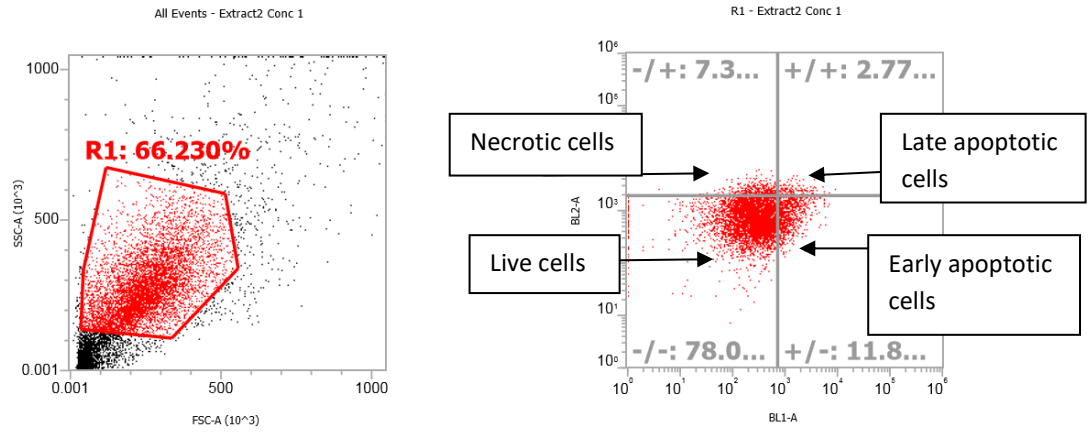

Fig 20: Cytotoxicity assay of *P. hysterophorus* at 30 µg/ml concentration
Key: (+) dead cells (-) live cells

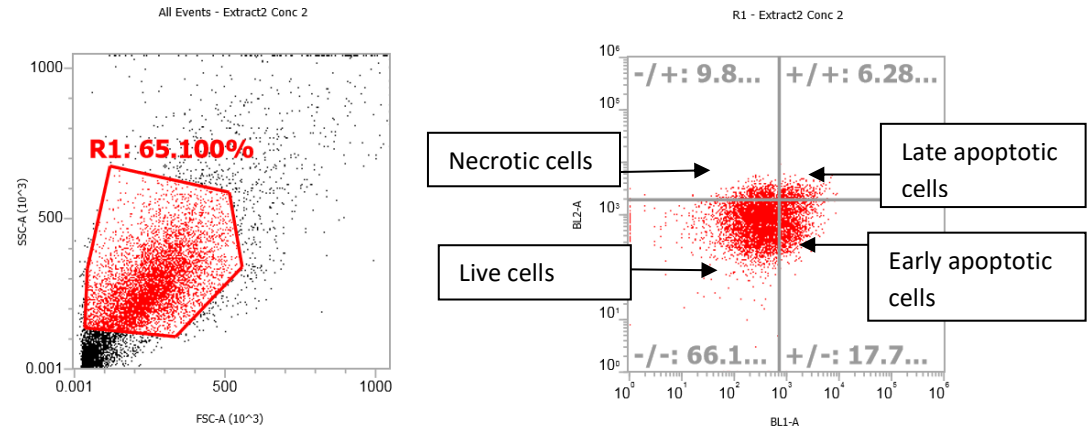

Fig 21: Cytotoxicity assay of *P. hysterophorus* at 50 µg/ml concentration
Key: (+) dead cells (-) live cells

Cytotoxicity assay was done using partially purified Parthenin compound. According to Akhtar *et al*., 2010, [11], Dermatitis is a T cell-mediated immune injury and the disease manifests as itchy erythematous papules and papulovesicular lesions on exposed areas of the body and according to the Narasimban *et al*., 1984, [12], these effects have been related to the cytotoxicity of the sesquiterpene lactone Parthenin. But the results of cytotoxicity assay of Parthenin are not in line with the previous report.

Table 14: Result of Cytotoxicity assay of *P. hysterophorus* at 30 µg/ml and 50µg/ml concentrations

Cells	Control	Concentration of Parthenin compound	
	-	30 µg/ml	50µg/ml
Total cells	50000	50000	50000
Targeted cells	33465	33000	32500
Alive cells	25433	25740	21450
Early apoptotic	7362	3960	5525
Late apoptotic	87	924	2048

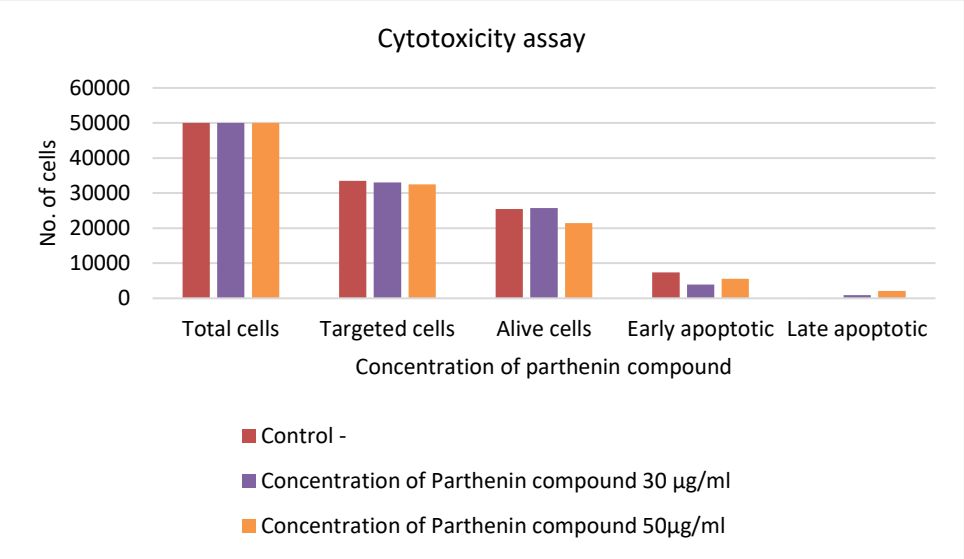

Fig 22: Bar diagram of result of Cytotoxicity assay of *P. hysterophorus* at 30 µg/ml and 50µg/ml concentrations

4. CONCLUSION:

The aqueous extract of *P. hysterophorus* contains alkaloids, flavonoids, steroids, phenols, carbohydrates, proteins etc. Due to its capability of absorbing all the nutrients from soil, it is very rich source of nutrients. It showed the more anti-microbial activity against human pathogens *Candida* and *Pseudomonas*. *P. hysterophorus* leaves extract showed significant thrombolytic activity, inhibition of α-amylase diffusion but least significant in glucose diffusion inhibition, anti-arthritic activity and anti-helminthic activity. Cytotoxicity assay of *P. hysterophorus* leaf extract showed less cytotoxic activity against MDMB231 cell line. *P. hysterophorus* is one of the dangerous weed which causes multiple harms to humans as well as animals but it also has the positive side of tremendous uses in environmental development. Weeds are cheap, readily available materials, so can be explored in herbal formulations and cosmetics.

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